## 343/W/Poster Board #1

**Increasing IKAP/ELP1 using transgenes improves splicing and extends development in Ikbkap null mice.** *Y. Chen[1], J. Mull[1], R. Shetty[1], L. Liu[1], M. Leyne[1], J. Pickel[2], S. Slaugenhaupt[1].* 1) Ctr Human Gen Res, HMS and Massachusetts General Hosp, Boston, MA; 2) National Institute of Mental Health, National Institutes of Health, Bethesda, MD.

Familial dysautonomia (FD) is one of the best known autosomal recessive sensory neuropathies. It is known that all FD patients carry at least one intronic mutation (IVS20+6T>C) in the IKBKAP gene that disrupts mRNA splicing and leads to tissue specific reduction of the IKBKAP/hELP1 protein, IKAP/hELP1. In FD patients, abnormalities in the nervous system suggest the importance of IKBKAP for neural development and function. To better understand the role of IKBKAP in vivo, and determine the underlying mechanism of FD, an Ikbkap knockout mouse line and several mouse lines that carry human wild-type or mutant IKBKAP transgene were created. We have recently reported that ablating mouse Ikbkap disrupts both neurulation and vascular development, and leads to embryonic lethality during gastrulation. To test whether the abnormalities caused by ablation of mouse Ikbkap could be rescued or ameliorated by the human wild-type or mutant IKBKAP gene, the transgenic lines that carry the human wild-type (TgWT) or different copy numbers of the mutant IKBKAP (TgFD) transgene were crossed with the Ikbkap knockout mouse lines. Results from our study show that embryonic lethality was rescued by TgWT and TgFD25, the line with the highest copy number of mutant IKBKAP transgene. These findings suggest not only the functional conservation of IKAP between human and mouse during development; more importantly, the rescue by TgFD25 indirectly demonstrates that FD results from abnormal reduction of IKAP and not from the presence of a truncated IKAP, as speculated by some researchers. Intriguingly, lower copy numbers of mutant IKBKAP transgene (TgFD1; TgFD3; TgFD4; TgFD9) could not overcome the lethality; however, they do prolong the survival of Ikbkap null embryos from 12.5 to 14.5 dpc. Further analyses indicated that increased expression of IKAP could transiently and differentially improve the embryonic development via a dosage-dependent manner. Treating pregnant mice with kinetin, a cytokinin that is capable of increasing the exon 20 inclusion in IKBKAP mRNA, could further improve the development of Ikbkap null embryos bearing mutant IKBKAP transgene, as revealed by the establishment of primitive cardiovascular and neural structures at 12.5 dpc. Results from current study demonstrate for the first time the existence of a stage-specific threshold for IKAP during development, and present a putative in vivo platform for testing drugs that might benefit FD patients in the future.

## 344/W/Poster Board #2

**A Defect in the N-Linked Glycosylation Pathway Causes Nonsyndromic Hearing Impairment in Mice.** *F. Probst[1], D. del Gaudio[1], A. Salinger[1], S. Gao[2], J. Oghalai[3], M. Justice[1].* 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Bioengineering, Rice University, Houston, TX; 3) Otolaryngology, Baylor College of Medicine, Houston, TX.

The study of mouse deafness mutants has lead to the identification of a number of human hearing impairment genes. The novel ENU-induced mouse deafness mutation *nse5* is a therefore a potential model of human hearing impairment. Homozygous *nse5* mutants have abnormal auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) to sounds up to 80 decibels (dB) at frequencies ranging from 4 to 90 kiloHertz (kHz), demonstrating a severe-to-profound hearing impairment. While most mouse deafness mutants show circling and head-tossing behaviors in addition to hearing impairment, *nse5* homozygotes behave normally and have no noticeable vestibular defects at up to 6 months of age. The gross appearance of the external and middle ears of mutant animals is normal, and only minor anomalies of the cochlea can be seen on light microscopy. Genetic analysis of several hundred backcross and intercross progeny has localized the mutant locus to a 715 kilobase (kb) interval on mouse chromosome 15. A missense mutation at a highly-conserved amino acid was found in the mouse homologue of the yeast asparagine-linked glycosylation-10 gene (*Alg10b*), which is within the critical interval for the *nse5* mutation. A 20 kb transgene containing a wildtype copy of the *Alg10b* gene rescues the phenotype *nse5*/*nse5* homozygous animals. Additional mutations in *Alg10b* will be isolated from the Harwell sperm bank to more clearly establish the biological function of this gene. This study provides the clearest example to date of the critical link between.

## 345/W/Poster Board #3

**Characterizing the Effect of Increased Expression of Glucosylceramide-Synthesizing Enzyme in Mouse Models of Gaucher Disease.** *S. Barnes, Y-H. Xu, Y. Sun, G.A. Grabowski.* Division of Human Genetics, Cincinnati Children's Hospital Medical Center and Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH.

Gaucher disease (GD) is a lysosomal storage disease caused by deficient acid β-glucosidase [GCase] enzyme activity. This deficiency leads to accumulation of toxic substrates mainly glucosylceramide (GC) in cells of monocytic origin, mainly macrophages. Several viable models of GD have been generated by introducing point mutations into the mouse GCase (gba) locus, including N370S, V394L, D409H, or D409V. These mouse models share only some characteristics of the human phenotypes. To create more severe variants, transgenic mice were developed to overexpress the glucosylceramide-synthesizing enzyme, UDP-glucose:ceramide glucosyltransferase (GCS). The transgene was driven by the ROSA promoter and crossbred into our D409V/null line. RT-PCR, Northern and western analyses (with GCS specific antibody) showed high level expression of GCStg in brain, liver, lung, and spleen. Immunofluorescence with GCS specific antibodies indicated GCStg has a similar pattern of localization as endogenous GCS in liver. In vitro assays using NBD-ceramide as a substrate showed high levels of GCS activity in liver compared to controls. LC/MS revealed accumulation of both GC and GS in lung tissues in 9 wk old D409V/null (9V/null) mice expressing the GCStg which is at least 11 weeks earlier than expected for 9V/null mice indicating the increased substrate load for GC and GS in these mice. Increased CD68 immunostaining (markers of activated macrophage) was observed in the liver and lung of 9V/null and 9V/9V mice expressing GCStg compared to their non-transgenic littermates. The 9V/9V mice without the transgene do not show macrophage infiltration into tissues before 8-9 months of age. Overall, the presence of GCStg increased the rapidity of disease development and its progression in this mouse model of Gaucher disease. (RO1 NS/DK 36681).

## 346/W/Poster Board #4

**Interrogation of the Pathogenesis of Loeys-Dietz Syndrome Using an Allelic Series of Mutant Mice.** *D. Loch[1], J. Habashi[1], H. Dietz[1,2].* 1) Johns Hopkins University School of Medicine, Baltimore, MD; 2) Howard Hughes Medical Institute.

Loeys-Dietz syndrome (LDS) is a recently described systemic connective tissue disorder with significant phenotypic overlap with Marfan syndrome (MFS) but significant differences including more widespread and aggressive vascular disease and frequent death in childhood, highlighting the need for elucidation of disease pathogenesis that would inform the development of novel medical therapies. LDS is caused by heterozygous mutations in the genes encoding transforming growth factor-beta receptors 1 and 2 (TGFBR1/Alk5 and TGFBR2, respectively). Expression of mutant receptor subunits in cells naïve for the corresponding receptor fails to support TGFβ signalling. However, a number of observations argue against a simple haploinsufficency/loss-of-function model of disease pathogenesis. First, MFS has been unequivocally associated with increased TGFβ signalling in many tissues including the aortic wall. Second, LDS is overwhelmingly associated with missense mutations in the kinase domains of either receptor subunit; the few nonsense mutations occur at the extreme 3'-end, precluding mutant transcript clearance by nonsense-mediated decay. Finally, analysis of LDS patient aortic tissue has shown paradoxically enhanced TGFβ signalling in the aortic wall. To definitively address this critical issue, we have created three mutant mouse models of LDS; two knock-in strains with missense mutations in Tgfbr1 (M318R) or Tgfbr2 (G357W) and a transgenic strain that ubiquitously over-expresses the mutant Tgfbr2 (G357W). We have also analyzed mouse strains heterozygous for null alleles of either Tgfbr1 or Tgfbr2. Initial phenotyping of both knock-in mouse strains has confirmed recapitulation of the LDS vascular phenotype, with widespread arterial disease including arterial tortuosity, aneurysm and dissection. G357W transgenic mice also develop vascular disease, with increased severity seen in homozygosity. In contrast, both haploinsufficient mouse strains show no evidence of vascular disease and normal longevity. Mice with severe vascular disease show increased canonical TGFβ signalling in the cardiovascular system (phosphorylated Smad2) and activation of noncanonical TGFβ signalling cascades (e.g. Erk1/2), concordant with our recent findings in MFS. Taken together, these data definitively exclude haploinsufficiency and are most suggestive of a gain-of-function mechanism of disease pathogenesis. On this basis, we are initiating pharmacologic trials in these mouse models of LDS.

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### 347/W/Poster Board #5

**The transcriptional pathways underlying heart development are defective in Ofd1 mutant animals.** *A. Vitale[1], A. Barra[1], A. Baldini[1,2], B. Franco[1,3].* 1) TIGEM, Telethon Institute of Genetics and Medicine, Naples, Italy; 2) Istituto di Genetica e Biofisica Adriano Buzzati Traverso via Pietro Castellino 111, 80131 Naples, Italy; 3) Medical Genetics, Department of Pediatrics, Federico II University, via Pansini 5, 80131 Naples, Italy.

Oral-facial-digital type I (OFDI; MIM 311200) syndrome is a male lethal X-linked dominant developmental disorder characterized by malformations of the face, oral cavity and digits. CNS abnormalities and cystic kidney disease can also be part of this condition. This rare genetic disorder is due to mutations in the OFD1 gene that encodes a centrosome/basal body protein necessary for primary cilium assembly and for left-right axis determination, thus ascribing OFDI to the growing number of disorders associated to ciliary dysfunction. We present the characterization of the cardiac phenotype observed in mutant animals bearing ubiquitous inactivation of the gene responsible for this rare genetic disease. Male mutants display randomization of heart-looping (47%) and an enlarged aortic sac revealed by intracardiac ink-injections. Female mutants displayed a reduced left pulmonary lobe. In situ hybridization analysis revealed aberrant expression of transcription factors critical for appropriate heart development including Nkx2.5, Tbx5, Mef2c, Hand1/2 and Wnt11, while markers strongly expressed in the cardiac field such as Mlc2v, Tbx20 and Anf displayed a normal pattern of expression. Taken together our results strongly suggest a specific alteration of the cardiac transcription program resulting in a severely compromised cardiac function in Ofd1 mutant animals. Immunoistochemical studies revealed increase of proliferating cells and reduction of apoptotic nuclei in the entire heart suggesting that Ofd1 may play a role in the cell proliferation/apoptotic events underlying heart morphogenesis. Our results indicate that Ofd1 has a critical role for the correct development and specification of the heart that goes beyond the expected involvement in left-right asymmetry.

### 348/W/Poster Board #6

**Loss of *Tbx22* causes submucous cleft palate, ankyloglossia and choanal atresia.** *P. Stanier[1], A. Hoshino[1], C. Keller[2], G.E. Moore[1], E. Pauws[1].* 1) Inst Child Hlth, Univ College London, London, United Kingdom; 2) Greehey Children's Cancer Research Institute, The University of Texas Health Science Center, San Antonio, TX, USA.

Cleft palate (CP) is a common birth defect affecting around 1 in 1,500 births. Submucous cleft palate (SMCP) forms a clinically important subgroup, which although more rarely reported, may be at least as common as open CP. X-linked cleft palate and ankyloglossia (CPX, MIM303400) is characterized by submucous or overt clefts of the secondary palate, showing that they are different manifestations of the same disorder. CPX is caused by loss-of-function mutations in the *TBX22* gene. Studies indicate that *TBX22* mutations are found in at least 4% of all CP. Here, we show that mice deficient for *Tbx22* have a classic SMCP or rarely an overt cleft palate. In addition to ankyloglossia, we also find oronasal defects including choanal atresia. Analysis of the craniofacial skeleton demonstrates reduced bone formation in the posterior hard palate, resulting in the classic 'notch', associated with SMCP. Osteogenesis is markedly reduced after condensation of the palatal mesenchyme, resulting in a significantly delay in the differentiation and/or maturation of osteoblasts. Rather than being involved in palatal shelf closure, we show that TBX22 is a key determinant for intramembranous bone formation at the posterior hard palate boundary, which is required for correct palate function. The *Tbx22* null mouse therefore offers an important and novel opportunity to study the molecular mechanisms underpinning SMCP.

### 349/W/Poster Board #7

**Implication of LGI1 in autosomal dominant temporal lobe epilepsy.** *E. Chabrol[1], G. Provenzano[1], V. Navaro[1,2], I. Cohen[1], R. Miles[1], M. Baulac[1,2], E. Leguern[1], S. Baulac[1].* 1) Inserm, UMR_S975,Centre de Recherche - Institut du Cerveau et de la Moelle,Hop. Pitie-Salpetriere, NEB, Paris, France; 2) Center for Epilepsy AP-HP, Hop. Pitie-Salpetriere, Paris, France.

Epilepsy consists in a wide range of syndromes, all associated with abnormal synchronous neuronal firing in one or more brain regions. Genetic factors play an increasingly recognized role in idiopathic epilepsies, i.e epilepsies without neurological abnormalities. To this date, a dozen of genes have been implicated in monogenic autosomal dominant idiopathic epilepsies. Until recently, human hereditary idiopathic epilepsies were thought to be exclusively channelopathies, since all mutated genes encoded subunits of neurotransmitter receptor (GABA and acetylcholine receptor) or ion channels (sodium and potassium channels). LGI1 (Leucine-rich glioma inactivated 1) gene was the first non-ion channel gene identified in monogenic human idiopathic epilepsy. Mutations in the LGI1 gene are the major cause of autosomal dominant lateral temporal epilepsy (ADLTE). ADLTE referrers to a familial epileptic syndrome characterized by an age at onset typically in late adolescence, focal and secondarily tonic-clonic generalized seizures with stereotyped auditory auras. We have investigated the functional consequences of several disease-causing LGI1 missense mutations and demonstrated that they markedly decrease protein secretion by immortalized cells in culture. Our findings suggest that LGI1-related epilepsy results from a haploinsufficiency of LGI1. In order to clarify the function of this newly identified protein in brain development, neuronal excitability and epileptogenesis, we generated a knockout mice model for LGI1. Heterozygote and homozygous mice were successfully obtained according to Mendelian expected ratio. We present the phenotype and morphological data of these animals.

### 350/W/Poster Board #8

**NEMO point mutation mouse models are more severe than human counterparts.** *Y. Gu, D.L. Nelson.* Dept Molec & Human Gen, Baylor Col Med, Houston, TX.

Several human disorders have been associated with mutations in NF-Kappa-B Essential Modulator (*NEMO* or *IKBKG*), including Incontinentia Pigmenti (IP), X-linked anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) and EDA-ID with osteopetrosis and lymphoedema (OL-EDA-ID). Molecular analysis demonstrates that IP is caused by the loss of the function of NEMO protein, while EDA-ID results from a variety of point mutations in *NEMO* gene. Intriguingly, OL-EDA-ID is associated with stop codon mutation, which results in translating the entire NEMO protein followed by 27 additional amino acids. Nemo deficient mouse models recapitulated the human IP disorder, including skin phenotypes of female heterozygous animals and embryonic lethality in males. In this study we created two point mutation mouse models by knockin mutations of nucleotides in exon 10 to model human EDA-ID and OL-EDA-ID. To our surprise, unlike human counterparts, males carrying either of these two point mutations die in the early stages of embryonic life and heterozygous females exhibit phenotypes. The EDA-ID model, with an insertion at the location corresponding to the human dup C 1161 in exon 10, resulting in a truncated NEMO-EGFP fusion protein showed normal skin and no macroscopic abnormalities in heterozygous females. In contrast, hemizygous males die between embryonic day 12.5 -13.5 due to apoptosis in liver, similar to Nemo deficient male mice. In the OL-EDA-ID model, where the stop codon is mutated to add a novel amino acid sequence to the C terminus of the protein, heterozygous females and chimeras showed skin phenotypes and growth retardation. Most died between postnatal days 10 and 14. Histological examination indicated that there are multiple organ development defects in these heterozygous females involving liver, spleen, intestines, heart and lung. Surviving females developed skin problems again between 3 and 4 months of age. Our initial data in these Nemo mouse models suggest that NEMO may play distinct roles in early embryonic development in humans compared to mice. Interestingly, the stop codon mutation exhibited more severe phenotypes than any other point mutation models, in accord with phenotypes seen in humans with NEMO mutations. These two murine models provide new tools to further study NEMO function in the canonical NFkB pathway and other non-canonical signal transduction pathways.

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## 351/W/Poster Board #9

**A new mouse model for Marfan Syndrome recapitules all the clinical variability of the disease.** *B.L. Lima, E.J.C. Santos, G.R. Fernandes, L.V. Pereira.* Department of Genetic, Instituto de Biociências - USP, São Paulo, SP, Brazil.

Marfan Syndrome is an autossomal dominant disorder associated with pleiotropic manifestations affecting the skeletal, ocular and cardiovascular systems. Mutations in the FBN1 gene, which encodes the fibrillin-1 protein, were associated with clinical manifestations on MFS patients. In 1997, a murine model for MFS - mgΔ lineage - was designed. Approximately 6 kb of Fbn1 encompassing exons 19-24 were replaced by a neomycin-resistance expression cassette (neo). Surprisingly, these animals were histologically indistinguishable from wild-type mice and no skeletal manifestation, lung emphysemas or aortic alterations were noted. It was postulated that the presence of the neo cassette sequence interfered with allele expression, consequently restricting the dominant-negative effect of the mutation. Here we report the generation of a variation of the mgΔ mouse model carrying the same mutant Fbn1 allele, but with neo flanked by lox-P sequences (mgΔloxPneo). Unexpectedly, heterozygous mgΔloxPneo mice present some of the MFS phenotypes, even before the removal of neo sequence. Moreover, these phenotypes presented significant variability in two different mouse strains, 129/Sv and C57Bl/6. We analyzed 45 mutant and 20 wild-type animals, on three different ages (3, 6 and 9 months). The main difference observed in the phenotypes of both strains, is on the onset of symptoms. While 129/Sv animals, with 3 months of age, presents severely affected individuals, not only on skeletal system, but also on aortic and pulmonary, all animals from B6 strain (3 months), presents mild alterations in pulmonary system, and are completely asymptomatic for bone changes. Immunofluorescence analysis of cultured fetal fibroblasts showed that, while wt cell cultures present an elaborated network of immunoreactive microfibrils, homozygous mgΔloxPneo cells present a more diffuse pattern. We also note that the mutant molecules are retained inside the cell, possibly in the endoplasmic reticulum. Several studies suggest that maintenance of proteins, inappropriately folded, inside the reticulum can result in an oxidative stress, which could be related to a patohgenical mechanism. Our results indicate that the mgΔloxPneo model seems to be an extraordinary tool in the screening of modifier genes in MFS, and to the study of its pathogenical mechanism.

## 352/W/Poster Board #10

**Neurodegeneration in mouse models of CMT4J and ALS.** *M.H. Meisler, C.J. Ferguson, G.M. Lenk.* Dept Human Gen, Univ Michigan, Ann Arbor, MI.

*FIG4* is a phospholipid phosphatase that functions as part of a protein complex regulating the cellular abundance of the signaling phosphoinositide $PI(3,5)P_2$. We described mutations of human *FIG4* in patients with Charcot-Marie-Tooth Type 4J and ALS (Chow et al, Nature 2007; Chow et al, AJHG 2009). We have recently identified several new disease mutations in *FIG4* and related genes. Mouse models of CMT4J and ALS with mutations in *Fig4* have been developed and are being characterized. The CMT4J mice express a transgene carrying the missense mutation I41T on a null background. The ALS model is heterozygous for a null allele of *Fig4*. The CMT4J allele I41T rescues the lethality of null mice, which usually occurs between 3 and 6 weeks of age. Mice from transgenic line 705 with 3x normal expression of the I41T transcript survive for 5 months. Mice from transgenic line 721 with 5x normal expression survive beyond 18 months. Thus, rescue by the partially functional I41T allele is dose-dependent. We also generated congenic lines carrying the *Fig4* null allele on strain C57BL/6J or strain C3HeB/FeJ. Survival on the C57BL/6J background is reduced to two hours postnatal. We are mapping the modifier loci responsible for the perinatal lethality of C57BL/6J null homozygotes. Analysis of these mouse models is providing insight into the cellular and neurological pathogenesis of CMT4J and ALS.

## 353/W/Poster Board #11

**AMPD3 Deficient Mice Exhibit Increased Erythrocyte ATP but Do Not Improve Anemia due to PK Deficiency.** *T. Morisaki[1,2], J. Cheng[1], K. Toyama[1,2], M. Ikawa[3], M. Okabe[3], H. Morisaki[1].* 1) Dept Bioscience, Natl Cardiovasc Ctr Res Inst, Suita, Osaka, Japan; 2) Dept Molecular Pathophysiology, Osaka Univ Grad Sch Pharm Sci, Suita, Osaka, Japan; 3) Genome Information Research Center, Osaka Univ, Suita, Osaka, Japan.

AMP deaminase (AMPD) catalyzes AMP to IMP and plays an important role in energy charge and nucleotide metabolism. Human AMPD3 deficiency are known as erythrocyte-specific enzyme AMPD deficiency and found in individuals without any clinical symptom, though the increased erythrocyte ATP level was reported. To better understand physiological and pathological role of AMPD3, we established AMPD3 deficient mouse [A3(-/-)]. No AMPD activity and high ATP level as well as high ADP and AMP level were observed in erythrocyte of this mouse like human AMPD3 deficiency. Also, A3(-/-) mouse was unremarkable otherwise. Then, we generated AMPD3 and pyruvate kinase (PK) double deficient mouse [PKA(-/-,-/-)] by mating A3(-/-) mice with CBA-Pk-1slc/Pk-1slc (PK(-/-); spontaneous PK deficient mice showing hemolytic anemia). In PKA(-/-,-/-) mice, the level of ATP in RBC was increased 1.5 times compared with that of PK(-/-) mice, though no improvement of hemolytic anemia was found in those animals. Next, we tested erythrocyte fragility. We observed the osmotic fragility of erythrocytes in A3(-/-) mice when mice was in the fasting condition, though the erythrocyte ATP level was found to be still higher in A3(-/-) mice than in the control. From these results, AMPD3 deficiency in murine model resulted in the increased erythrocyte ATP level as in human, but it could not improve anemia due to PK deficiency and rather caused erythrocyte dysfunction.

## 354/W/Poster Board #12

**A VCP R155H Knock-In Mouse Model mimics human IBMPFD (Inclusion Body Myopathy and Paget disease of bone and Frontotemporal Dementia) disease.** *M. Badadani[1], J. Vesa[1], H. Su[1], G.D. Watts[2], V.J. Caiozzo[3], V.E. Kimonis[1].* 1) Dept. of Pediatrics, Div. of Genetics and Metabolism, University of California, Irvine, CA; 2) Dept. of Orthopedic Surgery, Children's Hospital Boston, Harvard Medical School, Boston, MA; 3) Dept. of Physiology and Biophysics, and Dept. of Orthopedics, College of Health Sciences, University of California, Irvine, CA.

Dominant mutations in the Valosin Containing Protein (VCP/p97) gene cause Inclusion Body Myopathy Associated with Paget's Disease and Frontotemporal Dementia (IBMPFD), which is characterized by progressive muscle weakness, dysfunction in bone remodeling, and frontotemporal dementia. VCP plays an essential role in the endoplasmic reticulum associated degradation of misfolded proteins by binding to ubiquitinated substrates via co-factors and transferring them to the 26S proteasomes of the ubiquitin proteasome system. The majority of mutations are located in the ubiquitin-binding domain of VCP, R155H being the most common disease mutation. To elucidate the pathological mechanisms underlying disease progression, we have generated a knock-in mouse model with the R155H mutation and analyzed the progression of disease up to 15 months of age. Muscle strength measurements demonstrated progressive muscle weakness in mutant mice starting before the age of 6 months (6% decrease) and reaching the 18% decrease by the 15 months of age. Motor coordination and fatigue, analyzed by the Rotarod analysis showed progressive decline beginning at 6 months of age (10% decrease) and reaching 22% decline by the age of 15 months. Structural analyses of mutant muscle performed by hematoxylin and eosin staining and electron microscopy, showed vacuolization of myofibrils (2% of cells), centrally located nuclei (2% of cells), and disorganized muscle fibers. Further, immunohistochemical analyses of the quadriceps muscle tissues from mutant mice showed accumulation of TDP-43- and ubiquitin-positive inclusion bodies in 3.9% of quadriceps myofibrils, thus replicating the muscle pathology typically seen in patients. These results were confirmed by Western blotting using TDP-43 and ubiquitin antibodies. Increased apoptosis was observed by elevated Caspase-3 activity (12-fold increase) and increased number of TUNEL-positive nuclei in mutant quadriceps. Additionally, the micro PET and micro CT analyses and histological analysis of bone showed increased bone turnover and Pagetic lesions in mutant bones suggestive of Paget's disease of bone. In conclusion, the R155H knock-in generated mouse model replicates human disease, and therefore can be used to clarify the mechanisms causing IBMPFD. The knock-in mice can also be used in the development of novel therapies for this disease.

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## 355/W/Poster Board #13

**Loss of the Frizzled-4 receptor causes significant skeletal muscle atrophy: A potential new model for Facioscapulohumeral Muscular Dystrophy (FSHD)?** *E.M. MacDonald[1], J.L. Simmers[1], T.N. Burks[1], E. Andres-Mateos[1], R. Marx[1], R. Tawil[3], R.D. Cohn[1, 2].* 1) McKusic-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Department of Pediatrics and Neurology, Johns Hopkins University, Baltimore, MD; 3) University of Rochester Medical Center, Neuromuscular Disease Center, Rochester, NY.

The canonical Wnt pathway is characterized by signal transduction events that begin when the Wnt molecule binds to a Frizzled receptor on the cell surface. This binding induces a signaling cascade that leads to the stabilization of non-phosphorylated β-catenin and activation of target genes in the nucleus. Three phenotypically similar human primary vascular retinopathies are caused by loss of function mutations of Wnt pathway genes including the Frizzled-4 receptor gene as well as its co-receptor LRP5/6 and its ligand, Norrin. Interestingly, an identical retinal phenotype is also seen in Facioscapulohumeral Muscular Dystrophy (FSHD) raising the possibility the Wnt pathway is also involved in FSHD. Previous work has demonstrated that Wnt signaling plays a functionally significant role in muscle regeneration and in the maintenance of skeletal muscle mass. In order to further delineate the functional role of Wnt signaling in vivo, we performed a dedicated analysis of skeletal muscle from Frizzled-4 receptor knock-out mice. When compared to wild-type littermates, the Fz4(-/-) mice are smaller, appear to be significantly weaker, and have reduced survivability. Moreover, these mice also exhibit marked kyphosis throughout life which further interferes with their mobility. Biochemical analyses of steady-state muscle of Fz4(-/-) mice reveal an increase in the phosphorylated form of β-catenin. This indicates that knock down of the Frizzeled-4 receptor decreases the total amount of Wnt signaling in the skeletal muscle as compared to wild-type mice. Morphological assessment of skeletal muscle from the Fz4-null mice demonstrates significant variation in fiber size with multiple small and angulated muscle fibers. In addition, occasional fibers with centrally located nuclei are observed. Assessment of muscle fiber typing suggests a specific Type IIa (fast twitch) muscle fiber atrophy. In addition to the pathological changes in muscle and similar to human patients with FSHD, Fz4(-/-) mice also exhibit a progressive loss of auditory function throughout life. Thus, the combination of the retinal, auditory, and skeletal muscle phenotype suggests that Fz4-null mice may represent a novel animal model for studying the pathogenesis of FSHD.

## 356/W/Poster Board #14

**Residual laminin-binding activity and enhanced dystroglycan glycosylation by LARGE in novel model mice to dystroglycanopathy.** *M. Taniguchi[1,2], M. Kanagawa[1], S. Takeda[3], Y. Miyagoe-Suzuki[4], S. Takeda[4], T. Endo[5], K. Kobayashi[1], K.P. Campbell[6], T. Toda[1].* 1) Divison of Molecular Brain Science / Neurology, Kobe University Graduate School of Medicine, Hyogo, Japan; 2) Division of Clinical Genetics, Osaka University Graduate School of Medicine, Osaka, Japan; 3) Otsuka Pharmaceutical Co. Ltd, Otsuka GEN Research Institute, Tokushima, Japan; 4) Department of Molecular Therapy, National Institute of Neuroscience, National Center, Tokyo, Japan; 5) Glycobiology Research Group, Tokyo Metropolitan Institute of Gerontology, Foundation for Research on Aging and Promotion of Human Welfare, Tokyo , Japan; 6) Howard Hughes Medical Institute, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa, United States.

Hypoglycosylation and reduced laminin-binding activity of alpha-dystroglycan are common characteristics of dystroglycanopathy, which is a group of congenital and limb-girdle muscular dystrophies. Fukuyama-type congenital muscular dystrophy (FCMD), caused by a retrotransposal insertion in the fukutin gene, is a severe form of dystroglycanopathy. To better understand the molecular pathogenesis of dystroglycanopathies and to explore therapeutic strategies, we generated knock-in mice carrying the retrotransposal insertion in the mouse fukutin ortholog. Knock-in mice exhibited hypoglycosylated alpha-dystroglycan; however, no signs of muscular dystrophy were observed. More sensitive methods detected reduced levels of intact alpha-dystroglycan, and solid-phase assays determined laminin binding levels to be approximately 50% of normal. In contrast, intact alpha-dystroglycan is undetectable in the dystrophic Large(myd) mouse, and laminin-binding activity is markedly reduced. These data indicate that a small amount of intact alpha-dystroglycan is sufficient to maintain muscle cell integrity in knock-in mice, suggesting that the treatment of dystroglycanopathies might not require the full recovery of glycosylation. To examine whether glycosylation defects can be restored in vivo, we performed mouse gene transfer experiments. Transfer of fukutin into knock-in mice restored glycosylation of alpha-dystroglycan. In addition, transfer of LARGE produced laminin-binding forms of alpha-dystroglycan in both knock-in mice and the POMGnT1 mutant mouse, which is another model of dystroglycanopathy. Overall, these data suggest that even partial restoration of alpha-dystroglycan glycosylation and laminin-binding activity by replacing or augmenting glycosylation-related genes might effectively deter dystroglycanopathy progression and thus provide therapeutic benefits.

## 357/W/Poster Board #15

**Postnatal *Sim1*Deficiency Causes Hyperphagic Obesity.** *A.R. Zinn[1,2], K.P. Tolson[1], T. Gemelli[1], B.M. Kublaoui[1,3].* 1) McDermott Center for Human Growth & Development, University of Texas Southwestern Medical School, Dallas, TX; 2) Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas, TX; 3) Department of Pediatrics, University of Texas Southwestern Medical School, Dallas, TX.

Single-minded 1 (*SIM1*) encodes a hypothalamic transcription factor (1) and is one of only a handful of genes associated with nonsyndromic human monogenic obesity (2,3). *Sim1* homozygous mice (*Sim1*$^{-/-}$) die perinatally and lack paraventricular (PVN) and supraoptic (SON) hypothalamic nuclei (4). *Sim1* heterozygotes (*Sim1*$^{+/-}$) show hyperphagic obesity, increased linear growth and enhanced susceptibility to diet-induced obesity, consistent with defective melanocortin 4 receptor signaling (5,6,7). Whether the obesity phenotype of *Sim1*$^{-/-}$ mice is a caused by a developmental or post-developmental defect has yet to be determined. In order to dissociate the neuroanatomic and physiologic functions of *Sim1* in feeding regulation, we used a CamKII-cre transgene to conditionally delete *Sim1* in the postnatal brain. CamKII-cre/*Sim1*$^{+/flox}$ mice became obese on both high and low fat diets, and exhibited the same hyperphagic behavior previously observed in *Sim1*$^{+/-}$ mice. *Sim1* transcripts were decreased by about 50% in CamKII-cre/*Sim1*$^{+/flox}$ mice compared to control littermates, as were hypothalamic oxytocin levels, similar to *Sim1*$^{+/-}$ mice (8). Stereology data support the previous conclusion from less precise methods (7) that heterozygous *Sim1* deficiency does not affect PVN cellularity. Our results indicate that haploinsufficiency of *Sim1* after the PVN and SON are fully formed is sufficient to cause hyperphagic obesity, pointing to a post-developmental role of *Sim1* in feeding regulation.

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## 358/W/Poster Board #16

**Removal of Hsf4 leads to cataract development in mice through down-regulation of gamma S-crystallin and Bfsp expression.** *L. Hu, X. Shi.* Hlth Sci Ctr, Sibs, CAS, Shanghai, China.

BACKGROUND: Heat-shock transcription factor 4 (HSF4) mutations are associated with autosomal dominant lamellar cataract and Marner cataract. Disruptions of the Hsf4 gene cause lens defects in mice, indicating a requirement for HSF4 in fiber cell differentiation during lens development. However, neither the relationship between HSF4 and crystallins nor the detailed mechanism of maintenance of lens transparency by HSF4 is fully understood. RESULTS: In an attempt to determine how the underlying biomedical and physiological mechanisms resulting from loss of HSF4 contribute to cataract formation, we generated an Hsf4 knockout mouse model. We showed that the Hsf4 knockout mouse (Hsf4-/-) partially mimics the human cataract caused by HSF4 mutations. Q-PCR analysis revealed down-regulation of several cataract-relevant genes, including gamma S-crystallin (Crygs) and lens-specific beaded filament proteins 1 and 2 (Bfsp1 and Bfsp2), in the lens of the Hsf4-/- mouse. Transcription activity analysis using the dual-luciferase system suggested that these cataract-relevant genes are the direct downstream targets of HSF4. The effect of HSF4 on gamma S-crystallin is exemplified by the cataractogenesis seen in the Hsf4-/-,rncat intercross. The 2D electrophoretic analysis of whole-lens lysates revealed a different expression pattern in 8-week-old Hsf4-/- mice compared with their wild-type counterparts, including the loss of some alpha A-crystallin modifications and reduced expression of gamma-crystallin proteins. CONCLUSION: Our results indicate that HSF4 is sufficiently important to lens development and disruption of the Hsf4 gene leads to cataracts via at least three pathways: 1) down-regulation of gamma-crystallin, particularly gamma S-crystallin; 2) decreased lens beaded filament expression; and 3) loss of post-translational modification of alpha A-crystallin.

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### 359/W/Poster Board #17

**Down regulation of Hccs in medaka recapitulates the phenotype observed in Microphthalmia with linear skin lesions (MLS) syndrome.** *A. Indrieri[1,2], I. Conte[1], G. Chesi[1], P. Bovolenta[3], B. Franco[1,4].* 1) TIGEM, Telethon Institute of Genetics and Medicine, Naples, Italy; 2) European School of Molecular Medicine (SEMM), Naples, Italy; 3) Instituto Cajal, CSIC, Madrid, Spain; 4) Medical Genetics, Department of Pediatrics, Federico II University, Naples, Italy.

The Microphthalmia with linear skin defects (MLS) syndrome is an X-linked dominant male-lethal neuro-developmental disorder associated to mutations in the holocytochrome c-type synthetase (HCCS) transcript. Female patients display unilateral or bilateral microphthalmia and linear skin defects, additional features include CNS malformation and mental retardation. HCCS codifies a mitochondrial protein that catalyzes the attachment of heme to both apocytochrome c and c1 necessary for proper functioning of the mitochondrial respiratory chain. The molecular mechanisms underlying the eye and brain developmental anomalies in this disease are still unknown. Previous studies demonstrated the early lethality of mouse embryonic Hccs knock-out stem cells. We thus decided to generate a model for this disease in medaka fish (Oryzia latipes). This model will allow us to overcome the possible embryonic lethality using graded concentrations of either the mutated mRNA or the morpholinos. Specific morpholinos directed against the HCCS transcript have been designed and injected. Our experiments have determined that the morpholinos effectively downregulate the expression of the olhccs gene. Gain of function studies did not result in an aberrant phenotype. Instead injection of both the mutated protein (dominant-negative) and two different morpholinos resulted in a pathological phenotype, which resembles the human condition. As expected, morphants displayed microphthalmia, coloboma, and microcephaly. In addition, unexpectedly, absence of blood pigmentation was also observed. Analysis with specific markers (e.g, Pax6, Six3.2, Otx2, Rhodopsin, Crx, Chx10, Ath5, Syntaxin) showed an abnormal formation of the retinal-pigmented epithelium (RPE) and defects in differentiation of the ventral neural retina. RNA in situ hybridization studies revealed an abnormal domain of expression for connexin43, recently shown to be important for cell proliferation and apoptosis. Tunel and pHH3 assayes on morphants, revealed abnormalities in cell proliferation and programmed cell death. Altogether this data suggest a possible role of these mechanisms in the pathogenesis of MLS syndrome and further studies are ongoing to explain the pathogenetic link between Hccs inactivation and the eye abnormalities observed in morphants and affected patients.

### 360/W/Poster Board #18

**Improved colorimetric (TBA) sialic acid assay validated by GNE transduction of Gne-null Lec3 CHO cells.** *C. Saechao[1,4], Y. Valles-Ayoub[1,2], A. Haghighatgoo[1,2], Z. Khokher[1,3], D. No[1,3], S.L. Martinez[1], I. Shah[1,4], S. Esfandiarifard[1], A.C. Riley-Portuges[1], C. Jay[5], M. Pietruszka[1], D. Darvish[1,2].* 1) HIBM Research Group, Inc., Encino, CA; 2) VA Greater Los Angeles (VA-GLA/UCLA), Los Angeles, CA; 3) Los Angeles Mission College, Sylmar, CA; 4) California State University, Northridge (CSUN), Northridge, CA; 5) Gradalis, Inc., Dallas, TX.

To improve the sensitivity of colorimetric sialic acid (Sia) quantitation by a thiobarbituric acid (TBA) based assay, we optimized Leonard Warren's method for detection of physiologic tissue Sia levels. The role of Sia is diverse and essential to cellular stabilization, signaling functions, and biochemical modulations of cell membranes, lysosomal membranes, and glycol-proteins. UDP-GlcNAc 2-Epimerase/ManNAc Kinase (GNE) is the rate-limiting enzyme of Sia biosynthesis. In humans, GNE mutations may lead to either recessive muscle wasting disease (HIBM, IBM2, DMRV) caused by hypomorphic GNE, or dominant sialuria caused by hypermorphic GNE. Using our modified TBA assay, the results from triplicates of a standard curve show an R2 value of 0.994 in linear correlation with absorption at 549nm wavelength, allowing accurate lower detection limits of 0.5nmol/50μL (10μM) Neu5Ac. Subsequently, we transduced Gne-null Lec3 CHO cells with wild-type or sialuria (R266Q) GNE expression plasmids, and measured cell lysate Sia levels. The assay reliably and reproducibly showed an appropriate elevation of cellular Sia production in GNE transduced CHO cells. This assay is optimized at physiologic tissue levels of Sia (1-20nmol), and may be used as a rapid, economical, and reliable alternative to widely accepted HPLC methods.

### 361/W/Poster Board #19

**Molecular mechanisms underlying the skin disease erythrokeratoderma variabilis.** *C.A. Scott[1], D. Tattersall[1], T. Corbett-Jones[2], D.P. Kelsell[1].* 1) Centre for Cutaneous Research, Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, 4 Newark Street, London, United Kingdom; 2) Neuroscience Centre, Genome Biology Laboratory, Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, 4 Newark Street, London, United Kingdom.

Erythrokeratoderma variabilis (EKV) is a monogenic skin disease characterized by fixed hyperkeratotic plaques and transient erythema. Mutations in the genes encoding Connexin 31 (Cx31) and Cx30.3 are associated with EKV but can also cause hearing loss with/without neuropathy but no skin disease. Cx proteins form gap junctions, which are aqueous pores allowing the intercellular transfer of ions and small molecules between cells. Mutations in other Cx genes are implicated in a range of human disorders. The aims of this study are twofold, firstly, to identify new genetic loci associated with EKV and, secondly, characterize the mechanisms underlying different disease-associated Cx31 mutants. We and others have shown that approximately half of EKV patients do not harbour mutations in *GJB3* or *GJB4* encoding Cx31 and Cx30.3 respectively indicating genetic heterogeneity. We are performing a number of genetic studies to identify these other EKV associated gene(s). For example, cytogenetic investigations in one patient with recessive EKV reveals a chromosome translocation between chromosome 2 and 22. FISH mapping has reduced the break-point region significantly and may lead to the identification of a new genetic locus for EKV. The second aspect of this study is revealing both similar and distinct properties of Cx31 mutant proteins. When expressed *in vitro*, EKV associated mutants, for example, (R42P)Cx31 and (C86S)Cx31 plus the peripheral neuropathy and hearing loss mutant (66delD)Cx31), misstraffic and have a predominantly cytoplasmic localization in contrast to the wild-type protein which is characterized by punctuate aggregates between cells, indicative of gap junction plaques. In contrast, (R42P)Cx31 and (C86S)Cx31 but not (WT)Cx31 or (66delD)Cx31 cause elevated levels of cell-type specific cell death. Our immunocytochemical data suggests this skin mutant specific cell death phenotype is due to endoplasmic reticulum (ER) stress from the accumulation of unfolded proteins inducing the unfolded protein response (UPR) which can alleviate protein accumulation or lead to, in this case, cell death. This indicates that ER stress may result in aberrant keratinocyte differentiation and hyperproliferation in EKV patient skin *in vivo*.

### 362/W/Poster Board #20

**The $GABA_A$ receptor as a potential target for therapy of the fragile X syndrome.** *F. Kooy[1], C. D'Hulst[1], I. Heulens[1], K. Van Laere[2], C. Bagni[3], B. Hassan[3], P.P. De Deyn[4].* 1) Dept Medical Genetics, Univ Antwerp, Antwerp, Belgium; 2) Division of Nuclear Medicine, KU Leuven, Belgium; 3) Dept of Human Genetics & VIB, KU Leuven, Leuven, Belgium; 4) Dept of Neurochemistry and Behavior, Univ Antwerp, Antwerp, Belgium.

The fragile X syndrome is a common form of inherited mental retardation. Patients suffer from mild to severe cognitive impairment, recognizable facial features, behavioural abnormalities and an increased rate of spontaneous epilepsy. A mouse model has been developed that mimics the cognitive and clinical symptoms of the disorder. FMRP, the RNA-binding protein missing in fragile X syndrome, regulates mRNA localization to dendrites as well as mRNA translation and so influences local protein synthesis. Previously we have shown that a dysfunction of the GABAergic system is involved in the clinical presentation of the disorder. We demonstrated a 35-50% reduced expression of 8 of the 18 subunits that make up the GABA receptor and of enzymes involved in GABA synthesis (GAD), transport (GAT1 and GAT44) and degradation (SSADH). A reduction of corresponding genes was observed in the fragile X fly model, indicating decreased GABAergic expression is an evolutionary conserved hallmark of the fragile X syndrome. We demonstrated that FMRP binds several components of the GABAergic system resulting in an apparent increase in stability of these mRNA transcripts. In addition, we show that the previously reported under expression of specific subunits of the $GABA_A$ receptor can be corrected in a YAC transgenic mouse model, containing the full length human FMR1 gene in a knockout background. These results suggest that under expression of the $GABA_A$ receptor is a result of a direct interaction of FMRP with the encoding mRNAs. PET scans in human patients with labelled flumazenil were performed to measure the occupancy of the $GABA_A$ receptor in diverse brain regions. A comparison of 5 patients with 10 controls was indicative for a reduced quantity of $GABA_A$ receptors in specific cortical regions, mimicking our observations in animal models. We ague that the malfunction of the GABAergic system underlies many of the clinical symptoms of fragile X patients. Despite the altered composition of the $GABA_A$ receptor in the fragile X syndrome, rotarod and elevated plus maze experiments in mice performed by us showed that these are still functional. We postulate that the well described $GABA_A$ receptor pharmacology might open new powerful opportunities for treatment of the behavioural and epileptic phenotype associated with fragile X syndrome.

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## 363/W/Poster Board #21

**Differential expression of micro RNAs regulating cell cycle in facioscapulohumeral muscular dystrophy.** *Y.-W. Chen[1,2], N. Harafuji[1], R. Shi[1].* 1) Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 2) Department of Pediatrics, George Washington University Medical School, Washington DC.

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant muscular dystrophy that is characterized by progressive weakness and atrophy of the muscles from face, upper-arm and shoulder girdle to lower limb. The genetic cause of FSHD is associated with a shortening of the 3.3 kb D4Z4 repeat array from 11-100 to 1-10 copies on the subtelomeric region of chromosome 4q35. Several studies suggested that defects in cell cycle regulation might play a critical role in FSHD. In addition, microRNAs differentially expressed in FSHD muscles were reported previously. In this study, we hypothesized that some of the misregulated miRNAs are responsible for the cell cycle defects in FSHD. To identify miRNAs differentially regulated in FSHD myoblasts, we performed miRNA expression profiling using TaqMan Human MicroRNA Array v1.0 (ABI). Two time points, 0 hour and 48 hours post differentiation (PD), of eight primary human myoblasts (four controls and four FSHD) were analyzed. We identified 11 and 6 miRNAs significantly changed in expression in FSHD myoblasts at 0 and 48 hours PD, respectively. Among those, miR-411 was found up-regulated at both time points (2.1 and 1.5 fold). Expression levels of six miRNAs (let-7e, miR-99b, miR-143, miR-411, miR-532 and miR-601) were validated by individual TaqMan miRNA assay. The changes of let-7e, miR-99b, miR-411, and miR-601 were confirmed by the individual assay. To identify miRNA target genes that are differentially regulated in FSHD myoblasts, we expression profiled mRNA of the myoblasts collected at 48 hours PD using Affymetrix GeneChip Human Genome U133 Plus 2.0 Array. With p value less than 0.05, 1,090 transcripts were identified as differentially expressed in FSHD myoblasts. Among them, predictive miRNA targets of seven miRNAs (let-7e, miR-99b, miR-143, miR-369-3p, miR-411, miR-532, and miR-601) were searched using on-line target prediction software, miRNAMap. Potential mRNA targets were categorized into functional groups using DAVID/NIAID, NIH. The results showed that cell cycle-related genes were most over-represented in targets of let-7e, miR-411, and miR-532. The finding suggested that microRNAs might play an important role in the defects of cell cycle in FSHD.

## 364/W/Poster Board #22

**Mutation Analysis of PLOD1 gene in Ehlers-Danlos Syndrome Type VI.** *K. Damjanovich[1], T. Caine[1], L. Schwarz[1], M. Pasquali[1,2], N. Longo[1,2,3], P. Bayrak-Toydemir[1,2].* 1) ARUP Institute of Clinical and Experimental Pathology, Salt Lake City, UT; 2) Department of Pathology, University of Utah, Salt Lake City, Utah; 3) Department of Pediatrics, University of Utah, Salt Lake City, Utah.

Ehlers-Danlos syndrome type VI (EDS VI) is a recessively inherited connective tissue disorder characterized by kyphoscoliosis and muscular hypotonia starting at birth along with later signs of skin extensibility, tissue fragility, joint laxity, and ocular involvement. It is caused by a deficiency of collagen lysyl hydroxylase due to mutations in procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (PLOD1) gene. This enzyme is necessary for the formation collagen cross-links which provide the strength and elasticity to many body tissues. In this study, we characterize mutations in the *PLOD1* gene in 14 patients with EDS VI. Their diagnosis was established either by enzyme assay (decreased lysyl hydroxylase activity in fibroblasts) or by abnormal urinary pyridinium cross-links (increased doxypyridinoline with increased ratio deoxypyridinoline/pyridinoline). Primers were designed to detect mutations in the coding regions of the *PLOD1* gene and correlated with RNA and biochemical studies. DNA sequencing identified 8 novel mutations (p.Q49X, p.A72S, p.V380M, p.Y511X, p.R670X, c.580+1G>A, c.1651-2A>G, c.1677_1678dupC). Sequencing alone failed to identify mutations in patients with the duplication of exons 10-16. Most mutations introducing a premature STOP codon resulted in decreased levels of PLOD1 mRNA. There was no clear correlation between type of mutations and biochemical abnormalities in fibroblasts from these patients. These results show that DNA sequencing can be used to confirm the diagnosis of EDS VI and a multitier approach is required to identify causative mutations/rearrangements.

## 365/W/Poster Board #23

**Defective Mechanisms and Signaling Pathways in Inclusion Body Myopathy (IBMPFD) Muscle.** *J. Vesa[1], H. Su[1], G.D. Watts[2], S. Krause[3], M.C. Walter[3], D.C. Wallace[4,5,6], V.E. Kimonis[1].* 1) Dept. of Pediatrics, University of California, Irvine, Irvine, CA; 2) Dept. of Orthopedic Surgery, Children's Hospital Boston, Harvard Medical School, Boston, MA; 3) Dept. of Neurology, Ludwig-Maximillians-University, Munich, Germany; 4) Center for Molecular and Mitochondrial Medicine and Genetics, University of California, Irvine, CA; 5) Dept. of Biological Chemistry, University of California, Irvine, CA; 6) Depts of Ecology and Evolutionary Biology and Pediatrics, University of California, Irvine, CA.

Inclusion body myopathy associated with Paget's disease of the bone and frontotemporal dementia (IBMPFD) is caused by mutations in the Valosin Containing Protein (VCP) gene. Mutations typically in the ubiquitin binding domain of the gene result in progressive, proximal muscle weakness, inclusions and vacuoles in muscle fibers, malfunction in the bone remodeling, and premature frontotemporal dementia. VCP is involved in several cellular processes including the endoplasmic reticulum-associated degradation of defective proteins. We have studied molecular and cellular consequences of VCP mutations in primary myoblasts and muscle tissues from IBMPFD patients and control subjects. Our studies revealed that patients' myoblasts accumulate large ubiquitin-positive vacuoles that are able to fuse with lysosomes. Lysosomal membrane proteins Lamp1 and Lamp2 were defectively N-glycosylated in patients' myoblasts. The maturation processes of mutant cells to myotubes were also affected, which may be due to the down-regulation of the cell adhesion molecule M-cadherin. Additionally, mutant myoblasts demonstrated increased autophagy when cultured in the absence of nutrients, as well as increased apoptosis. To elucidate the affected signaling cascades in IBMPFD, we determined expression profiles by microarray technology using quadriceps muscle samples from patients and unaffected relatives. Statistical and pathway analyses of the obtained expression data revealed that 279 genes were differentially expressed in patients' muscle ($p<0.001$). Most of these dysregulated genes were shared with other muscle dystrophies. An exception was Platelet-Derived Growth Factor Receptor Alpha (PDGFR-$\alpha$), which showed 7.5-fold down-regulation in IBMPFD patients' muscle. This finding was confirmed by qRT-PCR and Western blotting. These findings, combined with the reports showing that defective PDGFR-$\alpha$ expression is involved in the progression of muscular dystrophies and the promotion of muscle fibrosis as well as in the active stage of tissue destruction, suggest that PDGFR-$\alpha$ may play a significant role in the development of progressive muscle pathology in IBMPFD patients. We hypothesize that affected PDGFR-$\alpha$-mediated signaling may result in defective autophagosomal functioning and accumulation of still uncharacterized storage material in patients' cells. This is associated with increased apoptosis and defective myotube formation, which eventually result in muscle weakness in IBMPFD patients.

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## 366/W/Poster Board #24

**The research on gene mutation analysis of SPG4 and SPG3A in Chinese hereditary spastic paraplegia patients.** *L. Shen[1,2], YG. Wang[1], C. Chen[1], J. Du[1], YY. Luo[1], JL. Wang[1], ZQ. Xiao[1], XX. Yan[1,2], BS. Tang[1,2,3].* 1) Department of Neurology, Central South University, Changsha, HUNAN, China; 2) Neurodegenerative Disorders Research Center, Central South University, Changsha, China;; 3) National Laboratory of Medical Genetics of China, Central South University, Changsha, China.

Hereditary spastic paraplegias (HSPs or SPGs) are a heterogeneous group of inherited neurodegenerative disorders characterized by slowly progressive spasticity and weakness of the lower extremities. They are divided into pure and complicated forms depend on whether the paraparesis exists in isolation or with other major clinical features, such as dementia, mental retardation, ataxia, deafness, epilepsy, peripheral neuropathy, extrapyramidal disturbances, and skin lesions. HSPs can be inherited in an autosomal dominant (AD), autosomal recessive (AR), or X-linked manner. So far, at least 37 distinct HSP loci designated SPG have been assigned with 19 disease-associated genes identified. SPG4 and SPG3A are the common subtypes, which account for 40% and 10% of AD-HSP patients, respectively. The rearrangment mutation on SPG4 gene have been reported abroad, however there was no rearrangment mutation detection of AD-HSP patients performed in Asia. Objective: To investigate the mutation frequencies of SPG4 and SPG3A gene in AD and sporadic HSP patients in China. Methods: PCR and direct sequencing were carried out to detect the micro-mutation of SPG4 and SPG3A gene in 21 Chinese AD-HSP patients and 44 isolated cases; MLPA technology and capillary electrophoresis were undertook to detect rearrangment mutation of SPG4 and SPG3A gene in 21 Chinese AD-HSP patients. Results: Five families had micro-mutations on SPG4 gene, and another five families carried rearrangment mutations of SPG4 gene. In addition, one individual had micro-mutation in 44 sporadic cases. Altogether, we found 10 mutations on SPG4 gene, which comprised of 1 missenses, 2 nonsenses, 2 micro-deletions, 1 micro-insertion, 3 exon deletions and 1 exon duplication, and five of them were novel. Among 11 SPG4 probands, 9 cases presented pure spastic paraplegia, and 2 cases showed complicated forms. One family and one isolated case had missense mutations on SPG3A gene, respectively. One of the two missense mutations was novel. Two SPG3A probands also presented pure spastic paraplegia, and the SPG3A family showed incomplete penetrance. Conclusions: The mutation frequencies of SPG4 were 47.6% in AD-HSP patients and 2.3% in isolated cases in Chinese Han people. The mutation frequencies of SPG3A were 4.8% in AD-HSP patients and 2.3% in isolated cases in Chinese Han people.

## 367/W/Poster Board #25

**Screening for the GJB2 c.-3170 G>A (IVS 1+1 G>A) mutation in Brazilian deaf individuals by Multiplex Ligation-Dependent Probe Amplification (MLPA).** *A.P. Marques-de-Faria[1], S.M. Silva-Costa[2], F.B. Coeli[2], C.R. Lincoln-de-Carvalho[1,2], M. Kurc[3], M.C.A. Pomilio[4], E.L. Sartorato[2].* 1) Department of Medical Genetics, State University of Campinas (UNICAMP) School of Medicine; 2) Center for Molecular Biology and Genetic Engineering (CBMEG) - UNICAMP; 3) Albert Einstein Hospital; 4) Therapeutic Association for Language and Auditory Stimulation.

Mutations in *GJB2* gene are the most common cause of nonsyndromic sensorineural recessive hearing loss. One specific mutation, c.35delG, is the most frequent in the majority of Caucasian populations and may account for up to 70% of all *GJB2* mutations. However, 10% to 40% of patients have a single pathogenic mutation in the *GJB2* gene. Deletions del (*GJB6*-D13S1830) and del (*GJB6*-D13S1854) truncating the *GJB6* gene have been detected in patients heterozygous *GJB2* in different populations. The IVS 1+1 G>A splice site mutation in the non-coding region of the *GJB2* gene has been found in heterozygous state in addition to c.35delG mutation. This mutation has not been reported in Brazilian deaf patients. In the present study we investigated the presence of the IVS 1+1 G>A mutation by Multiplex ligation-dependent probe amplification (MLPA) in 185 unrelated Brazilian patients with autosomal recessive nonsyndromic sensorineural hearing loss (43 heterozygous patients and 142 without any pathogenic mutation in the *GJB2* coding region). We have found two patients (4.6%) carrying the IVS 1+1 G>A mutation in compound heterozygous with c.35delG mutation. The use of this technique as a screening method is discussed. (*supported by CNPq*).

## 368/W/Poster Board #26

**Molecular Genetic Studies of Inherited Alopecia.** *A. Azhar[1], J. Klar[2], S. Nawaz[1], M. Tariq[1], A. Ali[1], I. Ahmad[1], M. Rasool[1], N. Dahl[2], S. Baig[1].* 1) Human Molecular Genetics Lab, National Inst for Biotech and Genet Eng (NIBGE), Faisalabad, Punjab, Pakistan; 2) Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden.

There are several forms of inherited isolated alopecias with variation in age of onset and severity. The main cause is consanguineous marriages in Pakistan. There are many consanguineous families with different types of alopecia including autosomal recessive hypotrichosis, congenital atrichia with papular lesions, autosomal recessive wooly hair and alopecia universalis etc. Unfortunately, there is no therapy for majority of such genetic disorders. However, these disorders can be controlled through carrier screening, genetic counseling, mutation detection and prenatal diagnosis. To establish such programs it is mandatory to understand the molecular basis of these disorders. In this study we ascertained thirteen consanguineous alopecia families with multiple affected members from northern part of Pakistan. The disease segregates in all families in autosomal recessive fashion. We used candidate gene approach for this study and selected five genes including HR, P2RY5, LIPH, DSG4 and FOXI3. Linkage analysis was performed by PCR using the STR (short tandem repeats) microsatellite markers for the known loci. Five families showed linkage to P2RY5 gene, two families with LIPH gene whereas only one family of Alopecia linked to HR gene. Direct sequencing of the PCR products was carried out and identified the pathogenic mutations. We found a mutation in P2RY5 gene in five families with autosomal recessive wooly hair (ARWH). Haplotype analysis identified same disease haplotype in all these families which showed the common ancestor for them. Along this mutation, we also identified three recurrent mutations in LIPH and HR genes. This molecular knowledge will help in the establishment of carrier screening and prenatal diagnosis tests for prevention of above diseases and to understand the molecular basis involved in the gene function.

## 369/W/Poster Board #27

**A novel homozygous missense variation in HR gene caused Atrichia with Papular lesion.** *S. Baig[1], J. Klar[2], A. Azhar[1], M. Tariq[1], A. Ali[1], I. Ahmad[1], J. Schuster[2], N. Dahl[2], S. Nawaz[1].* 1) Human Molecular Genetics Lab, Natl Inst Biotech/Genetic Eng, Faisalabad, Pakistan; 2) Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden.

Atrichia with papular lesions (APL) is a rare autosomal recessive form of congenital Alopecias characterized by onset of complete or near to complete irreversible alopecia or loss of hair soon after birth or in first few months of life. Only difference in Alopecia universalis Congenita (AUC) and Atrichia with popular lesions (APL) is the papules or milia in APL. APL has been mapped to 8p12 and identification of Human Hairless (HR) gene mutations are associated with its pathogenesis. Diagonisis of APL involves autosomal recessive form of transmittance of disease with possible consanguinity of parents, hair either present at birth or not that never regrows and presence of papules. Although cases with compound heterozygous mutations are emerged to challenge the criteria due to increasing number of sporadic cases. Atrichea with papules have equal prevalence in both men and women but its exact prevalence is still a question. Here we report a novel homozygous missense c.2427 C>T transition in exon10 of HR gene that results in p.A765V substitution and small variation of phenotype in a consanguineous Pakistani family in all affected individuals. This missense variant was excluded on 200 control chromosomes of Pakistani origin and 200 control chromosomes of Swedish origin.

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## 370/W/Poster Board #28

**A Novel Approach to Mapping Causal Variants in a Family with Extreme Iron Overload.** *J.M. Brew[1,2], J.A. Cavanaugh[2], M.L. Bassett[3].* 1) John Curtin School of Medical Research, Australian National Univ, Canberra, Australia; 2) Medical Genetics Research Unit, ANU School of Biology and ANU Medical School, Australian National University, Canberra, Australia; 3) ACT Health, Canberra, Australia.

Hereditary haemochromatosis (HH) is usually a late onset autosomal recessive disorder of excess iron storage that may lead to severe organ damage if left untreated. In 1996 two causative mutations (C282Y and H63D) in the *HFE* gene were shown to explain ~85% of HH cases. A recent recruit to our 20 year study of HH was an affected individual of Croatian origin who was diagnosed at ~55 years and had a serum ferritin of >9000 micrograms/L, which is significantly higher than in any other patients in our study (reference range for males is <300μg/L). Analysis of serum from his brother gave a similar result. DNA sequencing ruled out any known mutations or likely causal variants in the exons and 500bp in the 5' region of both *HFE* and *HAMP* (hepcidin antimicrobial peptide - sometimes called the master regulator of iron homeostasis). In addition, haplotype analysis across the *HFE* gene demonstrated that the brothers have only one haplotype in common. We hypothesized that other genes in the iron pathway may be responsible for the disorder in these two brothers. As no members of their extended family are available for traditional mapping, we have developed an innovative approach to identifying the causal variant(s) in these genes using lymphoblastoid cell lines (LCL) established from both brothers. Our results show that the expression levels of *HFE*, *TFRC* (transferrin receptor) and *HAMP* are more similar to those observed in LCLs from affected (YY) individuals than those from normal (CC) individuals. In particular, the expression of transferrin receptor 2 (*TFR2*) is significantly reduced in these two patient lines in comparison to all other samples. We have also assessed the impact of ferrous sulfate in the culture media and have demonstrated a similar mortality in the brothers' LCLs to that observed in LCLs from YY individuals (but not CC individuals). To follow up on these exciting results, we are currently sequencing other genes in the iron pathway, particularly focusing on variants in the *TFR2* gene as the likely explanation of the extreme iron overload in these brothers.

## 371/W/Poster Board #29

**Posterior polymorphous corneal dystrophy - copy number, gene expression and candidate gene analyses within the PPCD1 candidate region on chromosome 20p 11.2.** *L. Noskova[1], P. Liskova[2,4], V. Stranecky[1], H. Hartmannova[1], R. Ivanek[1,5], K. Jirsova[2,3], S. Merjava[2,3], M. Filipec[4,6], S. Kmoch[1,7].* 1) Institute of Inherited Metabolic Disorders, Charles University, Prague, Czech Republic; 2) Laboratory of Biology and Patology of the Eye, General Teaching Hospital, Charles University, Prague, Czech Republic; 3) Ocular Tissue Bank, General Teaching Hospital, Charles University, Prague, Czech Republic; 4) Department of Oftalmology, General Teaching Hospital, Charles University, Prague, Czech Republic; 5) Institute of Molecular Genetics, Academy of Sciences, Prague, Czech Republic; 6) Eye Clinic Lexum, Prague, Czech Republic; 7) Center for Applied Genomics, Prague, Czech Republic.

Posterior polymorphous corneal dystrophy (PPCD) is a genetically heterogeneous autosomal dominant disorder characterised by epithelisation of the endohelium and irregular thickening of Descemet's membrane. It often leads to irreversible corneal edema and requires corneal transplantation. The genetic heterogeneity of PPCD is currently known to be represented by three loci on chromosomes 20, 1, and 10 and several disease causing genes (COL8A2, ZEB1/TCF8) have been identified. We have previously shown linkage in two Czech PPCD1 families to chromosome 20p11.2. To further narrow the PPCD1 candidate interval we used Affymetrix Genome-Wide Human SNP Array 6.0 and genotyped family members demonstrating critical recombination events by STR analysis. Haplotype analysis narrowed the critical region to a 2.1Mb interval delimited by markers D20S114 and rs7509232. In parallel we used the genotyping data, assesed copy number status and found no indication for deletions/duplications within the candidate region. We have also manufactured custom oligonucleotide array and analysed expression changes of all genes located within the candidate region in samples of corneal tissues obtained from patients undergoing corneal transplantation and controls. This analysis showed significantly reduced amounts of destrin (DSTN) transcript in corneal tissues of four patients compared to healthy control tissue. However, subsequent sequencing analysis of the coding and promotor regions of DSTN did not reveal any potential disease causing mutation. As no mutations were found by sequencing of genomic coding regions of other genes in candidate region, we decided to sequence whole 2.1Mb candidate region by combination of NimbleGen Sequence Capture array and next-gen sequencing approach.

## 372/W/Poster Board #30

**Narrowing the critical regions associated with SHFM1 and SHFM3 forms of ectrodactyly through array-based comparative genomic hybridisation.** *S. Raskin[1,2], A. Bonalumi[1], J. Souza[1].* 1) Pontificia Universidade Catolica do Paraná, Curitiba, Parana, Brazil; 2) Laboratory Genetika, Curitiba, Parana, Brazil.

Introduction:Split-hand/foot malformation (SHFM), or ectrodactyly, is characterized by underdeveloped or absent central digital rays, clefts of the hands and feet, and variable syndactyly of the remaining digits. SHFM occurs as both an isolated finding and a component of many syndromes. SHFM is a heterogeneous condition caused by multiple loci, including SHFM1 (chromosome region 7q21-q22), SHFM2 (Xq26), SHFM3 (10q24), SHFM4 (3q27), and SHFM5 (2q31). The recent identification of submicroscopic tandem chromosome duplications affecting the SHFM3 locus in several families with non-syndromic SHFM has helped to further unravel the molecular basis of this malformation. We report two unrelated families, one with two brothers affected with non-syndromic SHFM and the other, a sporadic case with ectrodactyly-deafness syndrome, both investigated by CGH-array. Methods:Patients and their parents were investigated using a commercial DNA microarray constructed from large insert clones spaced across the genome. Array-based comparative genomic hybridization was performed with a targeted bacterial artificial chromosome (BAC) microarray. In addition to known clinical loci, other regions of the genome have been included on the microarray to help identify chromosome abnormalities outside of these known regions, for a total of 622 discrete loci. Conclusion: We found that the brothers with non-syndromic ectrodactyly have a small duplication of 273Kb at 10q24.32, including the genes BTRC, POLL, FBXW4, FGF8, NPM3 and MGEA5, therefore narrowing the citical region of SHFM3.Furthermore, the fact that their both parents are normal, shows that reduced penetrance should be considered in SHFM3. We speculate that one of the parents may have a somatic mosaicism for the mutation. In the second family, the finding of a deletion of 13.1 Mb at 7q21.11q21.3, including the genes SHFM1, DLX5, DLX6 and FZD1 also helps to narrow the critical region for SHFM1. These results show that array comparative genomic hybridisation should be considered to be an essential aspect of the genetic analysis of patients with different types of SHFM. Moreover, besides their importance for diagnosis and genetic counselling, they may allow the identification of critical regions associated with SHFM. Finally, the detailed molecular analysis of the rearranged regions may help to further unravel the molecular basis of this malformation and finally lead to the identification of SHFM causing genes.

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## 373/W/Poster Board #31

**A 944 kb duplication on chromosome 1p31.1 causes autosomal dominant omphalocele.** *R. Uppala[1,2,10], S.K Nath[3], K. McElreavey[4], U. Ratnamala[2], C. Sun[3], D. Hutchings[3], A.K. Maiti[3], H.L. Newkirk[5], D.B. Everman[6], A. Sharp[1], J. Murray[7], C. Schwartz[8], S.E. Antonarakis[1], M.G. Butler[9].* 1) Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland; 2) Cancer Ccenter, Creighton University, Omaha, NE. USA; 3) Arthritis and Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, USA; 4) Reproduction, Fertility and Populations, Department of Developmental Biology, Institut Pasteur, Paris, France; 5) Molecular Diagnostics, Clinical Reference Laboratory, Inc. - 8433 Quivira Road - Lenexa, Kansas, KS, USA; 6) Greenwood Genetic Center, Greenville Office, Greenville, SC, USA; 7) University of Iowa, Division of Neonatology, Department of Pediatrics Iowa City, IA, USA; 8) J.C. Self Research Institute, Center for Molecular Studies, Greenwood Genetic Center, Greenwood, SC, USA; 9) Departments of Psychiatry & Behavioral Sciences and Pediatrics, Kansas University Medical Center, Kansas City, KS, USA; 10) Green Cross Voluntary Blood Bank, Paldi, Ahmedabad, India.

Omphalocele is a congenital birth defect characterized by the presence of some of the internal organs located outside of the ventral wall. The size of the omphalocele varies, depending on the presence of which internal organs, (liver, spleen, intestines) are herniated into the umbilical cord. We have conducted a linkage study in a large family with autosomal dominant omphalocele using a genomewide SNP array. Our analysis revealed significant evidence of linkage (nonparametric NPL = 6.93, P = 0.0001; and parametric LOD = 2.70 under a fully penetrant dominant model) at chromosome 1p31.3. Haplotype analysis narrowed down the locus to a 2.74 Mb region between markers rs2886770 (63014807bp) and rs1343981 (65757349bp). Molecular characterization of this interval using array CGH followed by Quantitative Microsphere Hybridization (QMH) analysis revealed a 944 kb duplication between 63,561,322bp-64,505,749bp. All affected individuals who had an omphalocele and shared the affected haplotype were positive for this duplicated region and are not duplicated for probes around the region at chr1:63,495,050bp and chr1:65,004,750bp spanning 1509kb. Multipoint linkage analysis using the duplication as a marker yielded a maximum LOD score of 3.2 at 1p31.3 under a dominant model. The 944 kb duplication on 1p31.3 contains eight known genes including FOXD3, ALG6, ITGB3BP, KIAA1799, PGM1, ROR1, DLEU2L and UBE2U. The present study suggests that development of an omphalocele in this family is controlled by over expression of one or more genes in the duplicated region. To our knowledge, this is the first reported linkage and molecular characterization for the omphalocele phenotype.

## 374/W/Poster Board #32

**Identifying genes for cleft lip with or without palate using chromosomal abnormalities detected by array comparative genomic hybridization (CGH).** *K.M. Dipple[1, 2], J. Peredo[2], R. Jarrahy[3], J.P. Bradley[3], F. Quintero-Rivera[4].* 1) Deptartment of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA; 2) Department of Pediatrics, David Geffen School of Medicine at UCLA, Mattel Children's Hospital at UCLA, Los Angeles, CA; 3) Department of Surgery, Division of Plastic & Reconstructive Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA; 4) Department of Pathology & Laboratory Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA.

To identify novel genes predisposing families to cleft lip with or without cleft palate (CL/P), we have been analyzing patients with syndromic CL/P. We report a patient with multiple congenital anomalies including cleft lip and palate who had several genomic copy number imbalances. The proband presented at birth with multiple congenital anomalies including unilateral incomplete left cleft lip, a notch in the left alveolus, and a complete cleft of the secondary palate. In addition there was mild hypertelorism, bilateral undescended testes and a sacral dimple. He developed renal insufficiency due to obstructive uropathy secondary to posterior uretheral valves. He had bilateral hydronephrosis and bilateral grade 5 vesico-ureteral reflux. Echocardiogram revealed a patent foramen ovale but otherwise normal intracardiac anatomy. Head ultrasound showed some punctate lesions in the lateral ventricle. Spinal ultrasound showed no tethered cord. FISH analysis on interphase nuclei detected the presence of an extra X chromosome and there were no numerical aberrations of chromosomes Y, 13, 18 and 21. Chromosome analysis confirmed 47, XXY complement consistent with Klinefelter syndrome. Because of the atypical clinical presentation of Klinefelter's in this patient, a comparative genomic hybridization (CGH) array analysis was also performed. The aCGH detected the extra X chromosome, as well as a 5.8 Mb terminal deletion spanning bands 7p22.1-7p22.3, and a 2.6 Mb chromosome 16 terminal duplication at band 16p13.3. Parental chromosomes and CGH array were normal. Analysis of these genomic regions identified novel genes that may be involved in the formation of cleft lip with or without cleft palate, including *FAM20C, UNCX, LFNG,* and *UBE2I*.

## 375/W/Poster Board #33

**Mutations in *DNAH11* are exclusively seen in primary ciliary dyskinesia patients with normal ciliary ultrastructure: Deciphering the effect of splice mutations on the transcript.** *M. Zariwala[1], M. Leigh[2,5], J. Carson[2], M. Hazucha[3], S. Minnix[3], K. Burns[3], M. Armstrong[3], A. Lori[3], H. Metjian[3], N. Loges[4], H. Olbrich[4], A. Becker[4], M. Schmidts[4], H. Omran[4], M. Rosenfeld[5], T. Ferkol[5], S. Dell[5], K. Olivier[5], S. Sagel[5], M. Knowles[3,5].* 1) Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC; 2) Department of Pediatrics, University of North Carolina, Chapel Hill, NC; 3) UNC School of Medicine, University of North Carolina, Chapel Hill, NC; 4) Department of Pediatrics, University Hospital, Freiburg, Germany; 5) Genetic Disorders of Mucociliary Clearance Consortium, USA.

Primary ciliary dyskinesia (PCD)/Kartagener syndrome is an autosomal recessive, genetically heterogeneous disorder characterized by oto-sino-pulmonary disease due to the abnormal structure and function of cilia. The current method of diagnosis is ultrastructural analysis of the cilia and majority of the PCD patients (80-90%) have either defective outer dynein arm (ODA), or inner dynein arm (IDA) or both dynein arms (DA). The definitive diagnosis of PCD becomes challenging in patients who present with the compatible clinical phenotype, low nasal nitric oxide levels that are commonly seen in PCD, but normal ciliary ultrastructure. Although 10 PCD-causing genes have been described, two genes (*DNAI1* and *DNAH5*; both encode ODA proteins) account for 17-38% of PCD. The genetic etiology in majority of the PCD patient is still elusive. Two unrelated families with PCD and normal DA have been described to have biallelic mutations in *DNAH11* (encodes ODA heavy chain), but large scale studies were missing. Hence, to test *DNAH11* as a candidate gene for PCD, we carried out mutation analysis of 82 coding exons and intron/exon junctions in 164 well-characterized unrelated PCD patients (59 had normal DA, 74 had ODA defect, 8 had central pair defects and 23 ultrastructure unavailable). Biallelic mutations were seen in 13 of 59 (22%) patients with normal DA. Two additional patients (ciliary ultrastructure unavailable) had biallelic mutations. Of the total 31 mutant alleles, 9 were nonsense, 6 were frame-shift, 7 were splice site and 9 were missense mutations. We carried out the analysis of mRNA (from transformed lymphocytes) on 5 splice site mutations and found that splice mutations caused: 1) in-frame deletion of an exon (n=2); 2) out-of-frame deletion of an exon (n=2); and 3) out-of-frame deletion of the four exons (n=1). In conclusion, our results show that in the absence of ultrastructural defects, genetic analysis can confirm the diagnosis of PCD. In addition, we demonstrate that splice site mutations resulted in the abnormal transcripts that would be predicted to cause loss-of-function for protein. This abstract was funded by RR00046, 1 RO1 HL071798, 5 U54 RR019480. Resequencing was provided by NHLBI N01-HV-48194R99 NHLBI RS&G Services.

## 376/W/Poster Board #34

**Frequency of 35delG mutations in cochlear implant recipients.** *M. Falah[1], M. Houshmand[2], s. Akbaroghli[3], M. Farhadi[1].* 1) Department and Research Centr, Genetic, Tehran, Tehran, Iran; 2) National Institute for Genetic Engineering and Biotechnology, Tehran, Iran, Tehran, Iran, Islamic Republic of; 3) 3Tehran welfare Organization, Tehran, Iran, Islamic Republic of, 4National institute of genetic.

Hearing impairment is the most common sensory disorder, present in 1-4 of every 1000 newborns. Nonsyndromic sensorineural hearing loss(NSHL) impairment is inherited in a predominantly autosomal recessive manner in up to 70% of cases. It is also an extremely heterogeneous trait. The gene more often involved is GJB2, encoding the protein Connexin 26. In most populations a single mutation, 35delG, accounts for most cases of NSHI . 100 patients receiving cochlear implants were ascertained through research centre of ENT & head and neck surgery .After genetic counseling for them DNA isolated from the peripheral blood, all patients were molecularly evaluated for the presence of the 35delG mutation by ARMS /PCR. We investigated 100 patients , 45% male and 55% female , The age distribution was: Min.= 1 ; Max.= 28 and Mean:7±5 72% of these patients were born on consanguine family and 87%of them were nonsyndromic hearing loss. Among these patients 86% were normal, 10 % homozygote and 4% heterozygote for 35delG mutation. Conclusion The most frequent genes implicated in autosomal recessive nonsyndromic hearing loss are GJB2, which is responsible for more than half of cases.In our study, there was a significant relationship between consanguineous marriage in our evaluated group and express of NSHI .( PV#0/00 and O.R= 3.64 , 2.30< OR <5.79 ). but there was not any significant relationship between inheritance pattern and consanguineous marriage with 35delG mutation. These data can help genetic counselor and otologist for setting priority in evaluation, prevention and even treatment in these patients.

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## 377/W/Poster Board #35

**Further genetic heterogeneity in Emery-Dreifuss muscular dystrophy.**
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Emery-Dreifuss Muscular Dystrophy (EDMD) is a rare muscular disorder characterized by early joint contractures, slowly progressive muscular dystrophy, cardiac involvement including conduction defects and arrhythmias. So far only approx. 40% of the EDMD cases can be associated with LMNA, EMD, SYNE1 or SYNE2 gene mutations, suggesting the existence of additional genes involved. By a genome wide scan in a large German family, we found by multipoint linkage analysis a LOD score of 3.6 to the region Xq26. Using a candidate gene approach we found a mutation c.61559 T>C, p.C209R in the FHL1-gene. The mutation co-segregated with the disease in the family studied. The disease phenotype was compatible with EDMD unless a pronounced cardiac and shoulder girdle hypertrophy, which is compatible with a previously described phenotype - XMPMA - associated to a c.61559 T>C, p.C224W mutation in FHL1. Moreover, in our EMD and LMNA negative cohort of 198 patients, we identified the FHL1 mutation c.61606 C>G, p.C224W in four unrelated males suggesting a mutational hot spot. Thus FHL1 is a further major gene involved in the pathogenesis of an additional 2,5 % of our EDMD cases. It should strongly be considered as a diagnostic tool in all EMD and LMNA negative patients with EDMD or EDMD like phenotypes.

## 378/W/Poster Board #36

**Mapping of a novel locus for orbital innervation to chromosome 4q24-25.**
*N.A. Al Tassan[1], D.S. Khalil[1], L.J. Al Sharif[1], J.M.A. Shinwari[1], A.O. Khan[2].* 1) Department of Genetics, King Faisal Specialist Hospital & Research Center, P.O Box 3354 Riyadh 11211,Saudi Arabia; 2) Division of Pediatric Ophthalmology, King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia.

Orbital dysinnervation can cause incomitant strabismus and/or ptosis, such as congenital fibrosis of the extraocular muscles, Duane retraction syndrome (DRS), and Moebius syndrome. We identified a consanguinous family in which 3 siblings exhibited a unique form of orbital dysinnervation without other clinical findings. The oldest affected brother had right congenital ptosis. His sister had bilateral congenital ptosis. Their brother had DRS IV (synergistic divergence) in his right eye and DRS I in his left eye. Genome-wide linkage analysis using the Affymetrix GeneChip® Human Mapping 250K Sty Array suggested a novel recessive disease locus on chromosome 4q24-25 with a maximum multi-point LOD score of 2.5. This region comprises 40 genes of which selected candidate genes were sequenced. Several variants were identified. The genetic defect in this family likely affects a mechanism responsible for orbital innervation.

## 379/W/Poster Board #37

**Identification of a candidate locus in familial systemic lupus erythematosus by homozygosity mapping.**
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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by high fever, erythema, arthritis, renal dysfunction and elevated serum autoantibodies. SLE is a multifactorial disease in which both genetic and environmental factors are involved. Indeed, SLE patients with autosomal dominant or recessive inheritance have been reported. Recent studies have revealed many genetic predisposing factors from a large-scale candidate gene or genome wide association analysis in familial and sporadic cases. However, the genetic basis of SLE remains unclear. We identified one consanguineous Japanese family, which consists of 3 affected and 1 unaffected siblings. Affected siblings presented psychological disorders and multiple autoimmune diseases such as SLE, Sjögren's syndrome and Hashimoto thyroiditis. To identify an underlying gene, we performed a genome-wide homozygosity mapping using the high density SNP microarray (HumanCNV370-Quad, Illumina) in the four siblings and revealed two homozygous regions (FSLE-1, FSLE-2) that spanned 0.7 Mb and 1.1 Mb, respectively. Next, we confirmed and refined with flanking microsatellite markers spanning the 10-cM each homozygous region. Multipoint parametric linkage analysis and haplotype reconstruction were performed with the Genehunter program, under the assumption of autosomal recessive inheritance with complete penetrance and a disease allele frequency of 0.001. Haplotype reconstruction was consistent with autosomal recessive inheritance and showed that the affected siblings were homozygous by descent for the allele within the critical region. The FSLE-1 did not contain any gene, but the FSLE-2 region contained 40 known and predicted genes annotated in the UCSC database. We selected several genes from FSLE-2 region based on the available data on immunological functions and are now doing DNA sequence of those genes. The identification of the candidate gene of those SLE patients would provide crucial insights into not only pathogenesis of SLE but also regulation of immune systems.

## 380/W/Poster Board #38

**Disease mutation detection by next generation sequencing platforms.**
*H. Wang[1,2], K. Zhang[3], F. Yu[1,2], M. Bray[4], R. Lewis[2,5,6], J. Lupski[2,6,7], G. Mardon[2,7,8,9,10], D. Muzny[1,2], R. Gibbs[1,2,], R. Chen[1,2,10].* 1) Human Genome Sequencing Center, Baylor College of Medicine, houston, TX; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, houston, TX; 3) Department of Bioengineering, University of California at San Diego, La Jolla, California, USA; 4) Children's Nutrition Research Center, Baylor College of Medicine, houston, TX; 5) Department of Ophthalmology, Baylor College of Medicine, houston, TX; 6) Texas Children's Hospital, Baylor College of Medicine, houston, TX; 7) Department of Neurology, Baylor College of Medicine, houston, TX; 8) Department of Neuroscience, Baylor College of Medicine, houston, TX; 9) Department of Pathology, Baylor College of Medicine, houston, TX; 10) Program in Developmental Biology, Baylor College of Medicine, houston, TX.

Next-generation high-throughput DNA sequencing techniques have great potential to dramatically accelerate the discovery of mutations which cause human diseases. To test this approach, both targeted capture sequencing (TCS) and whole genome shotgun sequencing (WGS) strategies were tested on three sequencing platforms, including 454, Illumina GAII, and SOLiD, to identify novel mutations in patients with Leber congenital amaurosis (LCA). LCA is a clinically and genetically heterogeneous disorder characterized by severe vision loss at birth. Several novel LCA disease loci have been identified by our group via homozygosity mapping using genome high density SNP genotyping. The size of these loci ranges from 10Mb to 100Mb, making PCR followed by Sanger sequencing strategy cost prohibitive. Depending on the size of each locus, either TCS or WGS were performed. In the case of TCS, exons within the candidate locus were captured and sequenced using both the 454 and the Illumina GAII platform. Our preliminary data indicate that high sensitivity and specificity can be reached by this method. More than 90% of the exons can be captured and sequenced to sufficient depth. In addition, about 95% of sequencing reads can be mapped to the targeted regions, indicating very little off targets effect. In parallel, WGS was performed on two patient samples using the SOLiD platform. About 7x sequencing coverage were generated for each individual and a list of potential variations, including missense and nonsense changes, has been identified. Further validation and analysis of these potential changes are currently underway and will be reported. In addition, systematic comparison of TSC and WGS as well as the three NextGen sequencing platforms will also be performed on our data set.

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## 381/W/Poster Board #39

**Autosomal-Dominant Retinitis PIgmentosa is caused by mutations in a BTB-Kelch Protein, KLHL7.** *J.S. Friedman[1], J.W. Ray[2], N. Waseem[3], K. Johnson[4], M.J. Brooks[1], T. Hugosson[5], K.E. Branham[6], D.S. Krauth[1], S.J. Bowne[2], L.S. Sullivan[2], V. Ponjavic[5], A.R. Webster[3], D.G. Birch[7], G.R. Abecasis[8], Y. Fann[4], S.S. Bhattacharya[3], S.P. Daiger[2], J.R. Heckenlively[6], S. Andréasson[5], A. Swaroop[1,6,9].* 1) NEI/NNRL, NIH, Bethesda, MD; 2) Human Genetic Center, The University of Texas Health Science Center, Houston, TX; 3) Department of Molecular Genetics, Institute of Ophthalmology, London, UK; 4) Bioinformatics Section, Division of Intramural Research, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD; 5) Department of Ophthalmology, Lund University Hospital, Lund, Sweden; 6) Department of Ophthalmology, University of Michigan, Ann Arbor, MI; 7) Retina Foundation of the Southwest, Dallas, TX; 8) Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI; 9) Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI.

Retinitis pigmentosa (RP) encompasses a group of progressive retinal diseases that lead to the loss of photoreceptors in the retina. To date, 18 genes have been associated with autosomal dominant (ad) RP. Through linkage analysis of a large Scandinavian family, we identified an adRP locus (RP42) at chromosome 7p15. We determined the disease-causing mutation to be c.449G/A (p.S150N) in exon 6 of the KLHL7 gene. Six independent families with three different missense mutations were subsequently uncovered from a large screen of 502 retinopathy probands. KLHL7 is broadly expressed and encodes a protein with homology to the BTB-Kelch family. Homology modeling of the three mutations located in the conserved BACK domain suggests that mutant amino acid side chains can potentially fill the cleft between two helices. Gigaxonin, another BTB-Kelch protein, has been previously shown to cause giant axonal neuropathy. We are currently exploring KLHL7 protein interactors through immunoprecipitation and yeast-two hybrid experiments of bovine retina. Based on other BTB-Kelch family members' functions, we hypothesize that KLHL7 is involved in ubiquitin-proteasome protein degradation pathway and may interact with one or more E3 ligases. Further elucidation of KLHL7 interactors will assist in understanding of the biology and disease processes of the retina.

## 382/W/Poster Board #40

**Hunting down the last Meckel syndrome genes in the Finnish population.** *J. Tallila[1], E. Jakkula[1], T. Varilo[1,2], L. Peltonen[1,2,3], R. Salonen[4], M. Kestila[1].* 1) 1National Institute of Health and Welfare, Public Health Genomics Unit and FIMM, Institute for Molecular Medicine Finland, Helsinki 00290, Finland; 2) Department of Medical Genetics, University of Helsinki, Helsinki 00290, Finland; 3) The Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK; 4) Department of Medical Genetics, Väestöliitto, Helsinki 00100, Finland.

Meckel syndrome (MKS, [MIM 249000]) is an autosomal recessive, early lethal disorder characterized by a combination of severe malformations. Minimum diagnostic criteria are cystic dysplasia of the kidneys with fibrotic changes in the liver and occipital encephalocele or some other central nervous system malformation. Polydactyly is also a frequent finding in MKS. The number of disease genes and loci has increased rapidly in the past few years. To date, five genes, six loci and over 50 mutations have been implicated in MKS. These findings have demonstrated that MKS genes are not restricted to certain populations and that MKS is an allelic disorder with several other syndromes with overlapping phenotypes, e.g. Joubert syndrome. Unraveling the molecular background of MKS has revealed that it is caused by primary cilia dysfunction. These findings have had a great impact on studies of MKS as well as on studies of cilia. In the Finnish population 90% of the MKS cases are caused by homozygous mutations in either *MKS1* or *CC2D2A* genes: a 29 bp intronic deletion in the *MKS1* gene (c.1483-7_35del) and a C>T substitution in the coding region of *CC2D2A* (c.1762C>T), respectively. Both of these mutations result in abnormal splicing. In addition, systematic sequence analysis of the established MKS genes showed that one Finnish MKS case is a compound heterozygote for mutations in MKS1 and one for mutations in *CEP290* (*MKS4*). As we still have six typical MKS families without a known mutation, we are currently hunting for a new MKS locus (or loci). Instead of a traditional linkage analysis, we are applying homozygosity mapping with affected MKS cases using Illumina genome-wide SNP arrays, method which we have used successfully previously in identification of the fifth MKS gene, *CC2D2A* (*MKS6*). To evaluate which families would share the same founder mutation with a common homozygous region, we are genealogically tracing the consanguinities of the families and using dense SNP-data to estimate identity-by-descent (IBD) sharing between affected individuals. Combining of genealogical data, current genome-wide SNP technology and improved ciliary database information provide us effective tools to first identify additional MKS locus (or loci) and then proceed with further functional studies and improved knowledge of MKS pathogenesis.

## 383/W/Poster Board #41

**Non-syndromic X-linked deafness (DFN2) is caused by decreased functional mutations in PRPS1.** *H. Yuan[1], X. Liu[1,2], J. Li[1], B. Han[1], J. Cheng[1], Y. Wang[3], J. Chen[1], Y. Liu[3], P. Dai[1], D. Han[1].* 1) Inst Otolaryngology, Chinese PLA General Hospital, Beijing 100853, China; 2) Department of Otolaryngology, University of Miami, Miami, FL 33136, USA; 3) Hearing Center, Guizhou Provincial People's Hospital, GuiYang 550002, China.

Hereditary nonsyndromic hearing loss (NSHL) is extremely heterogeneous. During the past decade, remarkable progress has been made towards determining the deafness-causing genes. To date, over 110 loci have been mapped for NSHL, and 47 deafness genes from these loci have been identified. The vast majority of cases of NSHL are associated with mutations in autosomal genes. X-linked deafness is clinically and genetically a heterogeneous disorder accounting for about 5% of all congenital deafness, and for less than 2% of non-syndromic hearing impairment. The first deafness-causing gene, POU3F4, was identified in a family with X-linked non-syndromic hearing loss in 1995. Although seven non-syndromic deafness loci (DFN2-8) have been mapped to the X chromosome over the last 15 years, the second X-linked deafness gene has not yet been identified. The DFN2 locus was mapped to Xq13-q24 and previously defined by three different families. The age at detection of the hearing loss in affected males in these families varied from birth to 20 years of age, indicating that DFN2 can cause either congenital or post-lingual deafness. In the present study, we ascertained a five generation Chinese family characterized by X-linked postlingual, progressive, non-syndromic sensorineural hereditary hearing impairment. We mapped the disease locus to a 5.4 cM region on Xq22 between DXS8020 and DXS8055 overlapping with the known DFN2 locus. Mutations in the phosphoribosyl pyrophosphate synthetase 1 (PRPS1) gene were found in all identified DFN2 families, demonstrating that PRPS1 is likely the gene responsible for DFN2 deafness.

## 384/W/Poster Board #42

**Gene Scanning Of The Interleukin 2 Receptor Gamma (IL2RG) By High Resolution Melting.** *M.A. Liew[1], K.V. Voelkerding[1,2], C.T. Wittwer[1,2].* 1) ARUP Laboratories, Salt Lake City, UT; 2) University of Utah, Department of Pathology, Salt Lake City, UT.

X-linked Severe Combined Immunodeficiency Disease (X-SCID) affects approximately 1/50000-1/100000 newborns. It is characterized by the presence of persistent opportunistic infections. Individuals with X-SCID have mutations primarily in the interleukin 2 receptor gamma (IL2RG) gene, which leads to the loss of this receptor. IL2RG is an important part of the pathway that regulates lymphocyte maturation so without this receptor the immune system does not function properly and infections are able to persist and cause severe disease.

Primers for the 8 exons (8 amplicons), 3'-untranslated region (2 amplicons) and a gender typing control (amelogenin, 1 amplicon) were pre-spotted on 96-well plates and stored until needed. Genomic DNA was purified from whole blood by silica-based automated DNA extraction (40 min), followed by standard PCR in generic thermal cyclers (60 min). High resolution melting analysis was then performed on a dedicated 96-well platform (15 min) for increased throughput. Variants were detected by a change in shape of the melting curves compared to wild type controls. Common polymorphisms were distinguished from mutations by rapid cycle PCR and melting of small amplicons, unlabeled probes, or snapback genotyping (30 min). Only the rare variants were sequenced by standard dideoxytermination (45 min) and capillary electrophoresis (45 min).

A common PCR annealing temperature was identified for all amplicons using a gradient 96-well PCR instrument, melting analysis and agarose gel electrophoresis. Common variants were identified by analyzing 96 female control subjects. Based upon the workflow wild type samples are completed in <4 hours. Common variant genotyping requires an additional hour, and rare variant identification by sequencing requires an additional 2 hours. Scanning for mutations in IL2RG by high resolution melting enables a significant reduction in sequencing for improved turnaround time and cost reduction.

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## 385/W/Poster Board #43

**Homozygosity mapping of primary microcephaly in 86 Iranian families: novel mutations and phenotypes.** *H. Darvish[1], S. Esmaeeli Nieh[1,2], G. Bahrami Monajemi[1], M. Mohseni[1], F. Behjati[1], S. Ghasemi Firouzabadi[1], P. Jamali[1], S. Azimi[1], F. Mojahedi[1], A. Jankhah[1], I. Bahman[1], M. Ghani Kakhki[3], M. Garshasbi[2], S.S Abedini[1], A. Naghavi[1], A.W. Kuss[2], H.H. Ropers[2], H. Neitzel[3], K. Kahrizi[1], H. Najmabadi[1,4].* 1) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; 2) Max Planck Institute for Molecular Genetics, Berlin, Germany; 3) Institute of Human Genetics, Charité Medical University of Berlin, Germany; 4) Kariminejad-Najmabadi Pathology and Genetics Center, Tehran, Iran.

Primary microcephaly (MCPH) is a genetically heterogeneous disorder showing an autosomal recessive mode of inheritance in the majority of cases. Affected individuals present with head circumferences more than three standard deviations below the age- and sex-matched population mean, accompanied by mental retardation without further associated malformations. Five genes (*MCPH1*, *CDK5RAP2, ASPM, CENPJ* and *STIL*) and two additional genomic loci (MCPH2 and 4) have been identified so far. In this study, we investigated all six loci in patients with primary microcephaly and other family members from 86 Iranian families with 2 or more affected individuals. Thorough clinical characterization and karyotype analyses were performed for all patients. For linkage analyses, several microsatellite markers were selected for each locus and used for genotyping. Our investigation enabled us to detect linkage to the *ASPM* region in thirteen families. Three families showed linkage to MCPH2. Seven families were linked to *MCPH1* and four to *CENPJ*. The remaining 59 families were not linked to any of the six known loci. Subsequent sequencing revealed 10 novel mutations in *ASPM*, seven novel mutations in *MCPH1* and one novel mutation in *CENPJ*. Additional clinical features accompanying microcephaly and MR were observed in some patients. While confirming the general prevalence of *ASPM* mutations, our results indicate that these are comparatively less frequent in the Iranian population than in others. This and the high proportion of families without apparent linkage to known loci suggest the presence of additional, probably population specific loci.

## 386/W/Poster Board #44

**Refinement of the LGMD1 locus on 7q36 - genotyping new informative families.** *JP. Hackman[1], S. Sandell[2,3], J. Sarparanta[1], H. Luque[1], S. Huovinen[2,4], PH. Jonson[1], A. Paetau[5], I. Mahjneh[6], B. Udd[1,2,7].* 1) Folkhälsan Institute of Genetics and Department of Medical Genetics, University of Helsinki, Finland; 2) Neuromuscular Research Center, Dept of Neurology, University Hospital and University of Tampere, Finland; 3) Department of Neurology, Seinäjoki Central Hospital, Finland; 4) Department of Pathology, Centre for Laboratory Medicine, Tampere University Hospital, Finland; 5) Department of Pathology, Helsinki University Hospital, Finland; 6) Department of Neurology, University of Oulu, and MHSO hospital, Finland; 7) Vaasa Central Hospital, Finland.

We have described the clinical and morphological phenotype of an autosomal dominant limb-girdle muscular dystrophy in a large Finnish family. Molecular genetic analyses confirmed linkage of the disease to a locus on chromosome 7q36 with a LOD score 3.76 for marker D7S1823 and a LOD score 3.07 for marker D7S2465. Patients had onset of muscle weakness in the pelvic girdle between the fourth and the sixth decade, and the first symptoms were difficulties in running and climbing stairs. Later on the upper limb-girdle muscles were involved. The progression of the disease was relatively mild and patients were still able to walk with marked difficulty in the eighth decade. Muscle biopsy showed myopathic and/or dystrophic features with rimmed vacuolation and some minor myofibrillar disintegration and protein aggregations. We were now able to include three additional Finnish LGMD1 families with similar clinical genetic and morphological features in the study. They show linkage to the same locus, as identified by identically segregating alleles on a common haplotype in the patients. Genotyping members of these new informative families with additional microsatellite and SNP markers for the linked region, the locus on chromosome 7q36 was considerably narrowed down from >6 Mb to < 3.3 Mb. The region is now located between the marker D7S3037 and the telomere on chromosome 7q. Finemapping is ongoing to further reduce the linked area. Twelve described genes, including some fairly good candidate genes for LGMD1, as well as 15-17 hypothetical genes/pseudogenes are reported in this refined linked area. The sequencing of the genes is ongoing aiming to identify the underlying genetic defect of 7q36 linked LGMD1. Two American families have previously been linked to an overlapping locus on 7q36. ENMC reports and later reviews defined the 7q36 locus as LGMD1E while the OMIM database defines this locus as LGMD1D.

## 387/W/Poster Board #45

**Homozygosity mapping in patients with malformations of cortical development.** *K. Bilguvar[1,2], K. Yasuno[1,2], Z. Arlier[1,2], B. Tuysuz[3], C. Yalcinkaya[4], B. Korkmaz[4], S. Saygi[5], B. Tatli[6], M. Caliskan[6], N. Aydinli[6], M. Ozmen[6], H. Per[7], O. Caglayan[8], S. Kumandas[7], D. Yalnizoglu[9], M. Ozguc[10], M. Topcu[9], R.P. Lifton[11,12,13], M.W. State[2,13,14], M. Gunel[1,2,13,15].* 1) Neurosurgery, Yale University School of Medicine, New Haven, CT; 2) Program on Neurogenetics, Yale University School of Medicine, New Haven, CT; 3) Pediatrics, Istanbul University Cerrahpasa Faculty of Medicine, Istanbul, Turkey; 4) Norology, Istanbul University Cerrahpasa Faculty of Medicine, Istanbul, Turkey; 5) Norology, Hacettepe University Faculty of Medicine, Ankara, Turkey; 6) Pediatrics, Istanbul University Istanbul Faculty of Medicine, Istanbul, Turkey; 7) Pediatrics, Erciyes University Faculty of Medicine, Kayseri, Turkey; 8) Medical Genetics, Erciyes University Faculty of Medicine, Kayseri, Turkey; 9) Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey; 10) Medical Biology, Hacettepe University Faculty of Medicine, Ankara, Turkey; 11) Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT; 12) Internal Medicine, Yale University School of Medicine, New Haven, CT; 13) Genetics, Yale University School of Medicine, New Haven, CT; 14) Child Study Center, Yale University School of Medicine, New Haven, CT; 15) Neurobiology, Yale University School of Medicine, New Haven, CT.

The molecular mechanisms of brain development and function are largely unknown. The normal structural organization and maturation of the brain are the result of a precisely orchestrated series of developmental processes. Despite its fundamental importance, a mechanistic understanding of brain development at the level of the gene is generally lacking and only recently has begun to be elaborated in a broad fashion. One approach to obtain such insight is to identify genes that play a key role in this process specifically and directly within the human brain through molecular genetic studies of human brain malformations. Structural brain abnormalities represent disruptions of normal cerebral or cerebellar cytoarchitecture and often lead to severe neurological disorders including epilepsy, mental retardation and global developmental delays, as well as cognitive deficits and other motor and sensory neurological deficits. We collected 250 patients from consanguineous families mainly from Eastern Turkey, where the consanguineous marriages are common. Those patients consisted of malformations of cortical development (MCD), sub-classified into three broad categories reflecting the developmental steps at which the cortical development was first disrupted: (i) neural cell proliferation or apoptosis (ii) neuronal migration and (iii) cortical organization. We performed whole-genome genotyping using Illumina Human CNV370 or Human610 beadchips and then conducted homozygosity mapping. Using Plink Software, we performed runs of homozygosity analysis and determined individual homozygous segments greater than 1.5 million base pairs. We then sought for the overlap among the identified segments with and without shared haplotypes to evaluate both identity by decent and identity by state. We developed an R script to visualize the overlapping segments and plot them across the genome to manually evaluate the segments. We also used HapMap Phase III CEU genotype information to determine common regions of homozygosity and exclude them from further analysis. The analysis revealed multiple loci of homozygosity shared by more than 10 patients. To screen for mutations, we selected the genes that are expressed in the brain, especially during brain embryogenesis. Mutational screening is currently underway.

## 388/W/Poster Board #46

**Novel FGD1 Mutation Underlying Aarskog-Scott Syndrome with Myopathy and Distal Arthropathy.** *S. Majid[1], A. Al-Semari[2], M. Muhaizea[2], M. Dabobo[3], R. Omar[1], F. Al-Kuraya[1], B. Meyer[1].* 1) Genetics,Functional Genomics, King Faisal Specialist Hosp, Riyadh, Saudi Arabia; 2) Department of Neurosciences, King Faisal Specialist Hospital & Research Centre; 3) Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital & Research Centre.

Aarskog Scott Syndrome (AAS) is described as an X-linked disorder which is characterized by ocular hypertelorism anteverted nostrils, broad upper lip, digital abnormalities and peculiar penoscrotal morphology. Mutations in FGD1 the only known cauasative gene are found in about one fifth of AAS cases. FGD1 is a guanine nucleotide exchange factor that specifically activates the RhoGTPase Cdc42 via its RhoGEF domain. The Cdc42 pathway is involved in skeletal formation and multiple aspects of neuronal development. We describe a kindred with five affected males manifesting many of the well recognized features of AAS. Linkage analysis localized the disease in this family to Xp11.3-q13.3 with FGD1 present in the critical region. The diagnosis was confirmed by the identification of novel nonsense mutation which resulted in substitution of tryptophan by a stop residue 447 (W447X). This mutation segregated within the family in a manner fully consistent with an X-linked pattern of inheritance. In addition to the other features of AAS seen in this family, this novel mutation may underlie the electromyographic evidence of myopathy we observed in the affected members which we suggest may be an under-recognised feature of the syndrome.

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## 389/W/Poster Board #47

**Mutation of *ACTA2* Gene as a Major Cause for Non-Syndromatic Thoracic Aortic Aneurysm and/or Dissection in both Familial and Sporadic Cases.** *H. Morisaki[1], K. Akutsu[2], H. Ogino[3], N. Kondo[4], I. Yamanaka[1], Y. Tsutsumi[2], T. Yoshimuta[2], T. Okajima[2], H. Matsuda[3], K. Minatoya[3], K. Sasaki[3], H. Tanaka[3], H. Ueda[5], T. Morisaki[1,6].* 1) Dept Bioscience, Natl Cardiovasc Ctr Res Inst, Suita, Osaka, Japan; 2) Dept Cardiovascular Medicine, Natl Cardiovasc Ctr, Suita, Osaka, Japan; 3) Dept Cardiovascular Surgery, Natl Cardiovasc Ctr, Suita, Osaka, Japan; 4) Dept Cardiovascular Surgery, Aomori Municipal Hosp, Aomori, Japan; 5) Dept Pathology, Natl Cardiovasc Ctr, Suita, Osaka, Japan; 6) Dept Molecular Pathophysiology, Osaka Univ Grad Sch Pharm Sci, Suita, Osaka, Japan.

Approximately 20% of thoracic aortic aneurysm and/or dissection (TAAD) cases result from inherited disorders including several syndromatic connective-tissue disorders such as Marfan syndrome, Ehlers-Danlos syndrome and Loeys-Dietz syndrome, which are caused by mutations in the *FBN1*, *COL3A1*, and *TGFBR1*&*TGFBR2* genes, respectively. Also up to 20% of non-syndromatic TAAD has a familial background. Recent study revealed that mutations of *TGFBR2*, *MYH11*, *SLC2A10*, and *ACTA2* genes cause familial non-syndromatic TAAD. In order to elucidate the contribution of these genes to TAAD in the absence of known genetic syndromes, we conducted a genetic study of *FBN1*, *TGFBR1*, *TGFBR2*, *ACTA2* and *SLC2A10* genes, in 44 unrelated Japanese patients with non-syndromatic TAAD, 17 familial and 27 sporadic and young-onset (≤50 y.o.). By directly sequencing all exonic regions of those genes, we identified 4 mutations in *ACTA2* and one in *TGFBR2* in 17 probands with familial TAAD, and two mutations in *ACTA2* in 27 sporadic TAAD. No mutations in *FBN1*, *TGFBR1* or *SLC2A10* were identified in this study. All six mutations in *ACTA2*, including 4 novel, were expected to disturb the highly conserved structure of actin molecule. Reported cardinal features, iris cysts and livedo reticularis, were identified only in one pedigree with reported mutation, R149C. We did not find any specific clinical feature suggesting premature onset of coronary artery disease or premature ischemic stroke in 14 mutation carriers, though two of the family members with R212Q mutation died suddenly with chest pain in their 40s. In our study, genetic penetrance seemed to be higher than that in the previous report, since we found aortic involvement in all mutation carriers but one. Histopathologically, aortic tissues from affected individuals showed aortic medial degeneration and focal SMC loss or disarray, but we did not observe any SMC hyperplasia or occlusive/stenotic change of vasa vasorum as reported in the previous report. In conclusion, we confirmed that *ACTA2* mutations are the major cause in both familial and sporadic TAAD. Since the first and only clinical symptom can be life-threatening aortic aneurysm or dissection, genetic analysis of family members of patients with *ACTA2* mutations would be beneficial for improved surveillance and awareness of treatment. We think that genetic analysis of *ACTA2* should be recommended for all patients with familial or young-onset TAAD.

## 390/W/Poster Board #48

**A novel SNP genotyping array analysis yields linkage for a rare medial arterial calcification disorder in five siblings.** *S.G. Ziegler[1], M.P. Siegenthaler[2], D. Adams[1], C.E. Wahl[3,4], C. Groden[3,4], M. Anahtar[1], T.C. Markello[3,4], W.A. Gahl[1,3,4].* 1) Human Biochemical Genetics Section, Medical Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Office of the Clinical Director, NHLBI, NIH, Bethesda, MD; 3) Office of the Clinical Director, NHGRI, NIH, Bethesda, MD; 4) Undiagnosed Diseases Program, NIH, Bethesda, MD.

Sharp (1954) described a syndrome (OMIM 211800) in a pair of siblings from a consanguineous marriage with an unusual type of calcification of joint structures and medial walls of medium sized arteries. Two previous cases had been independently reported. Here we report five affected siblings with the same clinical findings who were examined as part of the NIH Undiagnosed Diseases Program. Two sisters were initially referred for peripheral vascular disease secondary to presumed “tumoral calcinosis”. Bone surveys revealed marked calcification following the distributions of the iliac, femoral, popliteal, and proximal tibial arteries. Additionally, there was subtle juxtaarticular calcification present in the wrists and ankles, the interphalangeal and metacarpophalangeal joints of the hands and feet, and the tarso-metatarsal joints. There was narrowing of interphalangeal joint spaces, without gross osteophyte formation or erosions. The medical history confirmed similar symptoms in three other siblings, but not in the parents, who are third cousins. DNA from siblings and parents was collected and genotyped on a 1 million multiplex SNP array. B allele plots (to determine genotype frequency at individual SNPs) identified one 22MB region on chromosome 6 with consecutive uninterrupted homozygosity for 7977 SNPs; this defined a potential region of identity by descent. Further analysis, using the ENT program (a genotype phasing algorithm based on entropy minimization) to find paternal and maternal haplotypes, confirmed linkage in this region; identical maternal and paternal haplotypes extended proximally across the centromere for an additional 40.3MB, indicating a linkage region of identical heterozygosity or identity by state (log score = 3.0 across this entire 62.3MB region). Sequencing studies on selected candidate genes are being performed to identify a causal mutation in this linked region. The molecular cause of calcification in medium sized vessels will provide new insights into extramedullary calcification of specialized connective tissue and explain this unique connection between the arterial wall and joint capsule ligament matrix.

## 391/W/Poster Board #49

**Molecular genetic analysis in app. 300 patients with Congenital Hyperinsulinism.** *K. Brusgaard[1], H. Christesen[6], K. Hussain[2], A. Molven[4], A. Angham[5], M. Melikian[3].* 1) Clinical Genetics, Odense University Hospital, Odense, Fyn, Denmark; 2) HC Andersen Children’s hospital, Odense University Hospital, Denmark;; 3) London Center for Paediatric Endocrinology and Metabolism, Great Ormond Street Hospital, London, UK; 4) Section for Pathology, the Gade Institute, University of Bergen, Norway; 5) Department of Molecular Pathology and Laboratory Medicine, King Abdulaziz Medical City, Riyadh, Saudi Arabia; 6) Endocrinology research centre, Moscow, Russia.

Background Congenital hypoglycemic hyperinsulinemia (CHI) is a clinical and genetic heterogeneous entity. Clinical manifestations can vary from serious life threatening to milder difficultly identifiable cases. Children who don’t react adequate to medical treatment are subject to pancreatic recession. The molecular ethiology are from recessive mutations of the ABCC8 (SUR1) and KCNJ11 (Kir6.2) to dominant mutations of the GCK or GDH genes. Focal dysplasia characterised by loos of maternal Chromosome 11 and hereby ABCC8 and KCNJ11 is a common cause of CHI. In some studies mutations in the ABCC8 promotor have been shown to cause CHI. In approximately 50% of the incidences the disease is still genetically unexplained necessitating the search for other genetic factors. 5n recent studies mutations in other MODY genes have been shown to cause severe forms of transient CHI. The purpose of the present study was to identify the genetic cause of CHI in a large group of patients with CHI. Materials: More than 300 children were tested for mutations in the ABCC8, KCNJ11 and/or GCK, GDH, HAD and TCF1, by sequencing. Results: We found mutations in ABCC8, KCNJ11, GCK, GLUD1 and TCF1. A number of previously undescribed mutations were detected. Several mutations including variants of ABCC8, KCNJ11 and GCK were functionally described expressing the mutated allele in in vitro systems using patch clamping, FISH staining and Rb+ flux. Discussion: ABCC8 mutations constituted the large majority of CHI causing mutations.The majority of ABCC8 positive individuals only carried one paternal mutation. Tissue collected from pancreaectomised individuals pointed to loss of maternal 11p15 in combination with the paternal mutation was the cause of the CHI phenotype. Still app. 50% of CHI cases are not explained by mutations in the genes analysed.

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## 392/W/Poster Board #50

**Both recessive and dominant INS gene mutations are a common cause of neonatal diabetes.** *S. Ellard, E.L. Edghill, J. Locke, S.E. Flanagan, A.-M. Patch, L.W. Harries, O. Rubio-Cabezas, A.T. Hattersley.* Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter, United Kingdom.

In 2008 we reported that heterozygous missense mutations in the INS gene are the second most common cause of permanent diabetes diagnosed before 6 months of life (PNDM). Mouse models suggest that these mutations cause misfolding of the proinsulin molecule, evoking the endoplasmic reticulum stress response which leads to apoptosis of the pancreatic beta cells. We hypothesised that homozygous loss-of-function INS mutations might cause PNDM in consanguineous pedigrees. We investigated 42 patients of consanguineous descent for mutations in the four genes known to cause isolated PNDM (INS, GCK, KCNJ11 and ABCC8). Mutations were identified in 22/42 probands. These included 7 homozygous GCK mutations, 4 homozygous ABCC8 mutations, 2 heterozygous KCNJ11 mutations and one heterozygous INS mutation. Eight patients had novel homozygous INS mutations; c.-331C>G (n=3), c.331C>A, M1I (n=2), Q62X and a large deletion (p.M1_Q62del) that was confirmed by MLPA analysis of the parental DNA samples. Patients with homozygous INS mutations were diagnosed earlier than those with heterozygous mutations (median age at diagnosis 6 days vs 9 weeks; p=0.005) and had a lower centile birth weight (<1st vs <5th; p>0.0001). Both recessive loss-of-function and dominantly acting misfolding mutations are common causes of isolated permanent neonatal diabetes. Recessive mutations were associated with an earlier age at diagnosis and lower birth weight, suggesting a more severe insulin secretion defect.

## 393/W/Poster Board #51

**Neonatal Hyperpigmentation - Diagnosis of FGD with a novel mutation in *MC2R*.** *Y. Anikster[1,2], E. Jacoby[1], O. Pinhas-Hamiel[1,2], S. Padeh[1,2], J. Laufer[1,2].* 1) Metabolic Disease Unit, Safra Children's Hosp, Tel-Hashomer Ramat-Gan, Israel; 2) Sackler Faculty of Medicine, Tel-Aviv University. Tel-Aviv.

Background: Familial Glucocorticoid Deficiency (FGD) is a rare autosomal recessive disorder of insensitivity to ACTH, characterized by isolated glucocorticoid deficiency while mineralocorticoid production is preserved. Clinical features include hypoglycemia and its sequels, hyperpigmentation, failure to thrive and recurrent infections. Genetic mutations have been identified in 40-45% of patients.

We describe a 3-month-old infant with hypoglycemia, anemia and hyperpigmentation. The patient's parents are first-degree cousins, and two out of 3 siblings died in infancy. The patient and both of the deceased siblings were noticed to have dark skin and mucous membranes present already at birth, while both parents and sister have a much lighter skin complexity. Diagnosis of FGD was suggested based on the clinical findings. Elevation of morning ACTH levels and low cortisol levels supported the diagnosis.

Methods: Genomic DNA was extracted from the family member's peripheral blood. The ACTH receptor exons were sequenced directly.

Results: The patient was found to be homozygous for a novel mutation in *MC2R* (635insC, I153-167X). His mother and sister are heterozygote for the mutation.

Follow up and Conclusions: Four months after initiation of corticosteroids treatment hyperpigmentation faded, the infant thrives without any hypoglycemia. A positive family history of neonatal hyperpigmentation and unexplained infant deaths should suggest the diagnosis of FGD. Early recognition and treatment prevent mental disability and fatal outcome. The finding of the mutation in this family enables prenatal diagnosis.

## 394/W/Poster Board #52

**Olfactory defects, pubertal delay, and erratic estrus cycles in *Chd7* deficient mice indicate underlying mechanisms of Kallmann-like features in CHARGE syndrome.** *W. Layman[1], E. Hurd[2], D. Martin[1,2].* 1) Dept Human Genetics, Univ Michigan, Ann Arbor, MI; 2) Dept Pediatrics, Univ Michigan, Ann Arbor, MI.

Mutations in *CHD7*, a chromodomain protein, are present in 60-80% of individuals with CHARGE syndrome, a multiple anomaly disorder characterized by ocular Coloboma, Heart defects, choanal Atresia, Retardation of growth and development, Genital hypoplasia with hypogonadotropic hypogonadism and delayed puberty, and inner Ear defects including hearing and balance disorders. CHARGE individuals also have olfactory impairments and delayed puberty, with decreased circulating levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Mutations in *CHD7* are present in patients with idiopathic hypogonadotropic hypogonadism (IHH) and olfactory impairment (Kallmann Syndrome), which is thought to result from deficiency of hypothalamic gonadotropin releasing hormone (GnRH) secretion or function. GnRH producing neurons originate in the olfactory placode in humans, and in the vomeronasal organ in mice. *Chd7* is highly expressed in the embryonic and adult mouse vomeronasal organ, hypothalamus and pituitary, suggesting it may have critical roles in these tissues. We recently showed that CHD7 is a positive regulator of neural stem cell proliferation and olfactory sensory neuron formation in the mouse olfactory epithelium, indicating that the deleterious effects of CHD7 deficiency may be mediated in part by defects in stem cell division and/or maintenance. Based on these observations, we further hypothesized that loss of CHD7 disrupts GnRH neuronal development and signaling, either directly or indirectly via defects in neural stem cells. We found that female mice with heterozygous loss of *Chd7* ($Chd7^{Gt/+}$) have delayed vaginal opening and erratic estrus cycles. GnRH neurons are present in the olfactory bulb in $Chd7^{Gt/+}$ mice, suggesting that initial formation and migration are unchanged. However, circulating levels of LH and FSH in six week old $Chd7^{Gt/+}$ mice are variable, and correlate with altered levels of hormone expression in the pituitary as measured by qRT-PCR. Collectively, these studies demonstrate that defects in GnRH signaling to the pituitary may contribute to the reproductive and endocrine dysfunction seen in humans with *CHD7* mutations.

## 395/W/Poster Board #53

**Identification of a novel SRY-mutation causing 46,XY complete gonadal dysgenesis in two half-sisters by paternal gonadal mosaicism.** *P. Miny[1], I. Filges[1], N. Boesch[1], C. Kunz[2], S. Tschudin[3], G. Szinnai[4], F. Wenzel[1], U. Zumsteg[4], K. Heinimann[1].* 1) Division of Medical Genetics, University Children's Hospital and Department of Biomedicine, Basel, Switzerland; 2) Molecular Genetics, Department of Biomedicine, University Basel, Basel, Switzerland; 3) Department of Obstetrics and Gynecology, University Hospital, Basel, Switzerland; 4) Division of Pediatric Endocrinology, University Children's Hospital, Basel, Switzerland.

Mutations and deletions in the SRY-gene have been identified in about 15% of individuals with 46,XY complete gonadal dysgenesis (CGD). The SRY gene (Yp11.3) encodes the testis- determining factor (TDF), a transcription factor that is a member of the high mobility group (HMG)-box family of DNA-binding proteins. Mutation of SRY results in complete gonadal dysgenesis. Familial cases with confirmed paternal mosaicism have been rarely reported. Here we describe the clinical, endocrinological and molecular characteristics of two half-sisters, related by their common but deceased father, with complete gonadal dysgenesis. Genetic investigations were performed after one sister was diagnosed for primary amenorrhea and infantilism of secondary sexual development, hypergonadotropic hypogonadism, infantile uterus and hypoplastic bilateral gonads. One sister delivered a healthy girl after egg donation. The karyotype revealed 46,XY. SRY deletion was excluded by FISH analysis of the Yp11.3 region. Direct SRY gene sequencing was performed according to standard PCR and DNA-sequencing methods. Direct sequencing of the SRY coding region revealed a novel, hemizygous missense mutation c.347T>C resulting in an amino acid replacement of Leucin to Serin at position 116 (p.Leu116Ser). The mutation is located in the HMG-domain, an evolutionary conservated region responsible for DNA-binding, with in silico analysis (PolyPhen software) predicting a pathogenic impact. Therefore, p.Leu116Ser is likely to adversely affect SRY's DNA-binding capacity. The same mutation could be identified in the affected half-sister, but not in 100 fertile male control individuals. DNA from the deceased father was available after extraction from formalin-fixed tissue of a biopsy of inflammatory infiltration after hip surgery. Mutation analysis of this DNA revealed the familial mutation in a mosaic state. Functional analysis of the mutation is ongoing. We describe a novel missense mutation in the SRY gene, p.Leu116Ser causing Swyer syndrome phenotype in two half-sisters related by their common father. The rare finding of paternal mosaicism has been confirmed in the father, so that the recurrent phenotype in this family is most likely due to paternal gonadal mosaicism.

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## 396/W/Poster Board #54

**Molecular analysis of the CYP21 gene in patiens with 21-hydroxylase deficiency in western Turkey.** *F. Ozkinay[1,2], H. Onay[2], F. Hazan[2], S. Ozen[3], O. Cogulu[1,2], S. Darcan[3], D. Goksen[3], C. Ozkinay[2].* 1) Department of Pediatrics, Ege University, Izmir, Turkey; 2) Department of Medical Genetics, Ege University Medical Faculty, Izmir, Turkey; 3) Department of Pediatric Endocrinology, Ege University Medical Faculty, Izmir, Turkey.

Congenital adrenal hyperplasia (CAH) caused by 21-hydroxylase deficiency is one of the most frequent autosomal recessive disorders. The aim of the study was to detect the frequencies of CYP21 gene mutations and to study the genotype-phenotype correlation in a group of 21-hydroxylase deficienct patients from western Turkey. Molecular analysis of CYP21 gene for the detection of 11 most common mutations (large deletion, P30L, I2G, del-8bp, I172N, E6 cluster, V281L, F30+T, Q318X, P453S and R356W) was performed in 20 clinically diagnosed CAH patients using real time PCR method. In 25 (25%) of the patients none of the 11 mutations analysed was detected. Six of the patients (30%) were homozygous, 1 patient (5%) compound heterozygus, 4 patients (20%) heterozygous for one of the mutations covered by the test. Four patients carried complex alleles. The most common mutation in the CYP21 gene of the study group was the I2G (in 35% of the patients). Genotype-phenotype correlations of the patients were similar to the results of previous studies from different populations except one case with homozygous I2G mutation which showed simple virilizing phenotype. To our knowledge, this is the first study showing the mutation spectrum of CYP21 gene in patients from western Turkey.

## 397/W/Poster Board #55

**Insights into the molecular mechanism of hypopituitarism caused by *Otx2* and *Prop1* mutations.** *A.H. Mortensen, S.A. Camper.* Human Genetics, University of Michigan Medical School, Ann Arbor, MI.

Mutations in a variety of transcription factors cause pituitary hormone deficiency. *HESX1* and *OTX2* mutations cause variable, syndromic effects, including brain and ocular malformations and pituitary hormone deficiencies, while *PROP1* and *POU1F1* mutations affect pituitary function specifically. The mechanism whereby *OTX2* mutations cause hypopituitarism is not clear, but regulation of *HESX1* and *POU1F1* transcription have been suggested. Mice with mutations in these genes reveal genetic background influences and generally recapitulate the human phenotypes. Both *Otx2* and *Prop1* mutant pituitaries are dysmorphic, but *Pou1f1* pituitaries have normal shape and size at birth. The pituitary hypoplasia characteristic of both adult *Prop1* and *Pou1f1* mutants arises from reduced cell proliferation in the anterior lobe after birth. We hypothesize that *Prop1* controls the expression of genes that regulate progenitor production, cell migration, survival, and differentiation, and vascularization. To identify genes involved in these processes we used microarray analysis of gene expression to compare pituitary RNA from newborn *Prop1* and *Pou1f1* mutants. Significant differences in gene expression were noted between the mutants. *Otx2* expression was elevated specifically in *Prop1* mutant pituitaries, suggesting that *Prop1* normally suppresses *Otx2* transcription. We analyzed the spatial and temporal expression of *Otx2*. It is normally expressed in the neural ectoderm and the prospective posterior pituitary when BMP, WNT, and FGF signaling are inducing anterior pituitary gland growth. *Otx2* is also transiently expressed with *Hesx1* in the anterior pituitary primordium, but it is extinguished long before the initiation of *Pou1f1* expression. This suggests that the ectopic posterior lobe and small anterior lobe in *Otx2* mutants result from early effects on pituitary gland induction and growth, with some potential downstream targets in the neural ectoderm. There is a temporal delay between normal *Prop1* transcriptional activation and the elevated, ectopic expression of *Otx2* in the anterior lobes of *Prop1* mutants, which suggests that *Prop1*-mediated suppression is indirect. This discovery provides support for our hypothesis that identifying molecular differences in mouse mutants will contribute to understanding the molecular mechanisms that control pituitary organogenesis and uncover candidate genes for human pituitary disease. NIH R01HD34283, R37HD30428.

## 398/W/Poster Board #56

**Pathways involved in Cayman Ataxia.** *K.M. Ito[1], V. Strumba[2], M. Hortsch[3], M. Burmeister[4].* 1) Program in Cellular and Molecular Biology, University of Michigan, Ann Arbor, MI; 2) Bioinformatics, University of Michigan, Ann Arbor, MI; 3) Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI; 4) Molecular & Behavioral Neuroscience Institute, Psychiatry, University of Michigan, Ann Arbor, MI.

Cayman Ataxia is a rare, non-progressive, congenital, recessive cerebellar ataxia that has no known treatment. Our group has found two mutations in the Cayman Ataxia gene, ATCAY/Atcay, in all individuals with Cayman Ataxia. This gene encodes the protein, Caytaxin, which we've shown to be absent in naturally-occurring ataxic mouse mutants that display a strong phenotype (sidewinder and jittery) and markedly decreased in mutants that display a mild phenotype (hesitant). In addition, we have also generated transgenic mice expressing a BAC that encodes human Caytaxin and rescues the mutant phenotype. Despite the 30 fold over-expression of the protein, these mice are healthy and have no indications of neurological dysfunction by 1.5 years. Mice that fail to express Caytaxin have normal brain weight, external brain morphology, regional and cellular architecture suggesting that this protein is not involved in brain development. We have generated a set of monoclonal antibodies, which detect Caytaxin as three different major proteins on a western blot, irrespective of developmental stage or brain region. Affinity purification of Caytaxin using one of these antibodies detected a direct association between Caytaxin and kidney-type glutaminase (KGA), an enzyme abundant in the brain that acts to hydrolyze glutamine to glutamate. Glutamate is the most abundant excitatory neurotransmitter in the brain and is required for synaptic transmission. These results confirm reports from Buschdorf et al (2006) who show this association through co-immunopreciptation. Immunocytochemistry in differentiated SH-SY5Y cells detects strong staining near the growth cones preceding synapse formation. We also used microarray analysis to identify genes whose expression is altered in response to a lack of Caytaxin. Interestingly, only very few genes were identified. The most significantly down-regulated gene is Carbonic Anhydrase Related protein 8 (Car8), which is mutated in the naturally occurring ataxic mouse mutant waddles. Recently, Robinson et al (2009) have described mutations in the Car8 gene in a consanguineous family in Iraq. All affected individuals display a form of congenital ataxia characterized by mild mental retardation and a quadrupedal gait. In addition to Car8, microarray analysis also identified several proteins in glutamate signaling pathways. Together, these results suggest that Caytaxin plays a role in glutaminase transport to the synapse or glutamate signaling.

## 399/W/Poster Board #57

***DFNA5*, a hearing loss gene, encodes an apoptosis-inducing protein.** *L. Van Laer[1,2], K. Op de Beeck[1], S. Thys[3], N. Cools[4], I. Callebaut[5], K. Vrijens[1], L. Van Nassauw[3,6], V.F.I. Van Tendeloo[4], J.P. Timmermans[3], G. Van Camp[1].* 1) Center of Medical Genetics, University of Antwerp, Antwerp, Belgium; 2) Present address: Center of Medical Genetics Ghent, University of Ghent, Ghent, Belgium; 3) Laboratory of Cell Biology & Histology, University of Antwerp, Antwerp, Belgium; 4) Vaccine & Infectious Disease Institute (VIDI), Laboratory of Experimental Hematology, University of Antwerp, Antwerp, Belgium; 5) Department of Structural Biology, Institute of Mineralogy and Physics of Condensed Media, Université Pierre et Marie Curie-Paris, Université Paris Diderot-Paris, Paris, France; 6) Laboratory of Human Anatomy & Embryology, University of Antwerp, Antwerp, Belgium.

Mutations in *DFNA5* are responsible for an autosomal dominant, nonsyndromic, sensorineural type of hearing loss with an onset age of 5-15 years. Hitherto, four *DFNA5* mutations have been described. Despite being different at the genomic DNA level, all mutations result in exon 8 skipping at the cDNA level. These findings have led to the hypothesis that DFNA5 associated hearing loss is attributable to a highly specific gain-of-function mutation. We previously provided the first experimental evidence for this hypothesis; transfection of mutant *DFNA5* caused cell death in both yeast and mammalian cells. Little is known about the molecular function of DFNA5, but a clear link with cancer exists. As such, DFNA5 was implicated in the p53-mediated cellular response to DNA damage and was epigenetically silenced in primary gastric tumors and colorectal carcinomas. In the current study, EGFP constructs containing different DFNA5 parts were transfected in HEK293T cells. Cell viability was assessed using flowcytometry and cytochemistry. The type of cell death was evaluated by annexin V and TUNEL assays. Interestingly, our experiments prove that the domain of DFNA5 responsible for cell death is located in a region that is shared by wild type and mutant protein, indicating that the observed toxicity is an intrinsic part of the physiological function of DFNA5. In addition, we show that sequences in exon 2 and exon 6 are essential for the toxic activity. Finally, we demonstrate that cells transfected with mutant DFNA5 are committed to an apoptotic cell fate. We conclude that DFNA5 contains an apoptosis inducing domain, suggesting a role in cell survival pathways. We hypothesize that this apoptosis inducing domain is activated by the hearing loss mutations, leading to accidental hair cell death.

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## 400/W/Poster Board #58

**Expression of CACNA2D3 in Gingival Fibroblasts.** *T.C. Hart[1], T. Han[1], X. Liu[2], I. Ambudkar[2], P.S. Hart[3].* 1) Human Craniofacial Genetics Section, NIDCR, NIH, DHHS, Bethesda, MD; 2) Molecular Physiology and Therapeutics Branch, NIDCR, NIH, DHHS, Bethesda, MD; 3) Office of the Clinical Director, NHGRI, NIH, DHHS, Bethesda, MD.

CACNA2D3 on chromosome 3p21.1-p14.3 encodes an alpha-2/delta subunit of a voltage-dependent calcium channel. Calcium channels control the influx of calcium ions into the cell upon membrane polarization and consist of a complex of alpha-1, alpha-2/delta, beta, and gamma subunits in a 1:1:1:1 ratio. Recently disruptions in CACNA2D3 have been reported in two patients with Zimmerman-Laband syndrome, a syndromic condition which includes gingival overgrowth. Because of this, we characterized expression of CACNA2D3 in normal gingival fibroblasts. Gingival fibroblasts were obtained from 3 control individuals undergoing gingivectomy. Three CACNA2D3 isoforms (A-C) have been reported in the literature. Isoform A is the refseq isoform and is encoded by the full length transcript. Isoform B has skipping of exon 20 with the introduction of an immediate stop codon. Isoform C has a novel first exon. These isoforms, including a novel isoform D, were evaluated in the control gingival tissues by quantitative PCR. The predominant isoform in all gingival samples was isoform B (~70%), followed by isoform D (~20%) and isoform A (~10%). The novel isoform D includes a portion of intron 20 that introduces an immediate stop codon. This isoform was found in several other tissues, including skin, kidney and brain. Isoform C was not present in gingival tissue. Next calcium signaling was investigated in the cells. $2.5 \times 10^5$ cells were plated in 35mm optical dishes in 2ml of media and incubated overnight. 1.5ml media was removed the next morning and 5μl Fura-2 AM dye was added. Plates were incubated for 1 hour at 37C and 5%CO2. Media was removed and cells were washed with PBS to remove excess Fura-2. Bayk-8644, an agonist for L-type calcium channels, was added to stimulate $Ca^{2+}$ entry into the cell. Fluorescence was measured. Two distinct populations of cells were noted in all 3 control lines. Both populations showed a rapid influx of calcium. The fist population had initial total fluorescence about half that of the other population followed by a rapid decrease in fluorescence back to baseline levels. The second population had a sustained response, with fluorescence levels slowly returning to baseline. Results indicate tissue specific isoforms and altered functioning of CACNA2D3 may contribute to gingival overgrowth.

## 401/W/Poster Board #59

**High-resolution breakpoint mapping of novel rearrangements involved in alpha- and beta-thalassemia using array-Comparative Genomic Hybridization (aCGH).** *M. Phylipsen[1], I.P. Vogelaar[1], Y. Ariyurek[2], J.T. den Dunnen[2], P.C. Giordano[1], C.L. Harteveld[1].* 1) Hemoglobinopathies Laboratory, Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands; 2) Leiden Genome Technology Center, Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands.

Thalassemias are hereditary microcytic hypochromic anemias characterized by abnormalities in hemoglobin production due to reduced expression of either the beta-globin gene, leading to beta-thalassemia, or the alpha-globin genes, giving rise to alpha-thalassemia. About 10% of the beta-thalassemias and 90% of the alpha-thalassemias are caused by deletions in either globin gene cluster. In a previous study, we applied Multiplex Ligation-dependent Probe Amplification (MLPA) to characterize large rearrangements in the alpha- and beta-globin gene cluster. Several new deletions and duplications were found, however, the exact breakpoint sequences are still unknown. To facilitate confirmation by breakpoint PCR and to gain more insight in the mechanisms causing these rearrangements we decided to determine the precise location of breakpoints. Array Comparative Genomic Hybridization (aCGH) measures DNA copy number differences between a reference and a patient's genome sample thereby detecting and mapping deletions and duplications. We used high resolution tiling arrays with 135,000 probes spaced at a density of ~15 bp to map the breakpoints to an interval that can be validated by PCR and sequencing. The array was hybridized to a set of 55 thalassemia patients who were found to carry a deletion in the alpha- or beta-globin gene cluster. The fine mapping results were used to design breakpoint PCRs and resulting fragments were sequenced to determine the precise breakpoint.

## 402/W/Poster Board #60

**Examination of PMP22 involvement in deafness by comparative analysis of protein localization and gene expression patterns throughout murine development.** *T.A. Carver, M.J. Kovach.* Biol & Enviro Sci/ 2653, Univ TN at Chattanooga, Chattanooga, TN.

A genetic variant of the Charcot-Marie-Tooth (CMT Type 1E) disease, which causes profound deafness in addition to progressive peripheral neuropathy, has been linked to a unique point mutation in the PMP22 gene rather than gene duplication. PMP22, also known as peripheral myelin protein-22, is a 22 kD glycoprotein classified as a Growth Arrest specific (Gas) gene. Members of the Gas gene family have been shown to regulate gene expression, cell division and cell death. Although PMP22 is largely expressed in Schwann cells, it also has been found in other tissues such as cochlea, lungs and intestines at critical times during development. Thus, PMP22 appears to play a role in myelination of neural tissues, as well as in cell growth regulation of non-neural tissues. This dual expression is consistent with the description of both neural and cochlear components to hearing loss in CMT1E patients. This study was designed in order to gain a better understanding of the cochlear involvement of PMP22 in the normal function and development of the inner ear. We hypothesize that in the cochlea, PMP22 is acting as a transcriptional regulator, and mutations in PMP22 lead to abnormal gene expression patterns that characterize the deafness phenotype. The Trembler-J mouse was chosen as a model for PMP22-associated auditory dysfunction. Differences in protein localization and gene expression profiles were characterized in control and experimental groups by immunohistochemistry (IHC) and differential display (DD), respectively. IHC of the cochlea confirms expression of PMP22 protein in non-neural regions. In general, staining of the PMP22 protein in the Tr-J mouse is reduced in the cochlear nerve but more pronounced in the sensory and non-neural tissues of the inner hair, hensen and spiral prominence cells in a developmental-specific manner. These cells have been proposed to be involved with the $K^+$ recycling pathway. Differences were also noted for Type IV spiral ligament and marginal cells of the stria vascularis, which are also involved with $K^+$ recycling and cochlear homeostasis. DD analysis of lung tissue collected at different time points during inner ear development has identified a number of differentially expressed genes in the Tr-J, with slightly more than 50% being upregulated. Sequence analysis of some of these transcripts has determined genes with functions important in neurogenesis and cell development such as apoptosis, signal transduction and $K^+$ transport.

## 403/W/Poster Board #61

**Decreased Expression of Heat Shock Protein 27 and phosphorylated Hsp27 in Cellular Models of MERRF (A8344G) disease.** *M. Hsieh[1], C.Y. Chen[1], S.J. Gi[1], Y.H. Wei[2].* 1) Department of Life Science, TungHai University, Taichung, Taiwan; 2) Department of Biochemistry and Center for Cellular and Molecular Biology, National Yang-Ming University, Taipei, Taiwan.

Mitochondrial DNA (mtDNA) mutations are responsible for human neuromuscular diseases caused by mitochondrial dysfunction. Myoclonus Epilepsy associated with Ragged-Red Fibers (MERRF) is a maternally inherited mitochondrial encephalomyopathy with various syndromes involving both muscular and nervous systems. The most common mutation in MERRF disease, A8344G, has been associated with severe defects in protein synthesis, which impaired assembly of complexes in Electron Transport Chain and resulted in decreased respiratory chain activity. In this study, we showed a dramatic decrease of Heat shock protein 27 (Hsp27) in lymphoblastoid cells derived from a MERRF patient and in cybrid cells with MERRF A8344G mutation. However, reverse transcriptase PCR showed no difference in the mRNA level between normal and mutant cell lines, indicating that alterations may occur in the protein level. In addition, normal cytoplasmic distributions of Hsp27 and normal heat shock responses were observed in both wild type and mutant cybrid cells. Overexpression of Hsp27 in mutant MERRF cells significantly decreased cell death under staurosporine treatment, suggesting a protective function of Hsp27 in cells harboring MERRF mutation. Meanwhile, it was noted that the phosphorylated Hsp27 was also markedly decreased in cells harboring MERRF mutation. Given that the phospho-Hsp27 in mutant cells has no response to exogenous ATP addition and the similar levels of phospho-ERK,-JNK and -p38 between MERRF and wild type cells, the possibility of low ATP content leads to decreased phospho-Hsp27 may be excluded. Furthermore, evidenced by the decreased levels of Hsp27 upon starvation, proteasome inhibitor and rapamycin treatments, Hsp27 may be degraded by the autophagic pathway. However, phospho-Hsp27 was accumulated under proteasome inhibition, and shown no alternation during starvation or rapamycin treatment. Thus, the degradation of Hsp27 and phospho-Hsp27 may go through different pathways. To further understand the relationship between phospho-Hsp27 and the cell viability, we are establishing stable cells expressing different forms of Hsp27 with mutations on the phosphorylated sites. Stable clones expressing mutant forms of Hsp27 will serve to better understand the roles of Hsp27 in MERRF disease.

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## 404/W/Poster Board #62

**Functional analysis of *UBE2A* c.382C-T mutation: evidence for functional specificity between the two human *RAD6* homologs.** *R.M.P. Nascimento, G. Monteiro, N.M. Vieira, L.E.S Netto, A.M. Vianna-Morgante.* Genetics and Evolutionary Biology, Institute of Bioscience, University of São Paulo, São Paulo, Brazil.

We have previously described a nonsense mutation (c.382C>T) in the *UBE2A/ HHR6A* gene (ubiquitin-conjugating enzyme 2A) as the cause of a novel X-linked mental retardation syndrome. It was the first description of a ubiquitin-conjugating enzyme mutation as the cause of a human disease. *UBE2A* is one of the two human homologs of the Saccharomyces cerevisiae RAD6 gene. In humans and other mammals, the RAD6 ortholog is duplicated, with one X-linked (*UBE2A*) and one autosomal (*UBE2B*) copy, both able to complement the rad6-null yeast strain phenotypes. The mutation found in our patients introduces a premature stop codon (Q128X) and abolishes the 25 C-terminal amino acids of the protein. Here we report on the effect of this mutation on UBE2A function. Functional complementation in a rad6-null yeast strain and *in vitro* ubiquitin-conjugating activity assays towards histone H2A revealed that the defective protein is toxic and, although capable of interacting with the ubiquitin molecule, it is unable to transfer ubiquitin to histones. Our data also indicate that the mutated protein undergoes poli-ubiquitination and proteasomal degradation *in vivo*. The fact that the loss of function of UBE2A causes a neurodevelopmental syndrome points to function specificity between the human paralogs, both expressed in brain. This conclusion is further supported by the finding of completely skewed X-chromosome inactivation, with the normal *UBE2A* allele being the active one in the heterozygous mothers, likely the result of selection against cells harboring the mutated allele on the active X, although having normal *UBE2B* expression.

## 405/W/Poster Board #63

**KIRREL3, a putative synaptic molecule, with a potential role in intellectual disability.** *A. Srivastava[1,2], Y. Luo[1].* 1) J.C. Self Research Institute of Human Genetics, Greenwood Genetic Center, Greenwood, South Carolina; 2) Department of Genetics and Biochemistry, Clemson University, Clemson, South Carolina.

Intellectual disability (ID), formerly known as mental retardation, is the most frequently reported developmental disability, affecting cognitive function in about 2-3% of the population. Growing evidence indicates that defects in synapse formation and plasticity are major causes of ID. We have recently identified a gene, *KIRREL3* at 11q24, encoding a putative synaptic molecule of the immunoglobulin (Ig) superfamily, that was disrupted by a balanced t(11;16) translocation in a patient with ID. We also identified three nonsynonymous *KIRREL3* variants that were apparently rare and unique to ID (Bhalla et al. Am J Hum Genet 83: 703-713, 2008). The *KIRREL3* gene is predicted to encode a type 1a membrane protein of 778 amino acids containing five Ig-like domains in its extracellular portion and a PDZ domain-binding motif in its cytoplasmic portion. We characterized the human KIRREL3 and determined the protein is primarily located in the neuronal cell membrane. *KIRREL3* mutations identified in patients with ID appear to have no effect on its cellular localization. Based on a previous report (Gerke et al. J Comp Neurol 498: 465-475, 2007), we showed that human KIRREL3 colocalizes and interacts with the synaptic scaffolding protein, CASK, recently implicated in X-linked brain malformation and ID conditions. In HT22 and PC12 neuronal cells, KIRREL3 and CASK both showed overlapping signals. We confirmed the presence of CASK in immunoprecipitates of KIRREL3. Furthermore, we determined that the extracellular domain of KIRREL3 is post-translationally modified by N-glycosylation and is cleaved after expression in neuronal cells. Thus, identification of the extracellular binding partner(s) of KIRREL3 in neuronal cells will help to further define the mechanism underlying its physiological function and the potential role of KIRREL3 in neuronal function.

## 406/W/Poster Board #64

**Functional characterization of mutations in the ATP-binding loop of ATP7B, the Wilson disease gene.** *L. Luoma, T.M.M Deeb, G. Macintyre, D.W. Cox.* Dept Med Gen, Univ Alberta, Edmonton, AB, Canada.

Wilson disease (WND) is an autosomal recessive disorder of copper homeostasis characterized by accumulation of excess copper in the liver, brain and kidneys, leading to tissue damage. Onset is highly variable and can be neurological, hepatic, renal or a combination of all. The primary means for maintaining copper homeostasis in mammals is through export of copper from the liver into the bile for excretion. The defective gene in WND is *ATP7B*. Previously WND diagnosis has been complicated by extensive clinical and biochemical heterogeneity of the disease. Complete sequencing of *ATP7B* has become a major means of WND diagnosis. Sequencing has resulted in the identification of over 580 *ATP7B* variants. As such novel missense variants can be diagnostically ambiguous. When functional data for missense variants is unavailable, predictive algorithms such as SIFT, PolyPhen and Align-GVGD, may be used to classify new ATP7B variants, although their accuracy is unknown. *ATP7B* encodes a P-type copper transporting ATPase, ATP7B. The yeast orthologue of ATP7B is Ccc2. If *Ccc2* is deleted, yeast cannot grow under iron limiting conditions. Their high affinity iron uptake complex is unable to assemble and function, as copper is an essential cofactor for iron oxidization and uptake. This growth deficit can be complemented by transformation of Δ*ccc2* yeast with *ATP7B*. Relative complementation of *ATP7B* variants identified in patients is a good indicator of the consequence of these variants on copper transport function. Twelve patient variants localized to the ATP-binding loop of ATP7B were tested in our yeast model system for their effect on copper transport. L1043P, G1000R, G1101R, I1102T, V1239G, D1267V were found to be deleterious to function, E1173G is temperature sensitive, G1176E and G1287S are intermediate. T991M and I1148T are mildly defective while R1228T is neutral. Protein modelling suggests possible consequences of specific amino acid changes. Comparison of functional results with predictive algorithms showed that PolyPhen was the most accurate, with 74% accuracy. SIFT and Align-GVGD were accurate 64% of the time. Through our functional studies we can determine whether novel patient variants are disease-causing or rare normal, assisting in diagnosis of WND. Furthermore, we find that computational predictive algorithms, while a useful adjunct, cannot replace functional data for designating the status of novel variants.

## 407/W/Poster Board #65

**Functional assessment of N-terminal *ATP7B* variants identified in patients with Wilson disease.** *G. Macintyre, J.J. Goodall, A.N. Szava-Kovats, M.A. Benusic, D.W. Cox.* Department of Medical Genetics, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Wilson disease (WND), an autosomal recessive disorder of copper transport, results in liver and/or neurological disease and can be fatal if left untreated. ATP7B, defective in Wilson disease, is the main copper transporter that eliminates copper via the liver and facilitates copper incorporation into copper-requiring proteins (eg. ceruloplasmin). Mutation studies have identified 642 *ATP7B* variants, listed in our WND database, http://www.wilsondisease.med.ualberta.ca. Functional testing is necessary to ensure identification of WND-causing variants. The ATP7B N-terminus (ATP7B-N) consists of 653 amino acids and contains six copper binding domains, each ~70 residues in length with an MXCXXC motif that binds copper. Additional motifs and domains, important for protein trafficking and copper transport, are located within ATP7B-N. Chinese Hamster Ovary (CHO) cells do not express endogenous ATP7B and are sensitive to copper. Using a tetracycline-inducible Flp-In FRT system (Invitrogen), we generated stable transfectants of *ATP7B* and *ATP7B-N* variants to measure CHO cell viability (CHO-CV) over a range of copper concentrations (0-500 micromolar), using Alamar Blue. Wild-type ATP7B protects CHO cells from copper. We tested 14 missense ATP7B-N variants, identified either by our group or others, for their ability to confer protection from high concentrations of copper. Nine exhibited normal function (N41S, G85V, D196E, I381S, A486S, E541K, R616W, L641S, M645R), four partial function (G96D, L492S, A604P, H639Y) and one, R616Q, a variable phenotype. The five defective ATP7B variants showed partial mislocalization by immunofluoresence microscopy (IFM), supporting their designation as WND-causing variants. Our findings agree with functional scores predicted by the Sorting Tolerant from Intolerant (SIFT) algorithm for 9 of 14 variants. However, three ATP7B variants, G85V, I381S and R616W, predicted to be defective, exhibited normal CHO-CV in the presence of copper. G96D and A604P, abnormal in the CHO-CV assay, were predicted to have normal ATP7B function. SIFT shows a poor success rate (65%) for the prediction of deficits in ATP7B-N. Four ATP nonsense variants, Q457X, C490X, L523X, Q544X, did not confer protection from high copper concentrations. Q457X and C490X produced truncated ATP7B proteins detectable by Western blot analysis, and were mislocalised by IFM. The contribution of stable truncated ATP7B proteins to WND severity requires further investigation.

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## 408/W/Poster Board #66

**Functional consequences of a germline mutation located in the leucin rich repeat domain of NLRP3 identified in an atypical form of autoinflammatory disorder.** *E. Cochet[1], I. Jéru[1,2], S. Marlin[1], G. Le Borgne[1,2], P. Duquesnoy[1], F. Dastot-Le Moal[1], L. Cuisset[3], V. Hentgen[4], R. Dhote[5, 6], G. Grateau[7,2], S. Amselem[1,2].* 1) Institut National de la Santé et de la Recherche Médicale (INSERM) U.933, Paris, France; 2) Université Paris 6 Pierre et Marie Curie, Paris, France; 3) Service de Biochimie et Génétique moléculaire, Hôpital Cochin, Assistance publique-Hôpitaux de Paris, Paris, France; 4) Service de Pédiatrie, Centre Hospitalier de Versailles, Le Chesnay, France; 5) Service de Médecine Interne, Hôpital Avicenne, Assistance publique-Hôpitaux de Paris, Bobigny, France; 6) Université Paris 13, Bobigny, France; 7) Service de Médecine Interne, Tenon, Assistance publique-Hôpitaux de Paris, Paris, France.

We investigated the pathophysiology of an atypical familial form of periodic fever syndrome (PFS) characterized by autosomal dominant sensorineural hearing loss, systemic inflammation but without any cutaneous manifestation. Microsatellites were used to test the segregation of the NLRP3 locus with the disease phenotype. All NLRP3 exons were screened for mutations by direct sequencing. A heterozygous missense mutation (p.Tyr859Cys) was identified. This mutation, which segregated with the disease phenotype within the family, is located in the leucin rich repeat (LRR) domain of the protein. Functional assays were performed in HEK293T cells to determine the effects of normal and mutated NLRP3 proteins on NF-kB activation and caspase 1 signaling. The mutation activates speck formation and procaspase 1 processing, but does not alter the inhibitory properties of NLRP3 on NF-kB signaling. This study identifies the first familial form of cryopyrinopathy associated with a mutation located outside exon 3 of NLRP3, thereby underlining the importance to screen other exons of the NLRP3 gene in patients presenting atypical manifestations. Our data, which assess the relevance and limitations of in vitro functional assays, also provide the first evaluation of the functional consequences of a missense mutation involving the LRR domain of NLRP3. This mutation readily explains, through a gain of function,the pathophysiology of this autoinflammatory syndrome.

## 409/W/Poster Board #67

**GLUT10 Located on the Mitochondria Facilitates Dehydroascorbate Transport and Protects Cells from Oxidative Stress: A New Insight into Arterial Tortuosity Syndrome.** *Y. Lee[1], H. Huang[1], C. Chang[1], C. Cheng[1], J. Wu[1], Y. Chen[1,2].* 1) Institute of Biomedical Sciences, Taipei, Taiwan; 2) Department of Pediatrics, Duke University Medical Center, Durham, NC, 27710, USA.

Glucose transporter 10 (GLUT10) is a member of the class III facilitative glucose transporter family. Mutations of the GLUT10 cause arterial tortuosity syndrome (ATS), an autosomal recessive disorder with tortuosity and elongation in the major arteries due to disorganization of elastic fibers in the arterial wall and laxity of skin and joints. However, the physiological function of GLUT10 and the molecular mechanisms of ATS caused by GLUT10 deficiency remain unclear. Here, we showed that GLUT10 mRNA was highly expressed in white adipose tissue and aortic smooth muscle cells. In addition, we found that GLUT10 was located on mitochondria of the aortic smooth muscle cells, and its subcellular location in adipocytes was on Golgi but translocated to mitochondria upon insulin stimulation. We identified the pheylalanin/tyrosine (YXXΦ)- based motif on C-terminus of GLUT10 is critical for mitochondria targeting. We further found that GLUT10 mediated transport of L-dehydroascorbic acid (DHA), an oxidized form of ascorbic acid (AA), but not glucose into mitochondria, and resulted in significant reduction of the reactive oxygen species (ROS) in cells under oxidative stress. We proposed that loss-of-function mutations of GLUT10 impair DHA transport into mitochondria, which affects the redox homeostasis and vitamin C recycling. These in turn lead to the disturbance of elastin and collagen synthesis and result in arterial tortuosity and laxity of skin and joint in human ATS.

## 410/W/Poster Board #68

**DUX4 Promotes FSHD-Associated Pathology *In Vivo.*** *L. Wallace[1,3], S. Garwick[3], W. Mei[4], A. Belayew[5], J. Yang[4], S.Q. Harper[1,2,3].* 1) MCDB, The Ohio State University, Columbus, OH; 2) Department of Pediatrics, The Ohio State University, Columbus, OH; 3) Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, OH; 4) Center for Cell and Developmental Biology, The Research Institute at Nationwide Children's Hospital, Columbus, OH; 5) Laboratoire de Biologie Moléculaire, Université de Mons-Hainaut, Académie Universitaire Wallonie-Bruxelles, Mons, Belgique.

Facioscapulohumeral muscular dystrophy (FSHD) is a dominant genetic disorder caused by D4Z4 repeat contraction on chromosome 4q35. Current evidence supports that FSHD is an epigenetic disorder, in which FSHD-associated deletions produce chromatin changes that aberrantly upregulate myotoxic genes. Although the primary mutation was identified nearly two decades ago, the pathogenic mechanisms underlying FSHD are unclear. We hypothesize that the DUX4 gene is involved in FSHD pathogenesis because of its location in the D4Z4 repeat, elevated expression in FSHD myoblast cultures, and pro-apoptotic function *in vitro*. However, the in vivo effects of DUX4 over-expression in muscle have not been reported. Here, we describe the first *in vivo* evidence that DUX4 over-expression causes histological and functional deficits consistent with muscular dystrophy in zebrafish and mice. In fish, muscle-specific DUX4 expression produced body malformations, impaired mobility, somite defects, and myofiber degeneration. These results are consistent with abnormalities reported in other zebrafish models of muscular dystrophy and indicate that DUX4 is toxic to developing eukaryotic muscle. To confirm DUX4 toxicity in a mammalian model more closely related to humans, we delivered adeno-associated viral vectors (AAV6) carrying DUX4 or control GFP vectors to neonate or adult mouse muscle. In neonatal mice, DUX4 caused significant muscle atrophy, increased central nucleation, myofiber size variability, fibrosis, fat replacement of muscle, and inflammatory cell infiltration, while control GFP or untransduced muscles were unaffected. These results confirm our findings in zebrafish, and indicate that DUX4 toxicity to developing muscle is conserved between eukaryotic species. In adult muscle, AAV6.DUX4 caused massive, dose-dependent myofiber degeneration, apoptosis, histological evidence of muscle turnover, mononuclear cell infiltration, and gross muscle weakness. Untransduced or GFP transduced myofibers were normal. Importantly, expression of structurally intact but functionally inactive DUX4 mutants eliminated DUX4 toxicity *in vitro* and *in vivo*, supporting our hypothesis that DUX4-associated dystrophic phenotypes are related to DUX4 function as a transcription factor. Together, these data support DUX4 muscle toxicity *in vivo*, and justify further investigation of DUX4 in FSHD pathogenesis.

## 411/W/Poster Board #69

**Transcriptional profile of the embryonic myosin heavy chain gene *MYH3* responsible for two congenital distal arthrogryposis syndrome subtypes.** *A.E. Beck[1], A.W. Ward[2], M.J. Rieder[3], M. Regnier[2], M.J. Bamshad[1].* 1) Dept Pediatrics, University of Washington, Seattle, WA; 2) Dept Bioengineering, University of Washington, Seattle, WA; 3) Dept Genome Sciences, University of Washington, Seattle, WA.

The distal arthrogryposis (DA) syndromes are a group of 10 dominantly inherited disorders characterized by multiple congenital contractures such as camptodactyly and clubfoot. DA syndromes are caused by mutations in at least seven different genes that encode components of the contractile apparatus of fast-twitch myofibers including genes for troponin I, troponin T, tropomyosin, and myosin heavy chains. Mutations in *MYH3*, which encodes embryonic myosin heavy chain, cause both the most common DA, Sheldon-Hall syndrome, and the most severe DA, Freeman-Sheldon syndrome. To better understand the mechanism by which *MYH3* mutations cause contractures, we sought to first describe the spatial and temporal expression patterns of *MYH3* in human fetal skeletal muscles in the upper and lower limbs. As a screen, we first examined mRNA transcripts derived from the skeletal muscles from both the lower arm and the lower leg of two human fetal samples aged 103 and 124 days gestation using an Illumina Human HT-12 whole transcriptome array. *MYH3* was the predominant myosin heavy chain gene expressed in all skeletal muscles studied. Next, we sought to determine which specific fetal muscle groups in the lower limb expressed *MYH3* and whether expression levels varied among muscles and/or gestational age. To this end, we prepared cDNA from individual skeletal muscles dissected from normal human fetal samples aged from 11 to 23 weeks gestation and "control" tissues including adult skeletal muscle. As assessed by amplifying intron-spanning amplicons, *MYH3* was highly expressed in all fetal skeletal muscles studied, not expressed in cardiac muscle, and expressed at a low level in kidney, spleen and liver. Surprisingly, *MYH3* was also expressed in adult human skeletal muscle. To verify the universality of this finding, we determined that *MYH3* expression is found in adult mouse and rabbit skeletal muscles as well. The demonstration that transcription of normal *MYH3* continues into adulthood suggests that mutations in *MYH3* may affect both prenatal and adult DA skeletal muscle. Understanding transcription patterns of the genes involved in DA syndromes may allow us to better understand the mechanism underlying the development of congenital contractures.

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## 412/W/Poster Board #70

**Tissue specific splicing of mutant transcript in patients with Hereditary Myopathy with Lactic acidosis (HML).** *A. Olsson[1], L.-E. Thornell[2], M. Holmberg[1].* 1) Department of Medical Bioscience, Umea University, Umea, Sweden; 2) Department of Integrative Medical Biology, Umea University, Umea, Sweden.

Hereditary Myopathy with Lactic acidosis (HML) is an autosomal recessive disorder with its origin in the northern part of Sweden. HML patients are exercise intolerant and will, even at a low work load, present symptoms such as muscle cramps, tachycardia, dyspnea and increased release of lactate and pyruvate. In severe cases, which can be triggered by fasting or hard exercise, the patients can develop severe acidosis which can prove fatal. We have previously identified an intronic mutation (G→C) in the *ISCU* gene in patients with HML. The mutation results in aberrant splicing of the IscU RNA introducing 100bp of intron RNA into the IscU mRNA. This in turn gives rise to a dysfunctional IscU protein which causes impaired energy metabolism. The IscU protein is involved in the assembly of iron-sulfur clusters, which are necessary for the function of several proteins in the respiratory chain and the TCA cycle. One would expect that a defect in such a general process would affect all energy demanding tissues but the phenotype is limited to the patients' skeletal muscles, while organs such as the heart and the brain are unaffected. We have shown by RT-PCR and western blot that the IscU mRNA and protein is expressed in all tissues examined, including heart. We therefore speculate that the fact that skeletal muscle is particularly vulnerable to the IscU defect is due to alternative splicing of the IscU RNA. We have studied the IscU transcripts in tissues from both patients and controls and preliminary data show that the mutant transcript is indeed alternatively spliced in a tissue specific manner in HML patients.

## 413/W/Poster Board #71

**Mitochondrial UBIAD1 Pore Function is Predicted to be Altered by Schnyder Corneal Dystrophy Mutations.** *M.L. Nickerson[1,5], B. Kostiha[2,5], W. Braun[3], W. Fredericks[4], B. Gold[5], B. Malkowicz[4], L. Wessjohann[3], H. Kruth[6], M. Dean[5], J.S. Weiss[7].* 1) Graduate Partnership Program, NIH, Bethesda, MD 20892, USA; Molecular Medicine Program, Institute for Biomedical Sciences, George Washington University, Washington, DC 20037, USA; 2) Biomedical Science Graduate Program, Hood College, Frederick, MD 21701, USA; 3) Leibniz-Institute of Plant Biochemistry Department of Bioorganic Chemistry, Weinberg 3,0612 0 Halle/Saale, Germany; 4) Department of Surgery, Division of Urology, University of Pennsylvania, Philadelphia, PA 19104, USA; 5) Cancer and Inflammation Program, National Cancer Institute, National Institutes of Health, Frederick, MD, 21702, United States of America; 6) Section of Experimental Atherosclerosis, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD, USA; 7) Kresge Eye Institute and Departments of Ophthalmology and Pathology, Wayne State University School of Medicine, Detroit, MI 48201, USA.

Schnyder corneal dystrophy (SCD, MIM: 121800) is a rare autosomal dominant disease characterized by abnormal deposition of cholesterol and phospholipids in the cornea leading to progressive corneal opacification and loss of visual acuity. A novel gene mutated in SCD, UBIAD1, was recently independently identified by two groups using classic positional cloning methodology. UBIAD1 was predicted to contain a prenyl-transferase domain and eight transmembrane spanning regions. Examination of protein homology showed that UBIAD1 is an outlier when compared to proteins involved in sterol and cholesterol metabolism. This may indicate a novel function for the enzyme. The current study presents examination of ten newly recruited SCD families. One represents a first report of SCD in a family of Native American ethnicity. Clinical manifestations of SCD in proband cornea are described. We report five novel UBIAD1 genetic alterations: A97T, D112N, V122E, V122G, and L188H. Five families possessed the hotspot alteration, N102S, bringing the total number of SCD families with this alteration to 41%. Mutated amino acids were observed to be highly conserved across species, and to date, 88% (15/17) of residues mutated in SCD are completely conserved from sea urchin to mammals. To assess the impact of SCD mutations on protein function, a three-dimensional model of UBIAD1 in a lipid bilayer was developed using protein threading. A model obtained using the Molecular Operating Environment was refined using the molecular dynamics refinement tool, YASARA. Stereochemical quality of the model was analyzed with PROCHECK. The model indicates the protein forms a potential pore structure composed of a circle of eight transmembrane helices. Amino acid, N102 appears to occupy a critical position within the pore as part of a previously identified enzyme active site. Docking simulations with various substrates were conducted and these indicate favorable interaction with oligoprenyl diphosphates. Naphthalin-1,4 diol also fit well when docked into the putative active site of the model. Significantly, changing N102 to N102S altered the binding of naphthalin-1,4 diol completely, rendering its prenylation impossible. Lastly, we demonstrate subcellular localization of UBIAD1 to the mitochondria in normal and SCD keratocyte cell lines.

## 414/W/Poster Board #72

**Gene expression signatures of primary colonic tissue from control, FAP and AFAP patients.** *D.W. Neklason[1,2], B.A. Milash[1], E.J. Manos[1], T.M. Tuohy[1], J. Lilley[1], R.W. Burt[1,3].* 1) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; 2) Department of Oncological Sciences, University of Utah, Salt Lake City, UT; 3) Department of Medicine, University of Utah, Salt Lake City, UT.

Familial adenomatous polyposis (FAP) is a colon cancer syndrome with a prevalence of 1:10:000. Patients have 100's to 1000's of precancerous colonic polyps and nearly 100% risk of developing colon cancer at an average age of 39 years in the absence of colon surveillance and surgery. Mutations in the *APC* gene lead to FAP as well as an attenuated form (AFAP) which presents with variable phenotypic expression, reduced polyp numbers and reduced cancer risk as compared to FAP. Current methods for the clinical diagnosis of genetic diseases most commonly involve analysis of germline DNA. Germline DNA-based diagnosis can be incomplete, for example no mutation is found in approximately 20% of FAP and 50% of AFAP patients. The objective of this study is to determine a molecular profile of the colonic epithelia from patients with *APC* mutations leading to FAP or AFAP then to use this information to establish a gene expression signature for diagnosis and for better understanding of the disease in the primary affected tissue. Aglient 44K RNA microarrays were run using mRNA from fresh normal-appearing colonic epithelia obtained as biopsies during endoscopy from FAP patients (n=6), AFAP patients (n=14) and control patients (n=12). Analysis using the Rank Product method found 48 mRNA probes with statistical significance (p<0.001) that consistently distinguish between control, FAP and AFAP normal appearing colonic tissue. These probes are up in FAP vs. control but low in AFAP vs. control or vice versa and will be tested for their accuracy to classify FAP and AFAP patients. Differential expression was also evaluated to better understand the phenotypic variability within AFAP using 5 individuals with > 100 adenomas versus 6 individuals with < 20 adenomas with the identical APC mutation. Differential expression identified 245 probes with a p-value of <0.05. The most striking were *DEFA5* and *DEFA6*, encoding microbicidal defensins involved in host defense, which consistently showed increased expression in the >100 adenoma group. It is not clear if this reflects a host or an environmental difference and will require further study. In conclusion, a distinct gene expression signature can be identified in FAP vs. AFAP patients that, in turn, can be applied to diagnostics. A separate set of genes can also distinguish phenotypic classes of individuals with the identical *APC* mutation.

## 415/W/Poster Board #73

**Molecular and Clinical Findings of Patients Referred for UGT1A1 Mutation Analysis.** *B. Durmaz[1], H. Onay[2], A. Alpman Durmaz[2], U.S. Akarca[3], F. Ozgenc[4], M. Baran[4], F. Ozkinay[1,2].* 1) Department of Pediatrics, Division of Genetics, Ege University, Izmir, Turkey; 2) Department of Medical Genetics, Ege University, Izmir, Turkey; 3) Department of Gastroenterology, Ege University, Izmir, Turkey; 4) Department of Pediatrics, Division of Gastroenterology, Ege University, Izmir, Turkey.

Gilbert syndrome (GS) is an inherited disorder characterized by unconjugated hyperbilirubinemia without any liver pathology or hemolysis. Hepatic UDP-glucuronosyltransferase enzyme activity is decreased to its 30% of normal levels leading to a defect of biluribin uptake into the liver resulting in unconjugated hyperbilirubinemia. UGT1A1 gene which is found to be associated with GS is located on 2q37. Increase in the number of TA repeats in the TATAA element of the promoter region of the UGT1A1 gene results in decrease of the enzyme activity leading to decreased bilirubin conjugation. A(TA)6TAA allele is seen in normal individuals whereas A(TA)7TAA or A(TA)8TAA alleles are found to be associated with hyperbilirubinemia. Here, we present 8 suspected Gilbert syndrome patients who were referred to us for molecular analysis of UGT1A1 gene. The sequence analysis of the promoter region of the UGT1A1 gene in these 8 patients revealed a normal A(TA)6TAA allele in homozygous state in 4 patients, (TA)7/(TA)7 in 2 patients, (TA)6/(TA)7 in one patient and (TA)7/(TA)8 in one patient. The clinical spectrum of the patients according to their genotypes of the UGT1A1 gene are discussed.

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## 416/W/Poster Board #74

**Novel mutations underlying familial enteropathies.** *D. Monies[1], Z. Rahbeeni[2], H. Al-Zaidan[2], A. Mehaidib[3], M. Al-Edreesi[4], M. Faqih[5], B.F. Meyer[1].* 1) Genetics, King Faisal Specialist Hospital and Research Centr, Riyadh, Riyadh, Saudi Arabia; 2) Medical Genetics, King Faisal Specialist Hospital and Research Centr, Riyadh, Riyadh, Saudi Arabia; 3) Pediatrics, King Faisal Specialist Hospital and Research Centr, Riyadh, Riyadh, Saudi Arabia; 4) Saudi Aramco Medical Services Organization; 5) Anatomic Pathology, King Faisal Specialist Hospital and Research Centr, Riyadh, Riyadh, Saudi Arabia.

Microvillous Inclusion Disease (MVID) and Congenital Tufting Enteropathy (CTE) are congenital disorders of the intestinal epithelial cells that cause an intractable watery diarrhea with usual onset near birth. MVID is characterized by lack of microvilli on the surface of enterocytes with the occurrence of intracellular vacuolar structures containing microvilli. Whereas pathologic studies for CTE patients demonstrate villi with crowded epithelial cells forming tufts. MVID and CTE are very rare disorders and are inherited as autosomal recessive traits. Recently, mutations of MYO5B and EpCAM were identified as the underlying lesion resulting in MVID and CTE, respectively. Four Saudi families were investigated, three with children affected by MVID and one with a child affected by CTE. Five patients and available unaffected individuals were subjected to genome-wide homozygosity scans using the Affymetrix 250K SNP array. Analysis with the copy number tool CNAG identified shared homozygous regions unique to the affected subjects. Of the three families with MVID, homozygosity was observed in two families at a locus on chromosome 18 which included MYO5B. Sequencing of MYO5B in individuals from these families identified two novel nonsense mutations in exons 24 and 36 (Q1047X and E1589X). In the other family homozygosity was absent at the MYO5B locus. However, a locus on chromosome 2 which included EpCAM was found to be homozygous in this family. Sequencing of EpCAM identified a 1bp insertion (c.499insC) in exon 5 resulting in premature truncation of the mature protein. This was consistent with this family being classified as having CTE rather than MVID. The fourth family studied was referred with a diagnosis of CTE and was found to have the same 1 bp insertion consistent with a common founder. The present study has identified novel nonsense mutations in MYO5B and EpCAM associated with autosomal recessive entheropathies in Saudi families. Our findings expand the limited spectrum of MYO5B and EpCAM mutations associated with gastrointestinal genetic disorders and provide an opportunity to investigate phenotype/ genotype correlations.

## 417/W/Poster Board #75

**Novel *SLC26A3* mutations in patients with congenital chloride diarrhea.** *S. Wedenoja[1,2], P. Höglund[1], C. Holmberg[2], J. Kere[1,3].* 1) Dept Medical Genetics, Univ Helsinki, Helsinki, Finland; 2) Hospital for Children and Adolescents, Univ Helsinki, Helsinki, Finland; 3) Department of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden.

The rare autosomal recessive disease congenital chloride diarrhea (CLD) is caused by mutations in the solute carrier family 26 member 3 (SLC26A3) gene. This gene resides on chromosome 7q31 and belongs to the human solute carrier family 26 (SLC26) with 11 structurally homologous anion exchangers, at least 3 of them causing rare autosomal recessive diseases. SLC26A3 encodes for an apical epithelial $Cl^-/HCO_3^-$ exchanger which shows expression in the surface epithelium of the ileum and colon, and in a few extraintestinal tissues including the sweat gland, male reproductive tract, and kidney. Intestinal loss of SLC26A3 results in profuse $Cl^-$-rich diarrhea, secondary loss of sodium, and a tendency to hypochloremic and hypokalemic metabolic alkalosis. The life-saving therapy for CLD, salt substitution with NaCl and KCl, allows favorable long-term outcome. CLD diagnosis is based on its typical clinical picture and a high concentration of fecal $Cl^-$, exceeding 90 mmol/L after correction of the fluid and electrolyte depletion. Although genetic testing for CLD is possible, the simple measurement of fecal $Cl^-$ is still sufficient to confirm the diagnosis in most of the cases. Differential diagnosis includes the rare entity of other inherited diarrheas. While most of the 250 cases reported so far arise from Finland, Poland, and Arabic countries, single cases with this rare autosomal recessive disorder appear worldwide. Such CLD infrequency makes diagnostics challenging but necessary, as untreated disease is usually lethal in early infancy. As a part of our research program, we have been characterizing mutations for CLD in patients from all over the world. After our mutation update in 2002, we have found altogether 16 novel mutations behind CLD by direct sequencing. The aim of this study is to report novel SLC26A3 mutations and assess their types and distribution in different regions of the SLC26A3 gene. These mutations add important data on SLC26A3 genetics but despite the spectrum of tens of mutations, evidence of genotype-phenotype differences remain non-existent. It is worth remembering, however, that most clinical data on CLD stem from a relatively homogenous population of Finnish patients with an identical genetic background for CLD (p.V317del mutation in 97%). Therefore, future work is necessary to assess possible phenotypical differences, if any, in CLD.

## 418/W/Poster Board #76

**Are endothelial nitric oxide synthase (eNOS) gene VNTR genotypes risk factors in the etiology of Necrotizing enterocolitis (NEC) in premature infants?** *K. Yanamandra[1], J. Aryama[1], D. Napper[1], H. Chen[1], S.A. Ursin[1], A. Pramanik[1], J.A. Bocchini Jr.[1], R. Dhanireddy[2].* 1) Dept Pediatrics, LSU Med Ctr, Shreveport, LA; 2) Dept Pediatrics, UT Health Science Center, Memphis, TN.

Necrotizing enterocolitis (NEC) is a severe GI disease of the premature infants. Although the precise etiology of NEC is unknown, suspected pathopyhisological mechanisms include prematurity, formula feeding, infection and bacterial translocation, ischemia, hyperosmolar load, and disruption of mucosal integrity in the GI tract. The disease could be fatal if untreated. It is a multifactorial disorder with fragile intestine susceptible to microbial infections culminating in ischemia and necrosis in premature infants. Nutritional factors were reported to be involved in the etiology of NEC, such as low Arginine. Also several investigators have reported the advantages of L-Arginine supplementation with a caution on the dose. L-Arginine is a precursor of endogenous nitric oxide (NO) through nitric oxide synthase (NOS). There are three forms of NOS, neuronal and endothelial being constitutive and the third inducible form. Endothelial nitric oxide (eNO) serves as a vasodilator, relaxes smooth muscle, prevents platelet aggregation, and facilitates improved blood flow, vascular tonicity. Reduced nitric oxide levels result in vasoconstriction and weak tone leading to decreased blood flow and hypoxia. Mutant eNO synthetase (eNOS) genotypes result in reduced nitric oxide levels by decreasing the enzyme activity. In the present investigation we have studied the association of eNOS variable number of tandem repeats (VNTR) of 27bp in intron 4 region of the gene. We have collected peripheral blood specimens from a total of 147 premature infants consecutively from our NICU facility and genotyped by PCR. The frequency of rear allele *a* consisting of 4 repeats was elevated over the common *b* allele consisting of 5 repeats in NEC patients as compared to controls (0.56 vs. 0.36, odds ratio 2.2). Clinical data of the infants from NICU with ethnic stratification and distribution of alleles and genotypes and their significance will be presented.

## 419/W/Poster Board #77

**Evaluation of 263 patients referred for molecular analysis of Alpha 1 antitrypsin gene.** *C. Ozkinay[1], E. Karaca[1], A. Alpman Durmaz[1], H. Onay[1], B. Durmaz[2], O. Cogulu[1,2], F. Ozkinay[1,2].* 1) Department of Medical Genetics, Ege University Medical Faculty, Izmir; 2) Department of Pediatrics, Ege University Medical Faculty, Izmir, Turkey.

Alpha 1 antitrypsin (AAT) enzyme deficiency is one of the most common autosomal-codominant genetic disorderand is characterized by early onset rapidly progressive emphysema. The variants within the AAT1 gene are classified according to the protease inhibitor (PI) system in which M is the normal allel and the inheritance of S and Z alleles is associated with decreased levels of the protein. The Z genotype is associated with liver cirrhosis in contrast to S genotype. The objctive of this study is to investigate the presence of S and Z alleles in patients with various indications. In this report we present the AAT1 genotypes of 263 patients referred for molecular analysis with various indications. The indications for AAT1 genotyping were chronic lung disease, chronic liver disease, cirrhosis, hepatitis, Wilson disease and some other entities. Among 263 patients, 252 patients were found to be normal MM genotype, whereas 4 pateints were PIMZ, 4 patients were PIZZ and 3 pateints were PIMS. The clinical features of the patients having mutant alleles are discussed. The frequencies of the mutant alleles were found to be rare among the patients referred to molecular analysis in the study group.

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## 420/W/Poster Board #78

**Adult-onset leukoencephalopathies with vanishing white matter with novel missense mutations in *EIF2B2* and *EIF2B5*, and decreased eIF2B activity.** *T. Matsukawa[1], X. Wang[2], R. Liu[2], A. Hida[1], A. Kubota[1], Y. Fukuda[1], H. Kowa[1], Y. Takahashi[1], S. Aoki[3], J. Shimizu[1], J. Goto[1], CG. Proud[2], S. Tsuji[1].* 1) Dept Neurology, Univ Tokyo, Tokyo, Japan; 2) Dept Biochemistry and Molecular Biology, Univ British Columbia, Vancouver, Canada; 3) Dept Radiology, Univ Juntendo, Tokyo, Japan.

Background: Leukoencephalopathy with vanishing white matter (VWM) is a leukoencephalopathy with autosomal recessive inheritance. MRI shows diffuse leukoencephalopathy with cystic lesions. The onset is usually at age 2-6 with cerebellar ataxia, spasticity and intellectual decline. The course is chronic-progressive with episodes of rapid deterioration following minor head trauma. Mutations in each of eIF2B subunit genes, *EIF2B1-5*, have been identified as causative for VWM. Thus far, the cases of 8 adult patients with VWM have been reported. Material & method: 10 Japanese adult patients with leukoencephalopathy of unkown origin in our hospital aged 28 to 70 years (mean age; 50.9) were investigated. Parents were first cousins in two patients. Brain MRI showed diffuse leukoencephalopathy with cystic lesions in 4 patients. We performed mutational analyses of *EIF2B1-5* of the patients using direct nucleotide sequence analysis. Functional assays of mutant eIF2B was accomplished by the analysis of the activity of complexes of eIF2B subunits containig the mutant eIF2B subunit. Results: We detected novel homozygous missense mutations in *EIF2B2* (p.Val85Glu) in one patient with severe intellectual decline and ataxia, and in *EIF2B5* (p.Asp270His) in one patient with only mild cognitive impairment, ataxia and spasticity. Both of their parents are consanguineous. In addition, we detected novel SNPs in introns of *EIF2B3-5*. These novel SNPs in introns are unlikely to be associated with splicing abnormalities. Analysis of eIF2B subunits containing the mutant eIF2B subunit was conducted in the adult case of VWM with a novel missense mutation in EIF2B2, and the results confirmed decreased activity of eIF2B. The decrease in the activity of the eIF2B containing the mutant eIF2B subunit is milder than observed for mutations tested previously. This was the second case among the adult-onset cases with the mutation in *EIF2B2*. The onset of the adult case of VWM with a novel missense mutation in *EIF2B5* was at age 50 and this was the latest among adult-onset cases of VWM. Conclusion: In our study, we detected 2 adult-onset cases of VWM out of 10 cases of leukoencephalopathy of unkown origin. Both of two adult-onset cases of VWM showed cystic lesions on MRI. To investigate the genotype-phenotype correlation of the cases of VWM and whether the level of residual activity is related to the variability of VWM phenotype, investigation on larger number of cases of VWM will be needed.

## 421/W/Poster Board #79

**Comprehensive functional analysis of human radiosensitive cell lines enables a molecular diagnosis of DNA repair disorders.** *S.A. Nahas, F. Fike, k. Nakamura, L. Du, R.A. Gatti.* Pathology and Lab Medicine, UCLA, Los Angeles, CA.

In an effort to unravel the many possible etiologies of human radiosensitivity, we studied a unique panel of 26 radiosensitive (RS) human lymphoblastoid cells (RS-LCLs) from individuals with undiagnosed diseases. Our comprehensive approach tested the integrity of known functional pathways for sensing and repair of DNA double strand breaks. We first categorized the RS-LCLs by assessing the kinetics of phosphorylation of SMC1 followed by radiation-induced DNA damage over 24-hours. We further interrogated cell cycle checkpoints, Non Homologous End Joining DNA ligation (NHEJ), mitochondrial respiratory integrity, and the chromatin ubiquitin ligase cascade. These results implicated specific repair pathways and protein defects. From among the cell lines with an S-phase checkpoint defect, we identified a Chk2- related deficiency that impaired the degradation of the Cdc25A phosphatase protein and phosphorylation of BRCA1 by Chk2. In another cell line absence of IR-induced 53BP1 foci implicated a portion of the recently described chromatin ubiquitin ligase pathway. A third RS-LCL unexpectedly lacked aprataxin, a deficiency not previously associated with RS. These studies provided proof of principle that a hypothesis-driven dissection of RS mechanisms can lead to improved diagnoses of DNA repair disorders.

## 422/W/Poster Board #80

**Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS): expanding the genotype in the French-Canadian population.** *I. Thiffault[1, 2], M. Tetreault[1,2], J. Demers-Lamarche[1,2], M.J. Dicaire[1,2], L. Loiselle[1,2], J. Mathieu[3], J.P. Bouchard[4], G.A. Rouleau[2], B. Brais[1,2].* 1) Lab Neurogenetics, M4211-L4, CRCHUM-Notre Dame Hospital, Montreal, PQ, Canada; 2) 2) Centre of Excellence in Neuromics of Université de Montréal, CRCHUM, Hospital Notre-Dame, Montreal, QC, Canada; 3) Carrefour de la Santé de Jonquière, Saguenay, QC, Canada; 4) Department of Neurology, CHUL, Quebec City, QC, Canada.

Recessive ataxias are a heterogeneous group of neurodegenerative diseases. Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS, MIM 270550) is one of the classical recessive diseases more frequent in the French-Canadian (FC) population due to a founder effect. Is has an estimated mutation carrier rate of 1/22 in the Saguenay-Lac Saint-Jean (SLSJ) region of Northern Quebec. First described in 1978, ARSACS manifests itself in childhood by a spastic paraparesis that evolves into a progressive spastic cerebellar ataxia accompanied by a sensory-motor neuropathy that ultimately leads to a loss of walking at a mean age of 41 (17-57 years). In 2000, two nonsense mutations in the SACS gene were found to be responsible for 92.6% and 3.7% of Quebec carrier chromosomes. Despite the fact that ARSACS is a common recessive disease in Quebec, our specific aim is to uncover the full spectrum of mutations in French Canadians to better delineate the clinical variability of the ARSACS phenotype. DESIGN/METHODS: RT-PCR, long-range PCR and extensive sequencing of all cDNA and genomic fragments were performed. We also designed two custom panels encompassing all variants reported in the literature and novel French-Canadian mutations using Sequenom iPLEX technology (N=63 variants) to investigate the frequency of SACS mutations in sporadic/idiopathic ataxia cases and validate it use for mutation detection. In order to better delineate rearrangement and asses the frequency of these events in SACS patients, we designed an Illumina GoldenGate array to interrogate 96 loci (SNPs and mutations). RESULTS: Mutation analysis was performed in 32 ARSACS patients with only one mutation identified or a classical phenotype and 127 spastic ataxia cases. We identified 18 mutations in the SACS gene. Most mutations are loss of function mutations (90%). Large deletion encompassing the entire SACS coding sequence and surrounding genes seems to be more common than expected (5/32 ARSACS cases). CONCLUSIONS/RELEVANCE: The prevalence of ARSACS in the French-Canadian population seems even higher than previously estimated. The phenotype of ARSACS patients seems less uniform than originally described. Multimodal molecular screening is required to improve the sensitivity of SACS mutation analysis. The Illumina Goldengate custom array that we designed could help international colleagues to better assess the role of SACS mutations in ataxia cohorts.

## 423/W/Poster Board #81

**FREQUENCY OF Q829X MUTATION IN OTOF GENE AMONG 650 INDIVIDUALS WITH NONSYNDROMIC DEAFNESS: GENOTYPE- PHENOTYPE CORRELATION.** *N. Gelvez[1], L. Morales[1], S. Florez[2], V. Rodriguez[3], M. Olarte[1], M.F. Leiva[4], M.L. Tamayo[1].* 1) Inst Genetica Humana, Univ Javeriana, Bogota, 1, Colombia; 2) Fundaciòn oftalmològica Nacional FUNDONAL, Bogotà, Colombia; 3) Hospital universitario San Ignacio, Bogotà, Colombia; 4) UDES, Universitaria de Santander, Cúcuta, Colombia.

Introduction: Non-syndromic deafness affects 1 to 1000 or 2000 newborn infants. The most common form is autosomal recessive with genetic heterogeneity. At least 16 genes have been implicated in the aetiology of deafness. The OTOF gene is one of the most important implicated in the nonsyndromic deafness of neural type, codifies for the Otoferlin protein with expression in cochlea, vestibule and brain. Alterations in this protein cause auditory neuropathy and deafness; the Q829X mutation is the third most frequent causing deafness. Objective: To determine the frequency of Q829X mutation among 650 individuals with nonsyndromic deafness; and to offer a genotype-phenotype correlation in positive cases. Methods: Complete clinical, genetic and ocular evaluation to 650 Colombian deaf individuals attending schools for the deaf throughout the country in order to confirm the diagnosis of Non-syndromic deafness. Blood samples were obtained from selected individuals, to identify the Q829X mutation, choosing children without mutation in the GJB2 and GJB6 genes (Cx26, Cx30), or deaf individuals heterozygous for any mutation in GJB2 or GJB6 genes. Results: Among 650 deaf children, the Q829X mutation was identified in 11 individuals: 7 homozygous and 4 heterozygous. The frequency for this mutation was 1.69% among deaf institutionalized population with Non-syndromic deafness. Discussion: These results indicate that Q829X mutation has a significant frequency among deaf Colombian population. It is important to include the Q829X mutation as a part of a future diagnostic panel in Colombia, based on molecular screening among hearing impaired individuals, in order to offer an early and reliable diagnosis, which is important to start an earlier rehabilitation in this population.

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## 424/W/Poster Board #82

**Computational Re-Examination of Tyrosinase Mutations in Oculocutaneous Albinism.** *D.R. Adams, W.A. Gahl.* Section on Human Biochemical Genetics, Medical Genetics Branch, NHGRI, NIH, Bethesda, MD.

INTRODUCTION: Oculocutaneous albinism (OCA) is an autosomal recessive condition causing decreased pigment production. Four genes are known to cause OCA, the most common being *TYR* (tyrosinase) and *OCA2* (OCA2). Sequence analysis in affected individuals has up to 85% sensitivity, with the remainder unexplained. Recent human whole-genome sequencing data suggests that rare, non-synonymous sequence variants (nsSNPs) are more common than previously thought. To help understand the pathogenicity of nsSNPs in OCA, we re-analyzed the published *TYR* mutations using pathogenicity prediction software. METHODS: The analyzed set of variants was collected from published literature, and included only non-frameshifting single amino acid changes. Each variant was tested using the published programs SNAP, PolyPhen and Panther. The results were compared with the published pathogenicity assessment, the proximity to tyrosinase protein functional elements, and the nsSNP frequency among affected individuals. The pathogenicity assessments of the three software programs was compared by ranking the assessments within each method and comparing the rankings using Spearman rank correlation. RESULTS: 20% of the variants that had been reported to be pathogenic were assessed as non-pathogenic. Another 20% were assessed as indeterminate. Pair wise correlations between the method rankings averaged 80% with a P-value of less than 1 x 10-7. 13/15 mutations that were reported separately in 3 or more papers were classified as pathogenic. Mutations that disrupted known structural elements were classified as pathogenic. CONCLUSIONS: Some reportedly pathogenic mutations found in persons with OCA are likely to be rare, non-pathogenic nsSNPs. Pathogenicity prediction software has false-positive and false-negative rates that are substantial (10-20%). In addition, there is significant overlap among the different programs' usage of assessment techniques such as multiple alignment. However, such programs would be expected to perform relatively well with TYR given a large number of available distant orthologs and a well characterized structure. While software pathogenicity prediction is not currently reliable enough for the assessment of individual nsSNPs, it can provide valuable categorization of groups of variants. Future refinement of pathogenicity assessment techniques, including functional assays, will be required to differentiate rare non-deleterious nsSNPs from deleterious mutations.

## 425/W/Poster Board #83

***ZNF469* mutations in patients with brittle cornea syndrome.** *F. Malfait[1], Ph. Vlummens[1], L. Van Laer[1], S. Ceylaner[2], G. Ogur[3], A. De Paepe[1].* 1) Centre for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 2) Zekai Tahir Burak Womens Health and Education Hospital, Department of Genetics, Ankara, Turkey; 3) Department of Paediatric Genetics, Medical Faculty, Ondokuz Mayis University, Samsun, Turkey.

Brittle cornea syndrome (BCS) is an autosomal recessive (AR) condition characterized by a thin, fragile cornea that tends to rupture upon minor trauma and blue sclerae. Additional systemic symptoms, such as joint and skin hyperlaxity place BCS among the heritable disorders of connective tissue (HDCT). BCS shows considerable overlap with the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS type VI), based on its AR transmission, joint and skin hyperlaxity and rupture of the eye. In general however, EDS VI patients have more severe muscle hypotonia, as well as kyphoscoliosis, atrophic scarring and arterial rupture. EDS VIA is caused by deficient activity of lysyl hydroxylase-1 (LH-1; encoded by *PLOD1*), which hydroxylates specific collagen lysines. EDS VIB patients have an identical phenotype but normal LH activity. Recently two homozygous 1 bp deletions were identified in the *ZNF469* gene in 6 unrelated BCS families. *ZNF469* encodes Zinc finger 469, a protein of unknown function which belongs to the C2H2-zinc finger (ZNF) family of proteins. ZNFs are abundant in eukaryotic proteins and generally function as sequence-specific DNA-binding motifs, although they can be involved in recognition of RNA and proteins. Here we present 2 consanguineous Turkish families, with respectively two (P1&2) and one (P3) affected children, all of them previously diagnosed with EDS VIB. They presented blue sclerae and corneal rupture after minor trauma, joint and skin hyperlaxity and marfanoid habitus. P1&2 were more severely affected with multiple dislocations, muscle hypotonia and kyphoscoliosis. LH-1 activity was normal in all patients. Direct sequencing of *ZNF469* revealed 2 homozygous duplications generating a premature stop codon: in P1&2 a c.6509_6512dupTCTT (p.Leu2171PhefsX99), and in P3 a c.9792dupT (p.Ala3265CysfsX6). Interestingly, molecular analysis of *PLOD1* revealed a previously unreported heterozygous variant c.79A>C (p.N27H) in P1&2. In conclusion, we identified 2 novel *ZNF469* mutations in 3 BCS patients. In one family, a potentially deleterious, heterozygous amino acid substitution in LH-1 was identified as well. Although LH-1 activity was normal, this variant may have an aggravating effect, accounting for the more severe EDS VI-like phenotype of P1&2. Further studies are needed to unravel the function of ZNF469. This will provide insight into the molecular pathways involved in the development of connective tissues and in the pathogenesis of HDCTs.

## 426/W/Poster Board #84

**Atlastin-1 (SPG3A) mutations in Portuguese families with dominantly inherited spastic paraplegia.** *A.F. Brandão[1,2], J.L. Loureiro[2,3], J. Pinto-Basto[1,2,4], P. Coutinho[2,3], J. Sequeiros[1,2,4], I. Alonso[1,2].* 1) CGPP, IBMC, Porto, Portugal; 2) UnIGENe, IBMC, Porto, Portugal; 3) Serviço de Neurologia, Hospital de São Sebastião, Feira, Portugal; 4) ICBAS, Universidade do Porto, Porto, Portugal.

Hereditary spastic paraplegias (HSPs) are a very heterogeneous group of neuro-degenerative disorders. Clinically, they are divided in pure and complex forms. Pure HSPs are characterized mainly by slowly progressive weakness and spasticity of the lower limbs; complex forms show additional neurological features, like ataxia, epilepsy and/or non-neurological signs. Genetically, HSPs are also very heterogeneous, and all modes of inheritance have been described: autosomal dominant (AD), autosomal recessive (AR) and X-linked (XL). Among the dominant forms, SPG3A is the second most common, responsible for about 10% of AD-HSP (SPG4 represents 40% of AD-HSP). Mutations in SPG3, encoding atlastin-1, a Golgi transmembrane GTPase involved in vesicle trafficking, are mainly associated with early onset. We ascertained 40 Portuguese families clinically with AD-HSP: 14 with pure HSP, 7 with complex form and 19 unclassified; and performed mutation analysis in SPG3A gene. Mutation analysis was carried out by PCR amplification of the entire coding region and consensus flanking sequences, followed by bidirectional direct sequencing. In patients in whom no mutation was found, MLPA was also performed to detect large deletions/duplications. Analysis identified two missense mutations in SPG3A: one replacing a highly conserved tyrosine by a histidine, at codon 336 (Y336H), while the second replaced an arginine by a cysteine, at codon 239 (R239C), in one family each. Both are predicted by Polyphen to be probably damaging to atlastin-1 function. The Y336H mutation was found in a family with onset on the first year of life and R239C mutation in a family with onset on the early infancy. No other mutations, including large deletions or duplications were identified in the other patients studied. We plan to enlarge our sample of HSP families to assess the frequency of SPG3A among the Portuguese families and functionally characterize the Y336H and R239C mutations identified.

## 427/W/Poster Board #85

**Legius Syndrome in the University of Utah Neurofibromatosis Clinic.** *TM. Muram-Zborovski[1], DA. Stevenson[3], AR. Wilson[2], M. Procter[2], BW. Shirts[1], L. Chou[2], D. Viskochil[3], R. Mao[1,2].* 1) Department of Pathology, The University of Utah, Salt Lake City, UT; 2) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; 3) Department of Pediatrics, The University of Utah, Salt Lake City, UT.

Legius syndrome, originally "neurofibromatosis type 1-like syndrome", is caused by SPRED1 mutations. The original report of Legius syndrome (Brems et al. 2007) suggested phenotypic overlap with NF1 without the more severe complications, specifically tumorigenesis. Patients often meet the diagnostic criteria for NF1 based on pigmentary findings, but appropriate classification is important since they are unlikely to require the same medical management. The aims of this study were to identify the frequency of Legius syndrome within an NF1 clinic population. The overlap of pigmentary findings in young children without other manifestations of NF1 may require the implementation of SPRED1 mutation analysis to establish the diagnosis of Legius syndrome. Patients fulfilling the NIH clinical diagnostic criteria for NF1 were enrolled from the University of Utah NF Clinic. Bidirectional Sanger sequencing for the coding regions and intron/exon boundaries of SPRED1 was performed. We sequenced 151 unselected patients with the clinical diagnosis of NF1 and identified 2 individuals (1.3%) with novel SPRED1 mutations, p.R18X and p.Q194X. The phenotype for the first patient (10 yrs.) included >10 café-au-lait spots, intertriginous freckling, learning disabilities, and no neurofibromas. The phenotype for the second patient (12 yrs.) included 10-20 café-au-lait spots, intertriginous freckling, single reported Lisch nodule, no learning disabilities, and no neurofibromas. A specific haplotype allele was identified in 27 individuals, allele frequency of 9.6%; (29/302). An unselected population showed a slightly decreased haplotype allele frequency of 6.3%; (4/64 alleles, p=0.049). However, because ethnicity data for the unselected population are not available, ethnicity may be a confounding factor. Statistical analysis, correcting for age, showed that the haplotype is associated with various phenotypes of NF1. The association was strongest for hypertension (p=0.003), but was also significant for the Riccardi severity score (p=0.049). We identified Legius syndrome in 2 of 151 patients who fulfilled the clinical diagnostic criteria of NF1 in our Neurofibromatosis Clinic. We identified a haplotype seen more frequently in our NF1 cohort than the unselected population. The association of a more severe NF1 phenotype in carriers of this SPRED1 haplotype suggests that it may have functional significance in Ras signaling or be linked to another modifier gene.

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## 428/W/Poster Board #86

**Spatacsin (SPG11) mutations in Portuguese patients with hereditary spastic paraplegia.** *M.C. Pereira[1], J.L. Loureiro[2,3], J. Pinto-Basto[1,3,4], P. Coutinho[2,3], J. Sequeiros[1,3,4], I. Alonso[1,3].* 1) CGPP-IBMC, Porto, Portugal; 2) Serv. Neurologia, Hosp. São Sebastião, Feira, Portugal; 3) UnIGENe, IBMC, Porto, Portugal; 4) ICBAS, Univ.Porto, Portugal.

Hereditary spastic paraplegias (HSPs) are a group of neurodegenerative diseases with clinical and genetic heterogeneity. SPG11 is the most frequent autosomal recessive form of these disorders, and is characterized by signs such as cognitive impairment, along with the traditional spasticity of the lower limbs. A thin corpus callosum (TCC) is a common feature of SPG11, proposed as a marker for its diagnosis. We selected 11 families with autosomal recessive pattern, as well as sporadic cases of spastic paraplegia with thin corpus callosum and/or consanguineous parents. Mutation screening was performed by direct sequencing of the SPG11 gene, to detect point mutations, followed by MLPA analysis for detection of large gene rearrangements, deletions or duplications. Sequencing was performed in 26 individuals (8 families and 14 sporadic cases), allowing the detection of five pathogenic mutations, previously described, which result in truncated spatacsin. These were found in 62.5% of the families, and only in 14.3% of the sporadic cases. Interestingly, there seem to be two recurrent mutations in the AR-HSP Portuguese families (c.733_734delAT [40%] and c.529_533delATATT [30%]). A thin corpus callosum was present in 60% of the families and 50% of the sporadic cases with SPG11 molecularly confirmed; mental impairment occurred in 40% of the SPG11 families and none of the sporadic cases. MLPA analysis, performed in 3/8 of the affected families and 7/14 sporadic cases, showed no gene rearrangements. Our results show that SPG11 is frequent among Portuguese patients with AR-HSP and two previously described mutations are highly recurrent in Portuguese families.

## 429/W/Poster Board #87

**Interstitial Lung Disease in Hermansky-Pudlak Syndrome-2.** *B. Gochuico[1], M. Huizing[1], G. Golas[2], R. Hess[1], W. Gahl[1].* 1) Section on Human Biochemical Genetics, Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Intramural Program of the Office of Rare Diseases, OD, NIH, Bethesda, MD.

There are eight known human subtypes of Hermansky-Pudlak syndrome (HPS), an autosomal recessive disorder characterized by aberrant biogenesis of lysosome related organelles. Progressive pulmonary fibrosis is a leading cause of mortality in people with HPS-1 and HPS-4, which are associated with defective Biogenesis of Lysosome-related Organelles Complex-3 (BLOC-3). HPS-2, which is associated with mutation of ADTB3A, is characterized by abnormal function of the adaptor-3 (AP-3) complex. Investigators have shown that the pearl mouse, a model for HPS-2, is highly susceptible to pulmonary fibrosis induced by bleomycin. Consistent with these data, we report that humans with HPS-2 have interstitial lung disease. Three individuals with HPS-2 were evaluated at the National Institutes of Health. Subject 1 is an 8-year old male with a history of neutropenia, sepsis, and severe pneumonias, including influenza, respiratory syncytial virus, and enterococcal sepsis. Subject 2 is a 27-year old male smoker with a history of neutropenia and no history of pneumonia. Subject 3 is a 22-year old nonsmoking male who is a brother of subject 2; he has a history of neutropenia and no history of severe infection, including pneumonia. None of these individuals has a history of dyspnea, cough, or significant environmental exposures associated with interstitial lung disease. Pulmonary function tests from subjects 2 and 3 reveal normal lung volumes and normal diffusion capacity. High-resolution computed tomography of the chest reveals bilateral ground glass opacification and thickening of interlobular septa in subjects 1 and 2; there is minimal thickening of interlobular septa in subject 3. Thus, human HPS-2 disease is associated with radiographic findings of interstitial lung disease. Taken together, these data indicate that children and young adults with HPS-2 are at risk for development of interstitial lung disease. The young age of these subjects may indicate that functional defects of AP-3 complex are associated with susceptibility to an aggressive form of interstitial lung disease.

## 430/W/Poster Board #88

**The deletion of *CCM2* is not uncommon in patients with Greig cephalopolysyndactyly contiguous gene syndrome: Recommendations for diagnosis and management.** *J.J. Johnston, J. Turner, J.C. Sapp, J.A. Butman, A. Gropman, L.G. Biesecker.* NHGRI, NIH, Bethesda, MD, USA.

The Greig cephalopolysyndactyly contiguous gene syndrome (GCPS-CGS) is a multiple malformation syndrome caused by haploinsufficiency of *GLI3* and adjacent genes. Deletions in our GCPS-CGS cohort range in size from less than 1 Mb to greater than 12 Mb with each patient having a unique set of haploinsufficient genes. Several of the genes in this region, including *TBX20*, *GCK*, and *CCM2*, have been implicated in disease phenotypes that are inherited in a dominant pattern and can be due to haploinsufficiency. A single individual in our cohort has a deletion that includes *TBX20*, a gene known to cause congenital heart disease, this individual presented with both a VSD and coarctation of the aorta. Nine individuals in our cohort have deletions that include both *GCK* and *CCM2*. Heterozygous mutations in *GCK* cause MODY, a form of NIDDM, however mutations in this gene typically lead to a mild form of nonprogressive hyperglycemia. In contrast, heterozygous mutations in *CCM2* cause cerebral cavernous malformations (CCM). CCMs are vascular malformations consisting of clustered malformed blood vessels without normal intervening brain tissue or mature vessel walls. Individuals with these malformations can present with a variety of symptoms including seizures, focal neurologic deficits, headaches and cerebral hemorrhage. In rare instances these malformations can cause cerebrovascular accidents leading to death. Approximately 50-75% of individuals with CCMs become symptomatic and it is therefore important when diagnosing an individual with GCPS-CGS to determine if *CCM2* is disrupted by the deletion. In cases where *CCM2* is deleted the patient should be followed for CCM development using susceptibility weighted MRI sensitive to the microhemorrhage associated with tiny CCMs. These data lead to several conclusions; 1) molecular diagnosis for patients with GCPS is important for management and prognosis 2) patients with deletion of *CCM2* must be followed closely with specific MRI protocols to anticipate and treat CCMs, 3) *TBX20* deletions may cause septal defects in patients with GCPS-CGS and 4) *TBX20* likely causes septal defects via haploinsufficiency in patients with GCPS-CGS.

## 431/W/Poster Board #89

**Genomic deletions in *OPA1* are common in Autosomal Dominant Optic Atrophy.** *G.J. Almind[1], M. Larsen[1,2], K. Brondum-Nielsen[1], J. Ek[1].* 1) Medical genetics lab , Kennedy Center, Glostrup, Glostrup, Denmark; 2) Department of Ophthalmolgy, Glostrup Hospital, University of Copenhagen, Denmark.

**Background:** Autosomal Dominant Optic Atrophy (ADOA, OMIN #165500), also known as Kjer's optic atrophy is the most common form of hereditary optic neuropathy. The characteristic expression is characterized by bilateral subnormal visual acuity, deficient color vision, central visual field defects, and optic nerve pallor. The prevalence is relatively high in Denmark (1/12,000) compared to other countries (1/50,000). Large family studies have found that the OPA1 locus on chromosome 3q28 is the predominant gene-locus for the disease and more than 200 mutations in *OPA1* have been identified. However, the mutations only explain between 60 and 80% of the cases of ADOA. Therefore we initiated a study of copy number variation and other genomic rearrangements at the OPA1 locus to supplement the results of direct sequencing.

**Aim/Purpose:** The aim of this study was to identify and report copy number variation in the *OPA1* gene in Danish patients with ADOA without demonstrable mutations in *OPA1* and to describe the phenotypic association with the rearrangements.

**Methods:** 42 unrelated probands clinically diagnosed with ADOA were analyzed for genomic rearrangements in *OPA1* by means of multiplex ligation probe amplification (MLPA). Abnormal results were confirmed by additional manually added probes and by long distance PCR. The results were compared with visual acuity and color vision data.

**Results:** Among 42 patients with clinically diagnosed ADOA but no identifiable mutation in *OPA1* we identified 9 patients (21%) with genomic rearrangements in OPA1. These included two independent probands with deletions of exon 26, two with deletions of exon 9-15, two with deletions of intron 28, and one patient with deletion of exon 17. In addition, we identified two probands harboring deletions of the entire OPA1 gene on one allele. Phenotypically, the patients were comparable to patients with point mutations in OPA1.

**Conclusion:** *OPA1* deletion is a frequent cause of ADOA, contributing substantially to the established linkage to 3q28.

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## 432/W/Poster Board #90

**Identification of Sulfonylurea Receptor SUR1 and Potassium Inward Rectifying Receptor Kir6.2 genes mutations in saudi patients with persistent hyperinsulenimic hypoglycemia of infancy (Nesidioblastosis).**

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Persistent hyperinsulinemic hypoglycemia of infancy (PHHI) (MIM 256450) formerly called nesidioblastosis is an autosomal recessive disorder of pancreatic B-cell function. It is mainly characterized by inadequate suppression of insulin secretion in the presence of severe fasting hypoglycemia. Clinical presentation of this disease, which occurs predominantly in neonates and infants under the age of one year, includes seizures and coma. Mutations within B-cell plasma membrane ATP-sensitive potassium (KATP) channel which is encoded by two adjacent genes on chromosome 11p15.1: SUR1 (sulfonyl urea receptor) ABCC8 and Kir6.2 (inward rectifying potassium channel) KCNJ11 gene were identified in patients with PHHI. Fifteen patients diagnosed with PHHI, were enrolled in this study. Screening of the whole open reading frame (ORF) for both SUR1 and Kir6.2 genes has been performed. Three patients showed small deletions in the ABCC8 gene that requires further investigations. Twenty five novel and reported misense, silent and splice variants were identified in those genes. Some of these variants were known to be associated with other disorders such as Type II diabetes mellitus and hyperinsulinemia.

## 433/W/Poster Board #91

**Modifying effect of common type 2 diabetes associated variants on HNF1A MODY age of onset.**

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Mutations in hepatocyte nuclear factor-1alpha (*HNF1A*) are the most common cause of maturity onset diabetes of the young (MODY). There is a substantial variation in the age of diabetes onset, even within families where diabetes is caused by the same mutation. Common genetic factors are likely to explain some of this variation but none have been described to date. We hypothesized that common genetic variants that predispose to type 2 diabetes might have a modifying effect on the age of diabetes diagnosis in *HNF1A* MODY.

Sixteen robustly associated type 2 diabetes variants were successfully genotyped in 407 individuals from 196 families, from two study centers in the UK and Norway. We assessed their individual and combined effect on the age at diagnosis, by summing up the number of risk alleles carried by each individual to form a genetic risk score.

We confirmed the strong effects of social, environmental and genetic factors known to modify the age at diabetes onset in HNF1A MODY, namely the age at study or genetic testing (0.31 years, $P=8.9x10^{-44}$), *HNF1A* mutation position (-5.1 years if at least two isoforms affected, $P=9.3x10^{-3}$), and intrauterine hyperglycemia (-5.9 years if present, $P=4.4x10^{-14}$). Additionally, there was a strong effect of gender in our data, with females diagnosed 3.7 years earlier than males ($P=1.0x10^{-4}$). We included these variables and study as covariates in the subsequent individual and joint SNP analyses.

There were no strong individual SNP effects, although the variants in *HNF1B*, *SLC30A8* and *CDKAL1*, known beta cell genes, showed strongest associations (all P<0.01, unadjusted for multiple testing). In the combined genetic score model, each additional risk allele was associated with 0.33 years earlier diabetes diagnosis ($P=4.4x10^{-3}$).

In summary, we show that type 2 diabetes risk variants of modest effect sizes reduce the age at diagnosis in HNF1A diabetes. This is one of the first studies to demonstrate that clinical characteristics of a monogenic disease can be influenced by common variants associated with the polygenic form of that disease.

## 434/W/Poster Board #92

**Characterization of intragenic deletions and duplications in the *NIPBL* gene in patients with Cornelia de Lange syndrome.**

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Cornelia de Lange syndrome (CdLS) is a rare developmental disorder characterized by distinctive facial features, growth retardation, hirsutism, and upper limb reduction defects. Mutations in the *NIPBL* and *SMC1A* genes have been identified in patients with CdLS with a frequency of ~50% and 3-5%, respectively. Intragenic deletions and duplications of the *NIPBL* gene are thought to contribute to the molecular basis of CdLS, although very few have been described to date. Our molecular diagnostic laboratory has identified 5 intragenic deletions and 1 intragenic duplication in 189 individuals tested by *NIPBL* deletion/duplication analysis, a detection rate of 3.2%. The incidence of intragenic *NIPBL* deletions and duplications in CdLS patients is likely to be higher as the patient population studied includes patients with a suspicion of CdLS and many of whom likely do not have this diagnosis. The 5 intragenic deletions and 1 duplication identified in our laboratory span from two to ten exons, and occur in different regions of the gene. An intragenic deletion spanning exons 46 and 47, the two terminal exons of the *NIPBL* gene, has been identified in two patients. As this class of mutations has not been well described in CdLS, we will present data further characterizing these intragenic deletions and duplications, including the size of each intragenic deletion or duplication and delineation of breakpoints, which may likely help with the understanding of the potential mechanisms mediating these mutation events. Detailed clinical information on patients with intragenic deletions or duplications in the *NIPBL* gene will also be presented. This clinical information is obtained via a questionnaire that is submitted to our laboratory with the patient sample at the time of test request. Our findings represent an update on the molecular basis of CdLS and highlight that intragenic deletions and duplications are a significant contributor to CdLS etiology.

## 435/W/Poster Board #93

**Toward comprehensive molecular diagnosis of Retinitis Pigmentosa.**

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Retinitis pigmentosa (RP) is a genetically heterogeneous group of inherited retinal disorders affecting the photoreceptors or retinal pigment epithelium (RPE) and usually leads to progressive visual loss. Clinical severity and onset of RP can be variable even in the same family. RP can be inherited along autosomal dominant (adRP), recessive (arRP) or X-linked recessive patterns. Molecular diagnosis of RP can use different approaches. We provided mutational analysis of patients with RP using a newer approach of simultaneously sequencing every coding exon from 18 genes in which mutations are known to be associated with RP as listed in RetNet. To analyze every coding exon and exon/intron borders from these genes, a total of 137 polymerase chain reactions (PCR) were performed and the products subjected to bi-directional DNA sequencing. Mutations and novel SNPs could be identified with this approach. We hypothesized that some of the novel SNPs cause clinical variability in patients. We present preliminary data from three families supporting our hypothesis. Family 1 is a large family with 4 generations affected; varying age of onset and phenotype. Families 2 and 3 are isolated cases and the proband in each family is an only child. A reported mutation, namely *RHO* p.A164V, was identified in the proband of Family 1; in addition, four novel variations also were identified in other retinal genes, one each in *IMPDH1*, *ROM1*, *RP9* and *SEMA4A*. A reported mutation, namely CRX p.A158T, was identified in the proband of Family 2; five novel variations also were also identified, one each in *CA4*, *FSCN2*, *PRPF8*, *ROM1* and *RP9*. No known mutation was identified in Family 3; however, seven novel variations including *CRX* p.G122D and *NR2E3* p.R125Q were identified in the proband. We are investigating the biological significance of these sequence changes in these three families by studying additional family members. Potential modifiers may also be present in genes associated with arRP. RP may be an excellent disease model to elucidate phenotypic modifiers due to the large numbers of known genes involved in the specific pathway.

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## 436/W/Poster Board #94

**Mutation analysis of the ATP7B gene in patients with Wilson disease from northeast China.** *S. Li[1], M. Sun[1,2], J. Niu[2], W. Xu[1], L. Wang[2], F. Wang[2].* 1) Dept Ped/Gen, BSEB 224, Univ Oklahoma Hlth Sci Ctr, Oklahoma City, OK; 2) The Department of Infectious Diseases, the First Hospital of Jilin University, Changchun, Jilin, P.R. China.

Introduction The worldwide prevalence of Wilson disease is estimated to be 1 in 30,000-100,000, and it is more frequent in China. The majority of patients with Wilson disease have a mutation of the ATP7B gene; this causes a defect of copper excretion, leading to copper accumulation in the liver, brain and cornea. Since ATP7B was cloned in 1993, more than 379 variants have been reported to be causative of Wilson disease; variant p.R778L was the most common mutation among Chinese people of the Han Nationality. The frequency of this mutation in patients with Wilson disease was between 28.8-39.4%. The majority of published mutation studies were based on the investigation of common mutations, not whole coding sequences. Here we present our sequencing findings of the whole coding region of the ATP7b gene in Chinese patients with Wilson disease. To determine the mutation spectrum of the ATP7b gene and the frequency of each mutation identified in our Chinese patients from northeastern China, as well as a possible correlation between gene mutation and disease severity. Materials and Methods A total of 17 patients with Wilson disease were included in this study; four of these were sporadic cases. All of the patients involved are of the Chinese Han Nationality. After our consent form was approved by the Ethnic, Medical Affair and Research committee of the First Hospital of Jilin University, 5 cc of peripheral blood was drawn from each patient, and DNA was extracted from these samples based on standard laboratory protocols. DNA samples were amplified by PCR using primers corresponding to all 21 exons and they were subsequently sequenced. Results: Thirteen out of 17 patients had two disease-causing mutations; another three had only one mutation found, one patient had no mutation identified Also, we have identified three new mutations and two new polymorphisms (Figure 1). Conclusions: Our results suggest that heterogeneity of the mutation exists in our small patient population in northeast China. At the same time, we have noticed there might be a correlation between the p.R778L mutation and liver impairment, and p.R778L homozygotes are associated with an early onset of hepatic symptoms. Another interesting finding is the fact that affected patients within the same family who have the same mutation can have very different phenotypes. This suggests that there are other factors impacting the presentation of the autosomal recessive disorder.

## 437/W/Poster Board #95

**Dysfunction of protein synthesis mediated by mTOR-dependent signaling in Fragile X syndrome.** *C. Hoeffer[1], E. Klann[1], H. Wong[1], R.J. Hagerman[2,3], F. Tassone[2,4].* 1) Center of Neural Science, New York University, NY , USA; 2) M.I.N.D. Institute, University of California Davis Medical Center, Sacramento, CA, USA; 3) Department of Pediatrics, University of California, School of Medicine, Davis, CA, USA; 4) Department of Biochemistry and Molecular Medicine, University of California, School of Medicine, Davis, CA, USA.

Multiple studies have revealed the important role played by the mTOR (mammalian target of rapamycin) signaling pathway in learning and memory. All components of the mTOR pathway, which is involved in protein synthesis-dependent phase of synaptic strengthening, are present in dendrites suggesting a role for mTOR in local translation. mTOR drives local translation through phosphorylation of its downstream targets, including the eukaryotic initiation factor 4E-binding protein (4E-BP)s, which permits eIF4E to bind to eIF4G and be phosphorylated by Mnk1. The 70kD ribosomal protein S6 kinase (S6K1) and the eukaryotic elongation factor 2 (eEF2) are two additional mTOR substrates involved in translation control. These substrates in turn regulate translational initiation and rates of peptidyl elongation. Hypophorylated 4E-BPs inhibit translation of a number of mRNAs, mRNAs by sequestering eIF4E. Loss of eIF4E activity especially impacts mRNAs with high CGG content and complex 5'UTR structure via steric hinderance. The mRNA encoded by the Fragile X gene (FMR1) bears these features. FMR1 contains a CGG repeat element in the 5'UTR. Expansion above 200 CGG repeats leads to Fragile X syndrome (FXS) through hypermethylation of the promoter, silencing and consequent absence of the encoded protein, FMRP. FMRP is an RNA-binding protein, which plays an important role in translational repression. Thus, we have investigated whether altered mTOR signaling is present in subjects with FXS. Our preliminary findings indicate that translation control mediated by the mTOR pathway is compromised in FXS. Peripheral blood leukocytes of FXS subjects, who lack of FMRP, displayed phosphorylation of translation factors and kinases involved in translation control. Specifically, a significant increase in the phosphorylation of both S6K1 and eIF4E, which is consistent with elevated basal translation, was detected in FXS subjects. The observed increases in translational signaling suggest excessive basal translation in FMRP-deficient cells, and this activity may contribute to the cognitive and behavioral deficits observed in subjects with FXS. Thus, altered phosphorylation of mTOR substrates and their effectors could represent putative biological markers of cognitive impairment in FXS and the assessment of their levels in FXS lymphocytes could complement existing molecular testing.

## 438/W/Poster Board #96

**A novel missence ABCA12 mutations lead to nonbullous congenital ichthyosiform erythroderma (NBCIE).** *S. Nawaz[1], J. Klar[2], A. Azhar[1], M. Tariq[1], A. Ali[1], I. Ahmad[1], J. Qureshi[1], J. Schuster[2], J. Qureshi[1], S. Baig[1].* 1) Human Molecular Genetics Lab, National Inst for Biotech and Genet Eng (NIBGE), Faisalabad, Punjab, Pakistan; 2) Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden.

Lamellar ichthyosis was a broad term initially used for all nonbullous autosomal recessive ichthyoses other then harlequin ichthyosis and ichthyosis syndromes, but now a days nonbullous autosomal recessive ichthyoses have been divided into 2 major clinical groups , nonbullous congenital ichthyosiform erythroderma NBCIE and Lamellar Ichthyosis (LI) with a prevalence of lamellar ichthyosis of approximately 1 in 200,000 persons. A considerable number of cases, however, show an intermediate phenotype, and neither histopathologic findings nor ultra structural features clearly distinguish between NCIE and LI. Nonbullous congenital ichthyosiform erythroderma (NBCIE) is autosomal recessive congenital ichthyosis with prominent features of generalized severe white scaling all over the body and erythrodermic skin without blister formation . ABC12, causal gene here, localized on chromosome 2q33-35 belongs to a subfamily of ATP-binding cassette (ABC) transporters which implicate in some autosomal recessive disorders pertaining to lipid metabolism. Here we report a large consanguineous Pakistani family exhibiting autosomal recessive inheritance and classic phenotype of NBCIE. A novel homozygous missense 1241 G to V transition in exon 31 of ABCA12 gene in all affected members was identified which showed exclusion to 200 Pakistani and 200 Swedish controls.

## 439/W/Poster Board #97

**Functional characterization of chronic pancreatitis-associated variations in the 5' regulatory region of the *SPINK1* gene.** *A. Boulling[1,2,3], J.M. Chen[1,2,3], C. Ferec[1,2,3,4].* 1) Institut National de la Santé et de la Recherche Médicale (INSERM), U613, Brest, France; 2) Université de Bretagne Occidentale (UBO), Faculté de Médecine et des Sciences de la Santé, Brest, France; 3) Etablissement Français du Sang (EFS) - Bretagne, Brest, France; 4) Centre Hospitalier Universitaire de Brest, Hôpital Morvan, Laboratoire de Génétique Moléculaire et d'Histocompatibilité, Brest, France.

Introduction: The SPINK1 gene, which encodes the pancreatic secretory trypsin inhibitor, is one of the major genes predisposing to chronic pancreatitis. To date, a dozen of variations have been described in the 5' regulatory region (RR) of SPINK1 but their functional effects remain unknown. The aim of this study was to systematically characterize all these currently known 5' RR variants. Method: The wild-type 5' RR of SPINK1 was firstly cloned into the pGL3-Basic Luciferase Reporter Vector. All the 5' RR variations in the SPINK1 gene were then introduced into the pGL3-SPINK1 construct, respectively, by means of site-directed mutagenesis. SPINK1 promoter activities were determined in human pancreatic COLO 357 cells. Functional relevance of some variants was further evaluated by EMSA. Results: The 5' RR variations can be divided into three categories in terms of luciferase expression, which correlated well with clinical findings. The variants that caused a decreased expression often show consistent disease association among different studies whilst those that had no effect on expression often show equal allele distribution between patients and controls. The variants that caused an increased expression are, in fact, in cis with a known disease-causing mutation. EMSA assay demonstrated that variations located in well defined regulatory motifs affected protein-DNA interactions. Conclusion: This work was the first to assess the functional impact of the 5'-RR's SPINK1 variations. Our finding clarified the role of the diverse SPINK1 5' RR variants in the etiology of chronic pancreatitis and resulted in a better understanding of the genotype/phenotype relationship.

## 440/W/Poster Board #98

**Molecular aspect of carnitine palmitoyltransferase II deficiency.** *J.H. Ding, N.H. McNeill, B.Z. Yang.* Inst Metabolic Disease, Baylor Res Inst, Dallas, TX.

Carnitine palmitoyltransferase II (CPT II) deficiency is an autosomal recessive disorder of mitochondrial long-chain fatty acid oxidation. CPT II deficiency has three distinct clinical forms: the adult-onset form characterized by episodes of muscle pain; milder infantile form, and severe neonatal form which may result in sudden unexplained death. In this report, eleven patients with CPT II deficiency had been investigated to identify the molecular defect. All five CPT II exons and their flanking intronic sequences were amplified from proband's DNA. The PCR products were purified and sequenced. The sequencing analysis revealed that ten novel mutations were identified, including R290X and D390fs. The novel mutations were also verified by DNA amplification/enzyme digestion method, but were not detected in the normal control subjects. In our group, The CPT II activities in patients' lymphocytes have also been tested and summarized with mutations and clinical presentation. This study demonstrated the genetic heterogeneity that may underlie the clinical variation in CPT II patients.

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### 441/W/Poster Board #99

**The Spectrum of Mutational Genes and Alleles in Chinese OCA Patients.** *W. Li[1], A. Wei[2], Y. Wang[2], Y. Long[3], Y. Wang[1], X. Guo[1], Z. Zhou[1], X. Bian[3], S. Lian[2].* 1) Insttitute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China; 2) Department of Dermatology, Xuan Wu Hospital, Capital Medical University, Beijing, China; 3) Department of Obstetrics and Gynecology, Union Hospital, Peking Union College, Beijing, China.

Oculocutaneous albinism (OCA) is a heterogeneous Mendelian recessive disorder with hypopigmentation in the skin, hair, and eyes. At least sixteen different genes have been identified as causative genes for human OCA. OCA1 is the most common type of OCA in some populations. Several novel mutational alleles of TYR, P and MATP have been identified in Chinese population. However, no comprehensive analysis has been conducted to reveal the spectral distribution of Chinese OCA. We have screened unrelated and unselected 127 Chinese OCA patients for the mutations in TYR, P, TYRP1, MATP, HPS1 gene. We found that OCA1 is the most common form of OCA in Chinese population (70.1%), while OCA2 and OCA4 account for 10.2%, 12.6% respectively. Only one HPS1 patient is identified in our OCA repository. No apparent pathological mutation on TYRP1 has been found in these OCA patients. In this study, 41 novel mutational alleles have been reported which were not found in 100 non-albinism subjects. Potential mutational hotspots on TYR and MATP have been characterized in Chinese OCA patients. Genotype-phenotype relationship analysis revealed that clinical diagnosed OCA1 or OCA2 patients are mixed types of OCA1, OCA2, OCA4 and HPS1. These results provide useful information for the establishment of an optimized strategy of gene diagnosis and genetic counseling of Chinese OCA patients. In addition, to simplify the conversion of a mutation from cDNA to protein by following the common rules in mutation nomenclature, we developed a user-friendly tool, MutConv, which is freely accessed at http://liweilab.genetics.ac.cn/mutconv/.

### 442/W/Poster Board #100

**DNA and RNA analysis of COL6A genes in a cohort of 73 patients with collagen type VI related myopathies.** *F. Gualandi[1], E. Martoni[1], C. Trabanelli[1], M. Bovolenta[1], M. Neri[1], A. Venturoli[1], M. Fabris[1], S. Falzarano[1], A. Urciuolo[2], P. Sabatelli[3], E. Bertini[4], E. Mercuri[5], P. Bonaldo[2], L. Merlini[1], A. Ferlini[1].* 1) Dept Exp Diagn Med, Medical Genetics Section, Ferrara, Italy; 2) Dipartimento di Istologia, Microbiologia e Biotecnologie Mediche, Università di Padova; 3) IGM-CNR, Unità di Bologna c/o IOR; 4) Unità di Medicina Molecolare, Ospedale Bambino Gesù, Roma; 5) Istituto di Neurologia, Università Cattolica, Roma.

The collagen VI related myopathies include Bethlem (BM) and Ullrich (UCMD) muscular dystrophies, a pure limb girdle presentation (LGMD) and the Myosclerosis Myopathy. We have fully screened by genomic sequencing COL6A1-2-3 genes in a total of 73 patients with collagen VI related myopathies (36 UCMD, 35 BM, 1 myosclerosis, 1 LGMD). The causative mutation was identified in 55 patients, with a detection rate approximating 75%; for both UCMD and BM patients. Among UCMD genotypes, 74% consisted of de novo heterozygous mutations. We identified 3 BM patients (11.5%) compound heterozygous for recessive mutations, pioneering autosomal recessive inheritance for Bethlem myopathy. 60 different mutations were detected, the large majority (52/60) representing previously unreported changes. The prevalent mutation type is represented by missense changes (66.6%), followed by mutations affecting splicing (20%), in frame deletions (5%), nonsense mutations (5%) and ins/dup causing frameshifting (1.7%). The identified mutations are distributed preferentially within N-Terminal and triple helix (TH) domains of COL6A1 and COL6A3 genes. In the COL6A2 gene, the mutations are clustered in the TH and C-Terminal domains. Among a total of 12 mutations affecting splicing, 9 were outside the invariant GT-AG dinucleotides. These non-canonical splicing mutations affect fairly degenerated sequences and the distinction between SNPs and pathogenic mutations is often challenging. RNA analysis was performed in all cases revealing unpredictable splicing phenotypes with single and multiple exons skipping, intron retention and cryptic splice site activation/usage. Non sense mediated decay of aberrant transcript turned out to represent a frequent event, often hampering the detection of mutated transcripts. A quantitative RNA assay revealed a reduced level of the in-frame mRNA originating from a COL6A2 mutation at intronic position +3. The transcription level of the in-frame mRNA originating from a genomic deletion removing the splicing sequences of COL6A1 exon 8 was normal. These findings support a different transcriptional efficiency of a regulatory splicing mutation compared to a genomic deletion causing a splicing defect and point to the reciprocal coupling between transcription and splicing as a possible modifier of pathogenic consequences of splicing mutations in collagen VI related myopathies. This study was supported by the Italian Telethon Foundation grant GUP07004 (to FG).

### 443/W/Poster Board #101

**Severe Factor V Deficiency caused by a Novel Frameshift Mutation, 5493insA.** *M.H. Al-Hamed, M. Al-Mansoori, H. Al-Saud, A. Al-Musa, B.F Meyer.* Genetics Department, King Faisal Specialist Hospital and Research Centr, Riyadh, Saudi Arabia.

Factor V deficiency is a rare autosomal recessive coagulation disorder (OMIM 227400) with an incidence of about 1 in 106, affecting both sexes equally and diagnosed in multiple ethnic groups. The factor V gene (F5) encodes a 330 kd single chain glycoprotein consisting of three homologous A-type domains, a heavily glycosylated B-domain and two smaller homologous C-type domains. We report on a family in which the proband had severe bleeding and a subnormal FV antigen level. The proband, a Saudi female whose parents were first cousins, presented at age two weeks with convulsions and a coagulopathy with a highly prolonged PT (71.4 seconds; control= 13.8 seconds) and APTT (150 seconds; control= 29.5-42.7 seconds). Plasma FVIII, vWF antigen and activity levels were within the reference (control) range. Factor V antigen was <0.01U/L in the proband's plasma. Computerized tomography scan (CT) and magnetic resonance imaging (MRI) revealed a massive intracranial haemorrhage (ICH) with no vascular malformations. She had three attacks of ICH within a 3-week interval all presenting with convulsions and coagulopathy. Sequencing of F5 in the proband identified a novel homoallelic single base insertion in exon 16 at nucleotide position 5493 (5493insA) predicting a premature stop codon at position 1776. Screening of members of the family for the mutation showed that both parents and the probands twin brother were heterozygous for the 5493insA mutation with both sisters homozygous for the wild type allele The premature stop at position 1776 predicts a 1775 residue protein lacking a significant part of the FV light chain and a molecular weight of approximately 200 kd. Presence of the heavily glycosylated B-chain in the truncated protein is consistent with FV antigen levels in the patient's plasma being severely reduced but detectable.

### 444/W/Poster Board #102

**Manifesting and asymptomatic females in families with OTC deficiency. Does X-inactivation status in blood cells correspond to phenotype?** *L. Dvorakova[1], M. Hrebicek[1], L. Stolnaja[1], J. Minks[1], E. Hruba[1], M. Bouckova[1], H. Treslova[1], H. Vlaskova[1], V. Stranecky[1], R. Ivanek[1], S. Kmoch[1], O. Luksan[2], M. Jirsa[2].* 1) Inst. Inherited Metabolic Disorders, 1st Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Czech Republic; 2) Laboratory of Experimental Hepatology, Inst.Clinical and Experimental Medicine, Prague, Czech Republic.

Ornithine carbamoyltransferase deficiency (OTCD;OMIM 311250) is the most common inherited defect of the urea cycle. Mutation analysis in OTC gene (Xp21.1) revealed a causative mutation in 27 patients from 24 families from Czech Republic and Slovakia (22), Japan (1) and Hungary (1). Patients from 8 and 10 families manifested neonatal and late onset form, respectively, 6 probands were heterozygous females. There were other 3 symptomatic heterozygotes among OTCD family members, while the other identified carriers were asymptomatic. Manifesting heterozygotes carried 4 missense, 3 nonsense and one splicing mutation and one gross deletion. Mutation analysis was based on direct sequencing, MLPA assay and DNA array analysis (Affymetrix SNP 6.0). We analyzed skewing of X-inactivation in OTCD heterozygotes by HUMARA assay in peripheral blood cells (PBC), although OTC is expressed almost exclusively in liver. We presume, that PBC can be used as the results of X-inactivation study performed in 21 necroptic tissues including liver and PBC from 5 individuals showed no substantial organ differences. To evaluate the results we classified the severity of mutations: severe (SM) - obviously deleterious mutations and missense mutations causing neonatal form of OTCD; mild mutations (MM) causing late onset form. Of the 5 informative symptomatic heterozygotes 4 carried SM with either random inactivation or preferential inactivation of WT allele and one was heterozygous for a MM with the X inactivation skewed in favor of the mutant allele. In 7 of the 12 asymptomatic carriers SMs were found. Six of them had substantial skewing (>75:25) favoring the wild type allele; the remaining carrier had random inactivation - this patient preferred diet with lower protein intake. Of the 5 heterozygotes with MM three had random inactivation patterns and in two the inactivation was skewed in favor of WT allele. Although the cohort is small, the results suggest that patients carrying SM do not develop symptoms only if the mutant allele is preferentially inactivated (with possible modification of the phenotype by dietary habits). Patients heterozygous for mild mutations tend to be asymptomatic with random inactivation and the symptoms may develop only if the WT allele is preferentially inactivated. More research is needed to better understand if this hypothesis is valid and can be included in phenotype prediction. Support: IGA MZ CR NR/9364-3, VZ MSM CR 0021620806 and VZ MZ CR 64165.

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## 445/W/Poster Board #103

**Molecular Analysis of AGPS in two probands with RCDP type 3 reveals two novel alleles.** *B.J. Itzkovitz[1], S. Jiralerspong[1], M. Potti[3], S. Steinberg[2], N. Braverman[1,3].* 1) Human Genetics,Mcgill University, Montreal , Quebec, Canada; 2) Neurogenetics, Kenedy Krieger Institute, Baltimore, MD USA; 3) Institute of Genetic Medicine, John Hopkins Medical Center, Baltimore, MD, USA.

Rhizomelic chondrodysplasia punctata (RCDP) type 3 is caused by a deficiency of alkylglycerone phosphate synthase (AGPS), the second enzyme in the committing steps of plasmalogen synthesis in the peroxisome. It physically interacts with the first enzyme of this pathway, glycerone-phosphate acyltransferase (GNPAT), deficient in RCDP type 2. RCDP types 2 and 3 are responsible for ~5% of RCDP. Thus far only 2 probands with RCDP3 have been reported. We report here the biochemical and molecular analysis of 2 additional probands. Fibroblasts from patient 1 had mean GNPAT activity of 2.2 nmol/2h/mg protein, or 61% of the simultaneous normal control (3.6). Plasmalogen synthesis was 1.5 (normal values are <1). RBC plasmalogen level was 0.028 for C16:0 DMA/C16:0 (normal .051-.090). Patient 2 had mean GNPAT activity of 1.8, or 56% of the normal control; plasmalogen synthesis was 18, and RBC plasmalogen level was 0.003. These values show that there is a similar reduction in GNPAT activity in both patients, but plasmalogen synthesis is more severely impaired in patient 2, compared to patient 1. PCR amplification of all exons and intronic flanking regions for the AGPS gene was performed according to our protocol, and directly sequenced. AGPS encodes a 3.3 kb mRNA and a 658 amino acid protein. Results in patients 1 and 2 showed presumed homozygous point mutations causing E471K and T568M substitutions, respectively. The parents of patient 1 are consanguineous. No other changes were identified in the coding regions and UTR. Immunoblotting of whole cell lysates from primary patient fibroblasts indicated patient 1 had complete absence of detectable AGPS protein, whereas patient 2 had near normal levels of mature protein. Homology analysis shows that the amino acids at the mutation site are evolutionarily conserved, and both are located in a putative FAD-linked oxidase domain. The absence of detectable AGPS protein in the E471K mutant suggests a destabilizing effect leading to protein degradation, possibly due to disruption of ionic interactions in the protein structure. These results, along with the measured reduction in GNPAT activity, confirm previous studies suggesting that AGPS protein is necessary to stabilize GNPAT in the peroxisome. Unexpectedly high levels of plasmalogen synthesis and comparable GNPAT activity in the E471K vs. T568M mutants suggest that the E471K protein has residual function, the cause of which is currently under investigation.

## 446/W/Poster Board #104

**Clinical and genetic heterogeneity in a Swedish family with Retinal Degeneration.** *F. Jonsson[1], O. Sandgren[2], A. Hollander[3], I. Golovleva[1].* 1) Dept Medical Biosciences/Medical and Clinical Genetics, Univ Hosp Umea, Umea, Sweden; 2) Dept Clinical Sciences/Ophthalmology, Univ Hosp Umea, Sweden; 3) Human Genetics, Nijmegen Center for Molecular Life Sciences, Nijmegen, Netherlands.

Retinal degenerations represent a heterogeneous group of disorders affecting the function of retina. The frequency of retinitis pigmentosa (RP) is 1/3500 worldwide, however, in northern Sweden it is 1/2000 due to limited migration and "founder" effect. Using large families of Swedish origin we identified underlying genetic mechanisms in a local form of RP (RLBP1 gene), cone dystrophy (PITPNM3 gene) and an autosomal dominant form of RP with reduced penetrance (PRPF31 gene). In this study we aimed to undercover genetic mechanisms underlying recessive form of retinal degeneration in one large family from northern Sweden. Patients admitted to the University Hospital were examined clinically and family history was collected. Genotyping of the DNA from three affected siblings done with SNP-array (Affymetrix) revealed homozygosity region on 1q31-q32.1 harbouring a gene known as a cause of a severe form of autosomal recessive RP and Leber Congenital Amaurosis (LCA). The gene, CRB1, 'crumbs' of Drosophila melanogaster is known to be involved in cell-cell interaction affecting of cell polarity of photoreceptors. Mutation analysis of the CRB1 gene done by sequencing resulted in a novel mutation c.2557C>T (p.Gln853X). Family testing showed that four individuals with clinical appearance of LCA were homozygous while two other carried only wild type alleles. One of these patients tested for 848 known mutations in 29 genes performed by arrayed primer extension (APEX) technology revealed presence of a mutation in the ATP-binding cassette transporter - retinal gene (ABCA4 IVS10-T>C) also known as a cause of autosomal recessive RP and Stargardt disease. Further analysis of the family members showed absence of the ABCA4 IVS10-T>C in carriers of the CRB1 c.2557C>T mutation, presence of biallelic mutation in one patient and one affected allele in another patient. Carriers of the ABCA4 IVS10-T>C have a distinct phenotype which was different from the phenotype of the CRB1 c.2557C>T mutation despite they belong to the same family. In conclusion, we identified a novel mutation in the CRB1 gene and demonstrated that in the same family two different genetic mechanisms causing different types of retinal degenerations can co-exist. Clinical and genetic heterogeneity can result in difficulties for both clinical geneticists and ophthalmologists.

## 447/W/Poster Board #105

**Serum cholinesterase activity: a phenotype-genotype study.** *M.L. Parnas[1], M. Procter[2], M.A. Schwarz[2], R. Mao[1,2], D.G. Grenache[1,2].* 1) Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT; 2) ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT.

Pseudocholinesterase (PChE), or serum cholinesterase, is one of two enzymes that hydrolyze choline esters. Encoded by the *BCHE* gene and synthesized in the liver, the function of PChE remains unknown although it is implicated in the metabolism of the neuromuscular blocking agent succinylcholine. Prolonged blockade and apnea with succinylcholine occurs in individuals with reduced PChE activity due to genetically determined variants. Phenotypic identification of variants is achieved by measuring PChE activity in the presence of the inhibitor dibucaine, which, together with total activity, is used to calculate a dibucaine number (DN). Assignment of PChE phenotypes requires well-defined, phenotype-specific PChE and DN reference intervals. Method-specific reference intervals are not available due to the number of biochemical methods for determining PChE activity and the lack of assay standardization. The goal of this study was to perform genotype-phenotype correlations of serum PChE to validate existing reference intervals and/or establish more appropriate intervals. Cell free DNA was extracted from 44 serum specimens for which total enzyme activity, DNs, and phenotypes were known. Phenotypes were assigned based on our laboratory criteria. PCR of the *BCHE* gene coding region was performed, followed by bidirectional DNA sequencing using BigDye® terminator chemistry. Sequencing data analysis was achieved using Mutation Surveyor® software. Phenotype-genotype agreement occurred in 36% of specimens. Of these, 38% were wild-type (UU) and 62% were heterozygous for a common PChE variant (UA). For 32% of specimens a PChE phenotype was not assigned due to inconsistencies between the PChE biochemistry and our interpretive criteria. Of these, 50% were genotypically UU or UA. Discordance between phenotype and genotype occurred in 32% of specimens, for which the inaccurately assigned phenotype either did not change the likelihood of succinylcholine susceptibility or implied a slightly increased risk when there was none. One discordant specimen was phenotyped as AS (high risk) and sequencing revealed a recently reported mutation that imparts low PChE activity. These data highlight the inability to assign an accurate phenotype based solely on PChE activity and the DN. Method-specific biochemical reference intervals based on PChE genotype are necessary. PChE gene sequencing combined with biochemical testing could provide improved and accurate assessment of patient risk.

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## 448/W/Poster Board #106

**Assessment of the Pathogenic Contribution of Individual Recessive Disease Alleles in *ABCA4*-Associated Retinal Degeneration.** *E.I. Schindler[1,2,3], A.C. Ko[3], A.V. Drack[3], A.V. Cideciyan[4], T.S. Aleman[4], S.G. Jacobson[4], R.G. Weleber[5], G.A. Fishman[6], V.C. Sheffield[1,7,8], E.M. Stone[1,3,8].* 1) Genetics Program, University of Iowa, Iowa City, IA; 2) Medical Scientist Training Program, University of Iowa, Iowa City, IA; 3) Department of Ophthalmology and Visual Sciences, University of Iowa, IA; 4) Department Ophthalmology, Scheie Eye Institute, University of Pennsylvania, Philadelphia, PA; 5) Macular Degeneration Center, Casey Eye Institute, Oregon Health and Science University, Portland, OR; 6) Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, IL; 7) Department of Pediatrics, University of Iowa, Iowa City, IA; 8) Howard Hughes Medical Institute, Iowa City, IA.

**Purpose:** Numerous studies have shown extensive allelic heterogeneity in *ABCA4*-related eye disease. Most individuals with disease-causing variations in this gene are compound heterozygotes and many of the variations they harbor are quite uncommon. For these reasons it has been difficult to establish genotype-phenotype correlations for *ABCA4* disease. However, if one has access to a sufficiently large number of subjects who share one disease allele, the phenotypic differences observed among the members of the group can be used to estimate the pathogenic contribution of their second disease alleles. **Methods:** An inexpensive high-throughput allele-specific assay was designed for the detection of 19 of the most common disease-causing alleles in the *ABCA4* gene. This assay was used to screen 351 patients with the clinical features of Stargardt disease, 238 with cone-rod dystrophy, 237 with retinitis pigmentosa (RP) and 253 controls. The clinical data from patients found to harbor two *ABCA4* mutations were then used to devise an empiric quantitative model of the relative pathogenic contributions of these 19 variations. An additional group of patients were found to harbor two disease-causing variations in *ABCA4* by using a combination of SSCP and automated DNA sequencing. These individuals were used to increase the resolution of the model as well as to estimate the pathogenicity of some more rare disease alleles. **Results:** Two of the most common disease-causing alleles (G1961E and G863A) were found to be the least pathogenic. These alleles rarely cause disease in the homozygous state or in the compound heterozygous state with each other. The four most virulent alleles (R152X, C1490Y, C1488R, and IVS14-1 G>A) were each found more commonly in cone-rod dystrophy or RP patients than in patients with Stargardt disease. **Conclusion:** The differences in disease severity among a group of patients who share one allele can be assigned, at least in relative terms, to their second allele. As the number of clinically well-characterized patients with two detectable alleles increases, the precision with which one can predict the pathogenicity of specific genotypes will also increase. Such predictions will be useful for a variety of purposes ranging from genetic counseling to the design and execution of clinical trials.

## 449/W/Poster Board #107

**Niemann-Pick type C1 patients carrying apparently only one mutant allele: Are there dominant negative variants?** *H. Vlaskova, M. Bouckova, L. Dvorakova, A. Hnizda, J. Sikora, M. Hrebicek, M. Elleder.* Institute of Inherited Metabolic Disorders, 1st Faculty of Medicine, Charles University and University Hospital, Prague, Czech Republic.

Niemann-Pick disease type C (NPC, OMIM 257220, 607625) is a severe autosomal recessive neuro-visceral disorder characterized by progressive neurological deterioration and hepatosplenomegaly. A defect in transport of unesterified cholesterol leads to its accumulation in lysosomes/late endosomes. The patients carry mutations either in NPC1 (18q11) or NPC2 (14q24.3). The molecular defect corresponds to two complementation groups NPC1 and NPC2, identified by biochemical assays. Both genes encode two late endosomal/lysosomal functionally linked proteins. We present the results of mutation analysis performed by sequencing of genomic PCR products in 42 probands from Czech and Slovak republic, Poland, Germany and Brasil. We have detected both mutant alleles in 39 patients and identified 43 different NPC1 mutations, 23 of them being novel. In 3 patients only one mutation was detected. One of these patients with severe clinical signs of NPC had classical biochemical phenotype of complementation group NPC1 (dr. M. T. Vanier, Lyon) and inherited mutation p.R1186H from her asymptomatic father; the maternal mutation was not identified. The patient was heterozygous for three common polymorphisms, two of them were exonic (p.Y129Y, p.I642M). The exonic polymorphisms as well heterozygous mutation p.R1186H were detected by direct sequencing of RT/PCR products. The presence of both alleles in the transcript analysis excluded splicing errors or deletions that could be missed by analysis of genomic DNA. This result also makes improbable the possibility that the level of transcription is affected. Although the complementation study indicated NPC1 group, we have also analysed NPC2 gene. The only change found by analysis of genomic DNA and cDNA was a novel polymorhism p.H150H. The presence of only one mutated allele in patients in whom NPC was proved by biochemical methods was reported by several authors. This raises the question if some of the mutations may not function as dominant-negative variants in this recessive disorder. Our data do not support this hypothesis as asymptomatic father of the patient carries the same mutation. Our data also do not support the possibility that polymorphisms p.Y129Y, p.I642M could modify the phenotype. Therefore, we suggest that some other process affecting NPC1 protein (e.g. translation defect) or defect in other gene may be in play. Support: VZ MSM CR 0021620806, VZ MZ CR 64165.

## 450/W/Poster Board #108

**A rare BCHE deficient allele, R386C, confirmed by phenotype-genotype correlation case study.** *J.E. Whittington[1], H.D. Pham[2], M. Procter[3], D.G. Grenache[1,3], R. Mao[1,3].* 1) Department of Pathology, University of Utah, Salt Lake City, UT; 2) Department of Anesthesiology, University of Minnesota, Minneapolis, MN; 3) ARUP Institute for Clinical and Experimental Pathology and ARUP Laboratories, Salt Lake City, UT.

*Background:* Pseudocholinesterase (PChE), or butyrylcholinesterase (BChE), catalyzes the hydrolysis of choline esters. Encoded by the *BCHE* gene and synthesized in the liver, PChE's function is unknown although it metabolizes the neuromuscular blocking agent succinylcholine. Extended blockade and apnea with succinylcholine occurs in individuals with reduced PChE activity due to genetically determined enzyme variants. Phenotypic identification of variants is achieved by measuring PChE activity in the presence of the inhibitor dibucaine, which, together with total activity, is used to calculate a dibucaine number (DN). Assignment of PChE phenotypes requires well-defined, phenotype-specific PChE and DN reference intervals which are lacking due to numerous biochemical methods for determining PChE activity and lack of assay standardization. *BCHE* sequencing can be used to infer a phenotype from the genotype. *Patient & Methods:* An adult Asian male underwent an emergent laparoscopic appendectomy. Intubation was facilitated with 1 mg defasciculating dose of vecuronium followed by 120 mg of succinylcholine. 1.7 hours later the patient remained paralyzed with no muscle twitches, small spontaneous inspiratory efforts, and no protective airway reflexes. Complete recovery was achieved 4 hours after succinylcholine administration. A serum specimen for PChE testing was collected 14 days later. The PChE activity and DN were determined using acetylthiocholine substrate at 37°C on a Roche cobas c501 analyzer and a PChE phenotype assigned based on these data. DNA was extracted from the serum by MagNAPure followed by bidirectional sequencing of the entire coding region and intron/exon boundaries to determine the *BCHE* genotype. *Results:* PChE activity was 89 U/L and the DN was <5 [reference intervals: 2900-7100 and 83-88, respectively]; consistent with a silent PChE phenotype. BCHE sequencing identified a unique apparent homozygous mutation: 1240 C>T (p.R414C, known as R386C) in exon 2. This rare mutation has been previously reported in a heterozygote with unknown clinical significance (Yen et. al, *Clin Chem* 2003; 49:1297). *Conclusions:* Genotype R386C is associated with a silent PChE phenotype and extended succinylcholine-induced paralysis. A complete family pedigree would allow further definition of the assigned mutation phenotype-genotype correlation. This case highlights the clinical significance of mutations in the *BCHE* gene and emphasizes the need for screening.

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## 451/W/Poster Board #109

**Systematic Classification of the Human Mitochondrial Phenome.** *C. Scharfe[1], H.H.S. Lu[2], J.K. Neuenburg[3], E.A. Allen[1], G.C. Li[4], T. Klopstock[5], T.M. Cowan[6], G.M. Enns[7], R.W. Davis[1].* 1) Genome Technology Ctr, Stanford Univ, Palo Alto, CA; 2) Institute of Statistics, National Chiao Tung University, Hsinchu, Taiwan; 3) BioMarin Pharmaceutical Inc. Novato, CA; 4) Department of Operations Research, University of California, Berkeley, CA; 5) Friedrich-Baur-Institute, Department of Neurology, Ludwig Maximilians University, Munich, Germany; 6) Departments of Pathology and Pediatrics, Stanford University, Stanford, CA; 7) Department of Pediatrics, Medical Genetics Division, Stanford University, Stanford, CA.

Mitochondrial diseases are caused by an abnormal function of mitochondria. They may be the result of spontaneous or inherited mutations in the mitochondrial genome or in nuclear genes that code for mitochondrial components, but may also be acquired secondary to adverse effects of drugs, infections, or other environmental causes. Even with years of experience, recognizing and diagnosing mitochondrial diseases is still a major hurdle in clinical medicine. Computational tools supporting clinicians not only help identify affected individuals, but also guide studies of the genetic and biological causes of these disorders. In the Mitochondria Phenome knowledgebase (www.mitophenome.org), we established a clinical phenotype catalog of 174 mitochondrial disease genes and study associations of diseases and genes. Phenotypic features such as clinical signs and symptoms were manually annotated from full-text medical articles and classified based on the hierarchical MeSH ontology. This classification of phenotypic features of each gene allowed for the comparison of diseases between different genes. In turn, we were then able to measure the phenotypic associations of disease genes for which we calculated a quantitative value that is based on their shared phenotypic features. The results showed that genes sharing more similar phenotypes have a stronger tendency for functional interactions, proving the usefulness of phenotype similarity values in disease gene network analysis. Our study of a large functional network of mitochondrial genes revealed distinct properties that differentiate disease and non-disease genes. Disease genes showed a lower average total connectivity but a tendency to interact with each other; a finding that we used to predict 168 high-probability disease candidates. Mitophenome allows for easy navigation between clinical phenotype and gene information and promises to aid in the recognition and diagnosis of mitochondrial disorders.

## 452/W/Poster Board #110

**Mutation analysis in patients with GSD III and correlation with clinical subtype.** *D. Bali[1], J. Goldstein[1], S. Austin[1], K. Boyette[1], A. Kanaly[2], C. Rehder[2], P. Kishnani[1].* 1) Division of Medical Genetics, Dept. of Pediatrics, Duke University Medical Center, Durham, NC; 2) Clinical Molecular Diagnostics Laboratory, Duke University Medical Center, Durham, NC.

Glycogen storage disease type III (GSD III; limit dextrinosis; Cori or Forbes disease) is an autosomal recessive disorder of glycogen metabolism caused by deficient activity of glycogen debranching enzyme (GDE). Patients with GDE deficiency in liver and muscle have GSD IIIa (85%), while those with only liver deficiency have GSD IIIb (15%). Clinical symptoms include hepatomegaly, hypoglycemia, short stature and, in patients with GSD IIIa, myopathy and cardiomyopathy. Both GSD IIIa and IIIb are caused by mutations in a single gene, amylo-1,6-glucosidase (AGL), which encodes GDE. The molecular basis behind differences in tissue-specific expression of GDE activity in patients with GSD IIIa and IIIb is not yet clearly understood. However, previous studies have shown that two mutations in exon 3, c.17_18delAG (p.Q6HfsX25) and c.16C>T (p.Q6X), are associated specifically with GSD IIIb. The large size of the AGL gene (35 exons) and the absence of common mutations, except those associated with GSD IIIb, present a diagnostic challenge. The goal of this study was to investigate clinical, biochemical and molecular characteristics of GSD III patients in order to examine genotype-phenotype correlation. We have sequenced the AGL gene in 21 patients with enzymatically confirmed GSD III. Eighteen patients had GSD IIIa and 3 had GSD IIIb. Two known or possible pathogenic mutations were found in 18 patients; 2 patients had 3 possible deleterious variants each, and a single mutation was found in another patient. In total, 26 different sequence changes were identified, 11 of which were previously cited. In patients with GSD IIIa, most mutations were nonsense (13 alleles), and splice site (11 alleles); whereas frameshift (5 alleles), missense (4 alleles), exon 3 deletion (2 alleles), and a duplication (1 allele) were seen less often. Each of the three GSD IIIb patients had one common exon 3 mutation (c.17_18delAG in two patients and p.Q6X in one), and other mutations elsewhere in the gene. Two mutations (p.W680X and p.R864X) were identified in patients with both GSD IIIa and IIIb. In conclusion, GSD III is a highly heterogeneous condition. Our results suggest that the GSD IIIb subtype can be predicted if the patient carries either the c.17_18delAG or p.Q6X mutation, however other mutations do not seem to be predictive of subtype or isolated to a specific part of the gene.

## 453/W/Poster Board #111

**Homozygous mutations in the 5' region of the *JUP* gene result in cutaneous disease but normal heart development.** *R. Cabral[1], L. Liu[8], C. Hogan[9], P.J.C. Dopping-Hepenstal[8], B.C. Winik[2], R.A. Asial[3], R. Dobson[4], C. Mein[4], P. Baselaga[5], J.E. Mellerio[7], A. Nanda[10], M.delC. Boente[6], D.P. Kelsell[1], J.A. McGrath[7], A.P. South[1,9].* 1) Centre for Cutaneous Research, Institute of Cell and Molecular Science, Barts and The London, Queen Mary's School of Medicine and Dentistry, University of London, London, UK; 2) Servicio de Microscopía Electrónica, INSIBIO, Universidad Nacional de Tucumán - CONICET, Tucumín, Argentina; 3) Facultad de Medicina, Universidad Nacional de Tucumán, Tucumán, Argentina; 4) Genome Centre, Barts and The London, Queen Mary's School of Medicine and Dentistry, University of London, London, UK; 5) Department of Cardiology, Hospital del Niño Jesús, Tucumán, Argentina; 6) Department of Dermatology, Hospital del Niño Jesús, Tucumán, Argentina; 7) Genetic Skin Disease Group, St John's Institute of Dermatology, The Guy's, King's College and St Thomas' School of Medicine, London, UK; 8) National Diagnostic Epidermolysis Bullosa Laboratory, St John's Institute of Dermatology, The Guy's, King's College and St Thomas' School of Medicine, London, UK; 9) Surgery & Molecular Oncology, University of Dundee, Ninewells Hospital & Medical School, Dundee, UK; 10) Asad Al-Hamad Dermatology Center, Salmiya, Kuwait.

Desmosomes are specialized cell junctions which mediate strong intercellular adhesion and connect intracellularly to the intermediate filament (IF) cytoskeleton. Desmosomes are prominent in the epidermis and heart, which are tissues that constantly experience physical stress. The constitutive desmosomal protein plakoglobin (PG) is involved in linking transmembrane desmosomal components to IFs. PG also contributes to intercellular adhesion through adherens junctions and has additional signaling roles. To date, two mutations have been found in the gene encoding PG (*JUP*), and in each case patients suffer from arrhythmogenic right ventricular cardiomyopathy (ARVC). Recently, reduced or absent PG expression in heart biopsy has been suggested as an ARVC diagnostic test. We have investigated the genetic basis of disease in three unrelated Argentinean patients and two patients from a Kuwaiti family. All patients had the distinct cutaneous phenotype of skin fragility, diffuse palmoplantar keratoderma and woolly hair (notably sparse in Kuwaiti patients) with no symptoms of cardiomyopathy. We describe two novel homozygous mutations in the 5' region of *JUP*; a nonsense mutation, p.S24X, in all three Argentinean patients and a splice site mutation, c.468G>A, in both Kuwaiti patients. By immunohistochemistry with three different PG antibodies, we show virtually absent PG expression in the skin of all patients. c.468G>A is predicted to abolish the donor splice site in exon three. RT-PCR and cDNA sequencing from p.S24X skin reveals normal 5' splicing of *JUP* and no loss of mutant transcript. Western blotting (WB) of skin proteins with a PG C-terminal antibody reveals low levels of a truncated protein in p.S24X patients and heterozygous carriers of the mutant allele. We demonstrate by WB and quantitative PCR that recombinant p.S24X PG is efficiently translated in dermal fibroblasts. These results show that p.S24X mRNA is expressed at similar levels to wild type mRNA and that an alternative translation start site, using an in-frame internal AUG codon downstream of the mutation, results in the expression of an N-terminal truncated protein. PG is required for correct maintenance of skin integrity and the absence of heart phenotype in patients suggests that aberrant PG expression does not compromise normal human heart development in children. Our findings provide new insight into the distinct roles PG plays in the epidermis and heart.

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## 454/W/Poster Board #112

**Evaluation of the RP1 gene in Chinese Patients with Retinitis Pigmentosa.** *L.J. Chen, P.O.S. Tam, X. Zhang, S.W.Y. Chiang, T.Y.Y. Lai, C.P. Pang.* Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong S.A.R.

Purpose: RP1 is a major gene responsible for both autosomal dominant (ADRP) and autosomal recessive retinitis pigmentosa (ARRP). In this study, we evaluated the contribution of RP1 gene mutations in Chinese patients with retinitis pigmentosa (RP). Methods: Genomic DNA of 56 RP patients were analyzed and the coding sequences and the exon-intron boundaries of RP1 were screened by direct sequencing. The amplicons containing the mutation candidates were resequenced in 190 normal controls. Pedigree analysis was performed after the identification of the nonsense mutations in a proband with ARRP. Computer programs were applied in silico for mutational analyses of the missense variants. Results: Sixteen sequence changes were identified, in which seven were novel and absent in controls. Two nonsense mutations (c.5_6delGT, c.4941_4942insT) were found to coexist in a proband with ARRP. In the patient's family, members compound heterozygous for the two nonsense mutations have early-onset and severe RP, while those with only one mutation did not develop RP. Two missense variants (K1370E and R1652L), which were found in patients with simplex RP, were predicted to have functional impact on the RP1 protein, being candidate disease-causing mutations. The other three variants (IVS3+34T>C, I408L and L2015L) were considered benign. Conclusions: In this study, we had for the first time reported ARRP associated with compound heterozygous nonsense mutations in RP1. Identification of the nonsense-mediated mRNA decay (NMD) sensitive mutation c.5_6delGT provided genetic evidence that haploinsufficiency of RP1 is not responsible for RP. We had also identified novel RP1 mutations likely to play a role in the genetics of simplex RP. Our results also indicate high mutability of the RP1 gene in the Chinese RP population.

## 455/W/Poster Board #113

**Evaluation of the NR2E3 and NRL genes in Chinese Patients with Retinitis Pigmentosa.** *P.O.S. Tam, X. Zhang, Y.P. Yang, L.J. Chen, S.W.Y. Chiang, T.Y.Y. Lai, C.P. Pang.* Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong S.A.R.

Purpose: Disease-causing mutations in the NR2E3 and NRL genes have been implicated in both autosomal dominant and autosomal recessive retinitis pigmentosa (RP) in non-Chinese populations. In this present study, the mutation profiles of these two genes were investigated in Chinese RP patients. Methods: 172 RP patients with varied inheritance patterns and 180 normal controls from Hong Kong, and an addition of 180 control subjects from Beijing were recruited. All received complete ophthalmic examinations. The coding exons and the exon-intron boundaries of NR2E3 and NRL were screened in the Hong Kong subjects by direct DNA sequencing. Any possible mutations detected were additionally screened in the control subjects from Beijing. Association analysis was performed for common SNPs while analysis of variants was performed for rare missense variants using in silico programs. Results: In NR2E3, a total of 20 sequence changes had been identified, with 14 being novel. Eight variants (IVS1-47C>T, IVS1-28T>C, IVS1-28_IVS1-13del, IVS2+75G>A, IVS3+8G>A, IVS8-87C>T, IVS8-47G>A, and c.1230+53C>T) were non-coding, four (I61I, L277L, P281P and C321C) were synonymous, and eight (G56R, V118M, E121K, E140G, M163T, T300M, V302I and P374L) were missense changes. Two missense variants, G56R (reported) and V118M (novel), were exclusively found in RP patients. Analysis of variants showed that these two missense variants might have functional impacts on the NR2E3 protein, suggesting a role of disease-causing, being responsible for 1.16% (2/172) and 1.74% (3/172) of patients, respectively. Notably, the E121K variant, which has been reported to be responsible for Enhanced S-cone Syndrome (ESCS), was found simultaneously in RP patients (13.4%) and control subjects from Hong Kong (10.0%) and Beijing (12.2%), being not associated with the disease. The rest missense changes were not significantly associated with RP. In NRL, seven sequence changes were identified, all were novel. Among them, four (W32S, P35P, T36T, S38P) and three (Y42Y, R233R, c.853C>T) were exclusively found in controls and RP patients, respectively. Conclusions: In this study, NR2E3 mutations (G56R, V118M) were found to be responsible for approximately 2.9% of overall RP. The ESCS-causative variant E121K was a common SNP in Chinese, suggesting an ethnic difference in the role of this variant on the pathogenesis of retinal degeneration. By contrast, none of the NRL variants was likely to be disease-causing.

## 456/W/Poster Board #114

**FAMILIAL EXUDATIVE VITREORETINOPATHY (FEVR): MOLECULAR AND CLINICAL-GENETIC CORRELATION IN A COLOMBIAN FAMILY.** *M. Tamayo[1], J.H. Montoya[1], N. Gelvez[1], C.L. Varón[2].* 1) Dept Genetics, Inst de Genetica Humana, Bogota, Colombia; 2) Fundación Oftalmológica de Santander, Clínica Ardila Lulle (FOSCAL). Bucaramanga, Santander. Colombia.

Introduction: The familiar exudative vitreoretinopaty, also called Criswick-Schepens disease, is caused by a genetic alteration producing a bilateral, asimetric and progressive ocular damage; this disorder has specially an autosomal dominant inheritance. OBJECTIVE: Genotype-phenotype correlation previous clinical analysis and molecular studies of the FZD4 gene to identify the causal mutation, in a extensive family from Colombia with confirmed diagnosis of familial exudative vitreoretinopathy disease (FEVR). METHODS: Initial complete ophthalmologic evaluation to the whole family; with special examination of the 32 individuals with diagnosis of FEVR. Ocular ecography, angiography and, optical coherent tomography (OCT) were performed to each one. DNA sample was taken after sign the informed consent, in order to perform DNA extraction and sequencing of the coding region of FZD4 gene. RESULTS: By ocular examination we defined that eleven individuals were affected, while the reminder 21 were non-affected. Molecular studies let us to identify the "1501delCT" mutation in the FZD4 gene, in all affected individuals. Autosomal Dominant inheritance was confirmed and, at least in this family, we can postulate the hypothesis that non-affected relatives did not present partial manifestations of the disease. CONCLUSION: We present here a complete clinical description with report of findings of angiography, optical coherent tomography and ocular echography. This exams showed the clasical peripheral changes in retinal vascularization. The Autosomal Dominant inheritance was defined in this family, as well as the causative mutation in the FZD4 gene. We were able to offer a complete genetic counseling in all evaluated individuals, after analysis of the clinical and molecular results.

## 457/W/Poster Board #115

**GLI3 Gene Mutation Spectrum of the patients with Pallister-Hall syndrome.** *Y-G. Xie[1,2,3], J. Cui[1], G. Sun[2], F-Y. Han[1,2].* 1) Discipline ofLaboratory Medicine, Memorial University, St John's, NL, Canada; 2) Discipline of Genetics, Memorial University,St John's, NL, Canada; 3) Discipline of Pediatrics, Memorial University,St John's, NL, Canada.

Pallister-Hall syndrome (PHS) is characterized with polydactyly, cutaneous syndactyly and hypothalamic hamartoma. Other clinical features include bifid epiglottis, imperforate anus, and kidney abnormalities. PHS is a rare autosomal dominant inherited disorder, and is caused by mutations in the GLI3 gene. Most cases are sporadic and the result of new mutations and the gene manifests variable expressivity. Although the typical patients can be diagnosed based on the classic signs of PHS, the patients with ambiguous or mild clinical findings can only be diagnosed by the detection of mutant GLI3 gene. The current known about the causative GLI3 mutations is very limited, and the genotype/phenotype correlation is even more not clear yet. Therefore, the study on more PHS patients for the causative mutations is urgently appreciated. To determine the mutation spectrum of GLI3 gene for PHS, we have collected DNA samples from 20 unrelated patients with clinical suspicion of PHS. The collected patients are originated from four countries including Canada, USA, Australia, and Turkey. Comprehensive mutation scan of entire GLI3 gene was carried out for all of the collected patients by using DNA direct sequencing analysis. Five nonsense mutations and one RNA processing mutation were identified from the six unrelated patients. The identified mutations include c4774 G>T (pE1592X); c4774 G>T (pE1592X), c2610 C> A (pC870X), c4774 G>T (pE1592X), c49 G>T (pE17X) and c1028+1G>T. Detailed clinical information from all patients is being further collected, and genotype /phenotype correlation analysis is under the way. Identification of as many as possible causative mutations for PHS will help to further understand the molecular pathogenesis of PHS and determine the genotype/phenotype correlation as well.

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## 458/W/Poster Board #116

**Evidence suggesting a new inheritance pattern for subgroups of patients with the Oculocutaneous Albinism Spectrum.** *P.-W. Chiang[1], R. Smith[2], R.E. Longman[3], E. Burner[4], C.S. Walton[5], E.B. Spector[1], A.C.-H. Tsai[5].* 1) UC Denver DNA Diagnostic Laboratory, Department of Pediatrics, UC Denver School of Medicine, Aurora, CO; 2) Maine Medical Center, Department of Pediatrics, Portland, ME; 3) Department of Pediatrics, Division of Genetics, University of Michigan, Ann Arbor, MI; 4) Duke Eye Center, Duke School of Medicine, Durham, NC; 5) The Children's Hospital, Department of Pediatrics, UC Denver School of Medicine, Aurora, CO.

Pigmentation is a polygenic trait determined primarily by the amount and type of pigment present in an individual. Many genes are involved and interactions among genes are essential in the determination of pigmentation. Disorders associated with Oculocutaneous albinism (OCA) spectrum are characterized by hypopigmentation of eyes, skin and hair, and are considered to be autosomal recessive disorders. One of the remaining unsolved puzzles for the study of the OCA spectrum is that a significant portion of Caucasian patients with OCA1 or OCA2 have only one identifiable mutation. Large deletions are unlikely due to the presence of heterozygous SNPs in most of these patients. Here, we provide evidence suggesting that a specific *OCA2* haplotype in *trans* to a severe mutation in *OCA2* or the *OCA1* p.R402Q/rs1126809 allele is associated with Caucasian patients with only one identifiable mutation in *OCA2* or *OCA1*, respectively. We studied four Caucasian patients with a clinical diagnosis of OCA2. DNA sequencing of the following 6 genes was performed on genomic DNA extracted from each patient; *OCA1*, *OCA2*, *OCA3*, *OCA4*, *GPR143* (X-linked OA) and *MITF*. A rare, severe mutation in *OCA2* was found in each patient, namely p.L734R, IVS10+2T>C, 2055insT and p.Q106X. An identical *OCA2* haplotype in trans to the mutation was also present in each patient. However, it appears that additional unknown modifier(s) is (are) also required in order to present the typical OCA eye phenotype, as shown by a sibs who inherited the same two OCA chromosomes but with only one sibling demonstrating the typical OCA eye findings. Similarly, even though the *OCA1* p.R402Q/rs1126809 allele is strongly associated with Caucasian OCA1 patients with only one identifiable mutation, the p.R402Q/rs1126809 allele cannot produce the typical OCA eye findings as shown by a sib who inherited the same two OCA chromosomes but with no typical OCA eye finding. These findings confirmed our previous hypothesis, namely the clinical spectrum of patients with OCA spectrum depends upon the combined effects of mutation(s) acting with various modifiers and crossing a threshold to produce a phenotype in a patient. In some Caucasian patients with OCA, the presence of one mutation, a hypomorphic allele/haplotype and extra unknown modifier(s) from other gene(s) are the likely cause of OCA spectrum in these patients. Therefore, OCA spectrum should not be treated as a purely recessive disease.

## 459/W/Poster Board #117

**Homozygous promotor mutation [-30/-30] of beta-globin gene caused by maternal uniparental isodisomy of chromosome 11 in a fetus.** *I. Keser[1,3], T. Bilgen[1], Y. Arikan[1], I. Mendilcioglu[2], A. Yesilipek[3], G. Luleci[1].* 1) Medical Genetics, Faculty of Medicine, Akdeniz University, Antalya, Turkey; 2) Perinatology, Faculty of Medicine, Akdeniz University, Antalya, Turkey; 3) Thalassemia Unit, Faculty of Medicine, Akdeniz University, Antalya, Turkey.

The homozygosity or compound heterozygosity for beta-globin gene mutations (HBB gene) can cause beta-thalassemia major phenotype. Most cases are inherited from parents who both have diseased alleles of the HBB gene. We report a fetus with homozygous -30 (T>A ) mutation that evolved from beta-thalassemia minor in which only one of its parents had the diseased HBB gene using DNA sequencing. To study the cause of homozygous -30 (T>A ) in this fetus, we performed the intragenic single nucleotide polymorphism genotyping assay, the STR analysis of the parental origin, and high resolution chromosome analysis of deletion of the HBB gene lokus. The results showed that homozygous -30 mutations were perfectly originated from one allele of its mother. Our study demonstrates that maternal uniparental isodisomy (iUPD) of chromosome 11 is associated with the homozygous -30/-30 mutation in this fetus.

## 460/W/Poster Board #118

**Verbal intelligence quotient (VIQ) is associated with FMR1 repeat size and reproductive cycling characteristics in women.** *E.G. Allen, A. Abramowitz, K. Charen, D. Hamilton, M. Leslie, R. Letz, G. Novak, M. Rusin, L. Shubeck, J. Juncos, S.L. Sherman.* Dept Human Genetics, Emory Univ, Atlanta, GA.

A CGG repeat sequence located in the 5' untranslated region of the FMR1 gene when expanded leads to fragile X-associated disorders. When greater than 200 repeats, the gene is silenced due to hypermethylation, and the lack of protein leads to fragile X syndrome (FXS). Alleles with 55-200 unmethylated repeats, termed premutation alleles, have been associated with ovarian insufficiency (FXPOI) and a late-onset tremor/ataxia syndrome (FXTAS). Several groups have reported neuropsychological deficits among carriers of the premutation, particularly among males with FXTAS. We have previously shown a subtle but significant effect of FMR1 repeat size on verbal intelligence quotient (VIQ) as measured by the Wechsler Adult Intelligence Scale - III (WAIS-III) among women between the ages of 18 and 50 years. We have confirmed this association, and expanded the model by including a variable for reproductive cycling characteristics in women. All subjects completed a neuropsychological test battery including WAIS-III, a reproductive questionnaire, and provided a blood sample for hormone measurement. We have completed testing on 232 women, and they have been categorized as having normal cycles (non-carriers n=48; premutation carriers n=52), irregular cycles (non-carriers n=43; premutation carriers n=44), and being menopausal (non-carriers n=4; premutation carriers n=41) based on their reproductive questionnaire. In a linear regression model, repeat size, as a continuous variable, explained 4% of the variation in VIQ ($p = 0.002$), and reproductive status explained 3% of the variation in VIQ ($p=0.018$) after adjusting for age, race, and education. Upon further examination of the VIQ index scores, Verbal Comprehension Index (VCI) and Working Memory Index (WMI), an association with repeat size and reproductive status ($p= 0.001$ and $p=0.003$, respectively) was identified for the VCI, but not the WMI. Finally, we stratified the dataset by women with normal cycles and those with irregular cycles and re-examined the model. A significant association with repeat size and VIQ was observed only among the group of women with normal cycles, with 10% of the variance in VIQ being explained (p-value for repeat size=0.002). Hormone replacement did not affect these results. We are in the process of finalizing hormone results and will use these to define reproductive stage in the next series of tests. These findings, including data on individual WAIS-III subtests, will be presented.

## 461/W/Poster Board #119

**Molecular etiology of Stargardt disease in Newfoundland and Labrador.** *J. Green[1], A. Sheaves[1], L. Wilkins[1], K. Hatch[1], D. Galutira[1], J. Whelan[2], D. Bautista[2], B. Younghusband[1], P. Parfrey[3], TL. Young[1].* 1) Discipline of Genetics, Memorial University of Newfoundland, St John's, NL, Canada; 2) Department of Surgery, Memorial University of Newfoundland, St John's, NL, Canada; 3) Department of Clinical Epidemiology, Memorial University of Newfoundland, St John's, NL, Canada.

**Objective**: Our goal was to identify mutations in the ABCA4 gene in Newfoundland (NL) families with Stargardt disease (STGD) and related retinal dystrophies. **Patients and Methods**: 31 families with STGD have been identified in NL since 1978 and members of 22 families were available for molecular analysis. DNA was collected from 37 affected and 27 unaffected consenting members of these families and sequenced on the ABI3130 automated sequencer. Variants were reviewed manually and with Mutation Surveyor, and compared with Asper Ophthalmics and Retina International mutation databases. Clinical records were reviewed for all affected individuals for up to 30 years, and phenotypes for each genotype were compared. The study was approved by the Human Investigation Committee of Memorial University Faculty of Medicine. **Results**: Ancestors of 13/22 families studied came from one isolate in eastern NL. Both disease-causing alleles have been identified in 28 patients, a single mutation in 4 individuals and no mutation for five individuals in five families. The common IVS40+5G>A mutation was homozygous in seven individuals and heterozygous in 11 others all from the same eastern bay. A second mutation, IVS38-10T>C, was present in seven individuals from this same bay. Twelve other mutations were identified in fewer individuals. **Conclusions**: Multiple mutations were identified in NL families, with IVS40+5G>A being the most common mutation. The IVS38-10T>C mutation was seen in seven individuals in two families with severe disease confirming the cone-rod dystrophy presentation previously reported for this mutation. As with other hereditary eye diseases in NL, founder effect and diversity of mutations for STGD was found with a high proportion (70%) of families having the disease-causing ABCA4 alleles identified.

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## 462/W/Poster Board #120

**A novel homozygous missense mutation in the HAX1 gene of severe congenital neutropenia patients (Kostmann disease).** *S.H.E. Zaidi[1], A. Al-Jefri[4], H. Abalkhail[2], M.A. Al-Muallimi[2], M. Toulimat[2], M.A. Pulicat[5], A. Gaafar[5], A.A. Alaiya[5], F. Al-Dayel[2], I. Peltekova[6], M. Faiyaz-Ul-Haque[2,3].* 1) Department of Medicine, University Health Network & University of Toronto, Toronto, Ontario, Canada; 2) Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital & Research; 3) College of Medicine, Alfaisal University, Riyadh, Saudi Arabia; 4) Department of Pediatric Hematology/Oncology, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 5) Department of Biological and Medical Research, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 6) Department of Medicine, Queen's University, Kingston, Ontario, Canada.

Autosomal recessive severe congenital neutropenia (SCN) is a rare inherited disorder, which is characterized by an early onset of recurrent infections in the presence of persistent agranulocytosis. This disease is caused by maturation arrest of granulopoiesis at the level of promyelocytes. In SCN patients, several mutations have been identified in the HAX1 and ELA2 genes. In the HAX1 gene, all of the mutations described to date are nonsense mutations that produce frame shifts of the coding region. The HAX1 gene produces two alternatively spliced transcripts, which differ in inclusion of sequences from exon 2. While nonsense mutations specific to the transcript variant 1 of the HAX1 gene only cause neutropenia, nonsense mutations that are common to both transcript variants produce neurological symptoms in addition to neutropenia. This study describes SCN patients who carry a novel homozygous missense, c.421T>C, mutation in the HAX1 gene. This mutation encodes for a p.Phe141Leu change in the HAX1 protein. The mutation affects both transcript variants of the HAX1 gene. No mutation was found in the ELA2 gene of these SCN patients. Interestingly, while patients in the present study exhibit neutropenia and apoptosis of neutrophils, there is no evidence of developmental delay or neurological impairment. We conclude that while p.Phe141Leu substitution is sufficient to produce SCN, this mutation does not cause developmental or neurological manifestations. This is the only known pathogenic missense mutation in the HAX1 gene of SCN patients.

## 463/W/Poster Board #121

**Molecular analysis of 6 Turkish Griscelli Syndrome Patients.** *A. Alpman Durmaz[1], F. Ozkinay[1, 2], H. Onay[1], M. Tombuloglu[3], A. Atay[4], E. Peker[5], M. Atmaca[5], H. Akin[1], C. Ozkinay[1].* 1) Department of Medical Genetics, Ege University Medical Faculty, Izmir, Turkey; 2) Department of Pediatrics, Ege University Medical Faculty, Izmir, Turkey; 3) Department of Internal Medicine, Ege University Medical Faculty, Izmir, Turkey; 4) Department of Pediatrics, Gulhane Military Medicine Faculty, Ankara, Turkey; 5) Department of Pediatrics, Van 100. Yil Medical Faculty, Van, Turkey.

Griscelli syndrome is a rare autosomal resessive disorder associated with various degrees of skin or hair hypopigmentation, hepatosplenomegaly, pansytopenia, immunological and central nervous system abnormalities. RAB27A coding a GTPase in terminal phase of cytotoxic granule/melanosome exocytosis is mutated in the most common GS subtype GSII which mainly is characterized by hemophagocytosis and variable immundeficiency. RAB27 gene is located on chromosome 15q21 and several mutations have been found in affected patients. We present RAB27A mutation analysis of 6 cases diagnosed as Griscelli syndrome type II which were missense mutations (L26P and L130P) in 2 cases, deletion of 5 bases (514delCAAGC) in 2 cases and one base deletion (148delA) in 2 cases. The 514delCAAGC deletion has been detected in only 2 previous cases and is close to a deletion hotspot found in GSII patients. The 2 cases having homozygous 148delA mutation which leads to premature stop codon also had homozygous R50T missense mutation. Mutation analysis of the parents revealed heterozygosity for these 2 mutations and it is considered to be linked changes within RAB27A gene. A similar previous case was reported from Iran and this is the second report defining this comlex genotype in unrelated 2 GS cases. The phenotype-genotype correlation of different types of mutations is also discussed in this presentation within the RAB27A gene in Griscelli Typr II syndrome.

## 464/W/Poster Board #122

**Mutation spectrum of the autoimmune regulator (AIRE) gene in Arab patients affected with autoimmune polyendocrinopathy syndrome type 1 (APS1).** *M. Faiyaz-Ul-Haque[1,2], B. Bin-Abbas[3], A. Al-Abdullatif[1], H. Abalkhail[1], M. Toulimat[1], S. Al-Gazlan[4], A.M. Almutawa[1], A. Al-Sagheir[3], I. Peltekova[5], F. Al-Dayel[1], S.H.E. Zaidi[6].* 1) Pathology & Lab Med, King Faisal Spec Hosp & Res Ctr, Riyadh, Saudi Arabia; 2) College of Medicine, Alfaisal University, Riyadh, Saudi Arabia; 3) Endocrine/Metabolism, Department of Pediatrics, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 4) Allergy/Immunology, Department of Medicine, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 5) Department of Medicine, Queen's University, Kingston, Ontario, Canada; 6) Department of Medicine, University Health Network, Toronto, Ontario, Canada.

Autoimmune polyendocrinopathy syndrome type 1 (APS1) is characterized by the manifestation of at least two out of three disease components, which comprise of Addison's disease, hypoparathyroidism, and chronic mucocutaneous candidiasis. Additional manifestations include alopecia universalis, diabetes mellitus, onychosis, growth hormone deficiency, nephrocalcinosis, keratopathy, vitamin B12 deficiency, gonadal failure, iridocyclitis, and celiac disease. This disorder is caused by mutations in the autoimmune regulator (AIRE) gene. While AIRE mutations have been described in APS1 patients of various ethnicities, the spectrum of AIRE mutations in Arab APS1 patients has not been determined. This study describes seven Arab families, in which eighteen individuals were diagnosed with APS1. DNA sequencing of these APS1 patients identified one recurrent and four novel mutations in the AIRE gene. These mutations likely result in loss of AIRE function in these patients. An interesting finding in this study is the manifestation of hypoparathyroidism and mucocutaneous candidiasis during the neonatal period in several patients. In addition, alopecia universalis was present in several patients with an onset during the ages of 3-10 years. Scalp biopsies from the patients showed decreased number of hair follicles and peribulbar lymphocytic inflammation. Presence of T-lymphocytes in the peribulbar area of hair follicles was confirmed by immunohistochemical staining for CD3 antigen. We conclude that the Arab APS1 patients carry novel and recurrent AIRE mutations, with early onsets of hypoparathyroidism and mucocutaneous candidiasis, and frequent occurrence of alopecia universalis, which is associated with inflammation of hair follicles.

## 465/W/Poster Board #123

**Genetic investigation of human natural killer lymphocytes deficiency.** *L. GINEAU[1,2], E. Jouanguy[1,2], J. Dunne[3], C. Cognet[4], L. Abel[1,2], S. Aoufouchi[5], E. Vivier[4], C. Feighery[3], JL. Casanova[1,2].* 1) Laboratory of Human Genetics of Infectious diseases, Necker Branch, University of Paris René Descartes INSERM U550, Necker Medical School, Paris, 75015, France, EU; 2) Laboratory of Human Genetics of Infectious diseases, Rockefeller Branch, The Rockefeller University, New York, 10065 NY, USA; 3) Departement of Immunology, St Jame's Hospital, Dublin, Irland, EU; 4) Centre d'immunologie de Marseille-Luminy, Université de la Méditerranée, INSERM U631-Centre National de la recherche scientifique UMR 6102, Marseille 13288 France, EU; 5) Genome plasticity and B cells, University of Paris-Sud, FRE2939, Institut de Cancérologie Gustave Roussy, 94805 Villejuif, France, EU.

Natural killer (NK) cells functions are well characterized in the mouse for their important role in the first line of defence in antiviral and anti-tumoral immunity. In humans, however, little is know mainly because there is no immunological defect restrictive to NK cells. We report six children with a novel primary immunodeficiency, consisting of a specific NK cell deficiency and susceptibility to viral diseases and tumoral proliferation. Indeed, one child developed an EBV-driven lymphoproliferative disorder, another one developed recurrent viral infections by HSV and VZV. The patients are related and they belong to a large inbred kindred of Irish nomadic descent, suggesting autosomal recessive inheritance of this defect. A genome-wide scan identified a single 12 Mb region on chromosome 8 linked to this immunodeficiency and containing 77 genes. The sequencing of the coding region of these genes conducted so far to identify a germline mutation in these patients. Our preliminary data show that the mutated gene seems to be specifically implicated in the development of a sub-population of NK cells. The functional characterization of this mutation will allow us to better understand a crucial regulatory step in NK cell differentiation.

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## 466/W/Poster Board #124

**Detection of copy number variation in the beta-globin locus potentially associated with beta thalassemia.** *M. Mikula, A. Buller, W. Sun, C. Strom.* Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.

There are an estimated 80 million carriers of beta thalassemia worldwide and more than 200 mutations are known affecting the beta-globin gene. Most mutations are point mutations within the gene or its immediate flanking sequences; deletions only rarely cause the disease. However, deletions account for approximately 20% of beta thalassemia alleles in some populations and more than 40 different beta-globin locus deletions have been reported. A newly developed beta-globin gene dosage assay, specifically a single tube multiplex semi-quantitative fluorescent PCR, can be used to detect deletions and duplications in the beta-globin cluster of genes and the associated locus control region (LCR). The assay detects mutations in 12 amplicons spanning the beta-globin locus and uses 2 internal controls to normalize signal for inter-sample comparison. Anonymized samples (n= 564) previously submitted for beta-globin sequencing from individuals with red cell indices suggestive of beta thalassemia were tested for deletions and duplications in the beta-globin gene cluster. The samples were chosen irrespective of sequencing results. Eleven deletions (2.0%) were detected: 5 deletions involved the beta- globin gene only (3 deletions of the entire gene and 2 deletions of the 5' portion of the gene) and 6 rearrangements that deleted both the delta-globin and beta-globin genes. No duplications were detected. Thus, testing for deletions in the beta-globin gene may reveal novel mutations associated with beta thalassemia. Clinical correlation and confirmation studies are underway to further characterize the detected deletions.

## 467/W/Poster Board #125

**TMPRSS6, the Serine Protease Mutated in Iron-Refractory, Iron Deficiency Anemia, Regulates Systemic Iron Homeostasis by Inhibiting Hepcidin Signaling Through Hemojuvelin.** *K.E. Finberg[1], R.L. Whittlesey[2], M.D. Fleming[3,4], N.C. Andrews[2,5].* 1) Dept Pathology, Duke Univ Med Ctr, Durham, NC; 2) Dept Pharmacology & Cancer Biology, Duke Univ Med Ctr, Durham, NC; 3) Dept Pathology, Children's Hosp Boston, Boston, MA; 4) Dept Pathology, Harvard Medical School, Boston, MA; 5) Dept Pediatrics, Duke Univ Med Ctr, Durham, NC.

Iron-Refractory, Iron Deficiency Anemia (IRIDA) is a familial disorder characterized by iron deficiency anemia unresponsive to oral iron treatment but partially responsive to intravenous iron therapy. Previously, we showed that IRIDA patients harbor loss-of-function mutations in TMPRSS6, a transmembrane serine protease expressed by the liver. Both humans and mice with Tmprss6 mutations show inappropriately elevated levels of hepcidin, a circulating hormone produced by the liver that inhibits iron absorption from the intestine and iron release from macrophage stores; these findings suggest that TMPRSS6 acts to negatively regulate hepcidin expression. We hypothesized that Tmprss6 may negatively regulate hepcidin expression by down-regulating hepcidin signaling through hemojuvelin (Hjv), a membrane-bound protein in hepatocytes that signals to promote hepcidin expression. To test this, we bred Tmprss6-deficient mice to Hjv-deficient mice; the latter are a mouse model of juvenile hemochromatosis characterized by low hepcidin levels and systemic iron overload. Compared to wild type controls, Tmprss6-deficient mice showed iron-deficiency related hair loss, decreased hemoglobin levels ($p<0.0005$), decreased transferrin saturation ($p<0.0005$), elevated hepatic hepcidin mRNA ($p<0.05$), and decreased hepatic iron stores ($p<0.0005$). Mice harboring one defective Tmprss6 allele had a normal coat but moderately decreased hepatic iron stores ($p<0.0005$ vs. wild type) and laboratory signs of iron-restricted erythropoiesis. Mice deficient for both Tmprss6 and Hjv, however, showed a normal coat and normal hemoglobin levels, and like mice deficient for Hjv alone, were systemically iron overloaded compared to wild type mice, displaying elevated transferrin saturation ($p<0.0005$), markedly decreased hepcidin levels ($p<0.005$), and markedly increased non-heme iron content of liver ($p<0.0005$), heart ($p<0.05$), pancreas ($p<0.005$), and muscle ($p<0.0005$). We conclude that TMPRSS6 is an essential regulator of mammalian systemic iron homeostasis that acts functionally upstream of hemojuvelin to negatively regulate hepcidin transcription by hepatocytes. In IRIDA, where TMPRSS6 function is lost, excess hemojuvelin signaling results in elevated hepcidin levels, which in turn promote systemic iron deficiency by reducing dietary iron absorption.

## 468/W/Poster Board #126

**A new concept of Alpha thalassemia: normal alpha locus and mutant Alpha hemoglobin stabilizing protein.** *S. Pissard[1,3], C. Vasseur[2], F. Toutain[5], M. Silva[4], E. Faubert-Laugé[1], MC. Marden[2], H. Wajcman[1,3], V. Baudin-Creuza[2].* 1) Lab Biochemistry & Genetics, Hosp Henri Mondor and Univ Paris 12, Creteil, France; 2) Inserm U779, CHU de Bicetre, Le kremlin Bicetre, France; 3) Inserm U 955 eq11, CHU H mondor, Creteil, France; 4) Hematologie Biologique, Groupe hospitalier du Havre, Le Havre, France; 5) service de Pediatrie, Groupe Hospitalier du Havre, Le Havre, France.

Alpha thalassemias ($\alpha$-thal) form a group of inherited diseases in which the deficit in $\alpha$-globin chain synthesis results in dyserythropoiesis. Due to the sequential activation of the $\alpha$-globin loci during the gestation and the degree of imbalance, the severity of the disease varies from a fetal death or, at birth, to a microcytic anemia with Hb Bart's detected by hemoglobin phenotype studies. Various molecular defects of the $\alpha$-globin locus result in $\alpha$-thal, the most frequent being deletions of the $\alpha$-loci (s). Point mutations are less frequent and, as rare etiologies, deletions of the activating sequence (HS-40) have been reported. $\alpha$-thal is a part of ATR-16 syndrome (large deletion within 16p) and ATR-X syndrome (mutation in the ATRX gene :Xq13) in which thalassemia is associated with mental retardation. Alpha hemoglobin stabilizing protein (AHSP) is a chaperon (16 p11.2) of $\alpha$-Hb described to have a major importance in the folding and stability of the $\alpha$-globin chains. In vitro studies and experiments on mice have shown that default in the folding process results in the degradation of the $\alpha$-chain and thus should lead to $\alpha$-thal. Up to now this has not been found in humans. We have studied the second child of a family originating from SE Asia, who was referred to the hospital one month after birth, because of palor and anemia. Biological tests demonstrated a hemolytic microcytic anemia (Hb 6.9 g/dl, MCV : 73.2 fL and MCH 25 pg), 3% Hb Bart's were detected by CE-HPLC. The molecular screening for $\alpha$-thal, including detection of common and rare $\alpha$ locus deletions using Gap-PCR and MLPA analysis, sequencing of $\alpha$ 1 and $\alpha$ 2 genes, and sequencing of the HS-40 core sequence revealed no abnormality. We then sequenced the AHSP gene and its promoter; the proband was found to be homozygous for a mutation in exon 3 (c.167 T>G) and the parents heterozygous for it. In vitro studies demonstrated a 20% -30% decrease in the association between normal $\alpha$-globin and the mutated recombinant AHSPV56G suggesting that the mutated AHSP could be responsible for the $\alpha$-thal, thus explaining the child's phenotype. Up to now, only 3 mutants have been described in the AHSP gene but none found to impair the association with normal $\alpha$-chain. This case is thus the first evidence of an isolated $\alpha$-thal not linked with $\alpha$-locus but with a mutated AHSP.

## 469/W/Poster Board #127

**Novel and recurrent mutations in the complement component 1 inhibitor (C1NH) gene of Arab patients affected with hereditary angioedema.** *H. Abalkhail[1], S. Al-Gazlan[3], A. Al-Abdullatif[1], M. Toulimat[1], I. Peltekova[4], A.M.R. Khaliq[3], F. Al-Dayel[1], S.H.E. Zaidi[5], M. Faiyaz-Ul-Haque[1,2].* 1) Department of Pathology & Labo, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 2) College of Medicine, Alfaisal University, Riyadh, Saudi Arabia; 3) Allergy/Immunology, Department of Medicine, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 4) Department of Medicine, Queen's University, Kingston, Ontario, Canada; 5) Department of Medicine, University Health Network, Toronto, Ontario, Canada.

Autosomal dominant hereditary angioedema (HAE) is characterized by episodes of subcutaneous edema in various body parts and/or submucosal edema of the upper respiratory or gastrointestinal tracts. This disorder is caused by mutations in the complement component 1 inhibitor (C1NH) gene. While C1NH mutations have been described primarily in European patients, the spectrum of C1NH gene mutations in Arab HAE patients has not yet been determined. In this study, we describe four unrelated Arab families, in which fifteen patients were diagnosed with HAE. These patients exhibited recurrent swelling of the face, lips, limbs, tongue and other body parts, abdominal pain, laryngeal edema, and respiratory distress. DNA from thirteen patients was analyzed for mutations in the C1NH gene by DNA sequencing. Three novel and two recurrent C1NH mutations were found in the HAE patients, which include the c.856C>T, c.1361T>A, c.509C>T, and c.1142delC novel mutations, and the c.1397G>A recurrent mutation. These mutations encode for p.Arg264Cys, p.Val432Glu, p.Ser148Phe, p.Ala359AlafsX15, and p.Arg444His changes in the AIRE protein, respectively. In one family, a single patient was found to be compound heterozygous for a novel and a recurrent missense mutation encoding for p.Arg264Cys and p.Val432Glu, respectively. The five mutations in the present study were not found in 119 control chromosomes of Arab ethnicity. All identified mutations belong to the micro C1NH gene mutation class. This is the first ever report of C1NH gene mutations in Middle Eastern Arab patients. Our study suggests that despite the numerous reports of mutations in the C1NH gene, there exist novel and recurrent mutations in HAE patients of non-European ethnic origins.

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## 470/W/Poster Board #128

**Mutation Spectrums of PRF1, MUNC13-4 and STX11 genes in patients with Familial Hemophagocytic Lymphohistiocytosis in North America.**
*K. Zhang[1], J. Meller[3,4], J. Biroschak[1], JA. Johnson[1], Q. Wei[8], C. Liu[1], EM. Uzvolgyi[1], R. Marsh[2], S. Satake[7], A. Grom[5], K. Risma[6], J. Bleesing[2], AH. Filipovich[2].* 1) Division of Human Genetics, Cinncinnati Children's Hospital, Cincinnati, OH; 2) Division of Hematology /Oncology, Cinncinnati Children's Hospital, Cincinnati, OH; 3) Depts. of Environmental Health and Biomedical Engineering, University of Cincinnati College of Medicine, Cincinnati, OH; 4) Division of Biomedical Informatics, Cinncinnati Children's Hospital, Cincinnati, OH; 5) Division of Reumatology, Cinncinnati Children's Hospital, Cincinnati, OH; 6) Division of Allergy, Cinncinnati Children's Hospital, Cincinnati, OH; 7) Section of Hematology/Oncology, University of California, Davis School of Medicine, Davis Medical Center, Sacramento, CA; 8) Department of Biomedical Engineer, Columbia University, New York, NY.

Familial hemophagocytic lymphohistiocytosis (FHL-familial HLH) is a rare disorder of immune regulation, characterized by defects in cell-mediated cytotoxicity that results in fever, hepatosplenomegaly and cytopenias. It is often rapidly fatal unless treated with chemotherapy, immune suppression and followed by bone marrow transplant. FHL is an autosomal recessive disorder. Three genes have been identified to be associated with FHL: PRF1, MUNC13-4 and STX11. From 2004 to 2008, we tested a total of 1,145 patients from North America with the clinical diagnosis of HLH. We found 1) PRF1 mutations in 190 families. PRF1-50delT is found in 32 families (17%). it is the most common mutation in PRF1. All of these patients are of African American descent. 2) For the 845 families available for MUNC13-4 sequence analysis, bi-allelic mutations were found in 38 families (4.5%), and more strikingly, single MUNC13-4 mutations were found in 94 families (11%). MLPA analyses were performed in these patients, and did not find any evidence of gross deletions. Careful examination of the patient's sequences in MUNC13-4, revealed more than three dozen haplotypes in MUNC13-4. One unique haplotype is associated with macrophage activation syndrome, which resembles HLH clinically. We predict that some of these haplotypes might host pathological mutations and contribute to the development of FHL and associated disorders. 3) Mutational analyses of STX11 by direct sequencing were done in the remaining 306 unrelated patients. Two novel nonsense STX11 mutations were identified in two families. Two previously unreported missense mutations are presented in another family. 4) More interestingly, there is an affected individual who is heterozygous for a nonsense STX11 mutation, and also carries heterozygous missense mutation in MUNC13-4 gene. As both STX11 and MUNC13-4 are involved in vesicle trafficking and membrane fusion in perforin mediated cytotoxic pathway, the finding of double heterozygosity of mutations in both STX11 and MUNC13-4 suggests that digenic effects may also lead to FHL. In summary, this is the first large scale genetic study of HLH patients in North America. Single MUNC13-4 mutations were found in a large portion of FHL patients. Certain haplotypes in the MUNC13-4 locus might be associated with FHL and associated disorders. And for the first time, bi-allelic pathological mutations in STX11 can be found in FHL patient without a Turkish/Kurdish background.

## 471/W/Poster Board #129

**Genetic disorders in Tunisia. A systematic review.**
*L. Romdhane, I. Manai, S. Romdhane, S. Abdelhak.* Institut Pasteur de Tunis, Tunis Belvédère, Tunisia.

Tunisia is a North African country, geographically situated at the crossroad between Africa and Europe. Little information is available about genetic diseases in developing countries, especially in Tunisia although hereditary disorders are considered as a real public health problem because of the high rates of consanguinity which may reach 60%. The purpose of the present study is to evaluate the burden of genetic diseases through a review of the literature. A research in OMIM texts was performed. A systematic review of publications using key-words "genetic disease Tunisia" and "Tunisian patient" was done using PubMed. Data from "gray literature" were retrieved through national and international meeting reports, local journals, thesis and monographs. Reports dealing with clinical, epidemiological, genetic and molecular features were selected. Data on genetic diseases among Tunisian population are from patients living in Tunisia and immigrant Tunisians. A non exhaustive list of 251 genetic disorders has been identified. Among those, 66.93 % are autosomal recessive, 21.51 % autosomal dominant, 5.18 % X-linked, 0.4 % Y-linked, 0.8 % mitochondrial, 2 % mixed, 2.4 % sporadic and 0.8 % of unknown mode of transmission. Fifty five per cent of genetic diseases had a defined molecular aetiology and were caused by at least one mutation. No molecular data were available for the remaining diseases with unknown gene or in case mutations are not yet identified in the Tunisian population. A founder effect was noticed in 7.17 %, about 50 % of them are specific to the Tunisian population, the remaining others are founder alleles shared with North African populations. In many cases, genetic heterogeneity is responsible for their high frequency in the Tunisian population. Allelic heterogeneity is also noticed that lead to expression of a single morbid phenotype. As a consequence of the high inbreeding rate in Tunisia combined with a founder effect, many genetic disorders emerged with autosomal recessive inheritance being the most prevalent. Currently in Tunisia, there are several studies on prevention of disabilities through pluridisciplinary networks. Health education early at scholar course seems to be a suitable tool for a developing country as Tunisia in order to prevent such disorders. Because of geographical, historical and socio-cultural reasons, our study has an impact at the regional level, as the population structure is very similar to the neighbouring countries.

## 472/W/Poster Board #130

**Molecular characterization of three Taiwanese families with Lowe syndrome.**
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Lowe syndrome is a rare and panethnic X-linked disorder characterized by anomalies in the eye, nervous system and kidney with an incidence of approximately 1 in 500,000 people. Mutations in OCRL gene (localized at Xq26.1) are responsible for the disease. We performed the molecular analysis to screen for mutations in all the coding sequences and exon-intron boundaries of the OCRL gene in three unrelated Taiwanese families. Three novel mutations, including two frameshift mutations (c.1854dupA on the exon 17and c.2309_2312delATTG on the exon 21) and one missense mutation c.1501A>T (p.I501F) on the exon 15 were identified. Familial analyses showed mutations in two of three probands (hemizygous c.1854dupA and c.1501A>T) were maternal origin. The remaining hemizygous frameshift mutation c.2309_2312delATTG (p.Asp619ValfsX54) in a sporadic case is a de novo mutation because it was not found in his parents. Western blotting analyses showed reduction or absence of the OCRL protein expression in all probands, providing evidence that all the three mutations we detected here are disease-causing defects.

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## 473/W/Poster Board #131

**Bifid Nose, Renal Agenesis, and Anorectal Malformations Syndrome is Caused by Mutation of *FREM1*.** *A.M. Alazami[1], L. Al-Gazali[2], K. Snape[3], F.S. Alkuraya[1,4,5].* 1) Dept of Genetics, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 2) Department of Pediatrics, United Arab Emirates University, Al-Ain, United Arab Emirates; 3) North East Thames Regional Genetics Service, Clinical Genetics Unit, Great Ormond Street Hospital NHS Trust, London; 4) Department of Pediatrics, King Khalid University Hospital and College of Medicine, King Saud University, Riyadh, Saudi Arabia; 5) Department of Anatomy and Cell Biology, College of Medicine, Alfaisal University, Riyadh, Saudi Arabia.

In 2002, Al-Gazali et al described an apparently novel autosomal recessive multiple congenital anomalies syndrome comprising bifid nose (with no associated hypertelorism), renal agenesis, and anorectal malformations (MIM 608980). The consanguineous nature of the parents allowed us to implement homozygosity mapping to identify two blocks of shared homozygosity on chromosomes 5 and 9. We hypothesized that the candidate gene will be expressed in the developing kidney and although *FREM1* fit this description, this gene is known to be one of several that are responsible for the spontaneous *bleb* mutant phenotypes in mice, which collectively are believed to be mouse models for Fraser syndrome (MIM 219000), an autosomal recessive condition in which cryptophthalmos and syndactyly are major features. To our surprise, we uncovered two mutations in *FREM1* in both the original family and a new unrelated family. FREM1 is an extracellular matrix protein that is found in close association with two other related extracellular matrix proteins, FRAS1 and FREM2. Only mutations in *FRAS1* and *FREM2* have been identified in human patients with Fraser syndrome and, while specifically pursued, *FREM1* mutations have never been reported in this disorder or any other human disease in general. A re-assessment of the original paper describing the spontaneous *Frem1* mouse mutant revealed no evidence of cryptophthalmos. *In situ* hybridization demonstrated *Frem1* gene expression in the midline of E11.5 embryos, in agreement with the observed cleft nose phenotype of our patients. Given that two of the defining traits of Fraser syndrome, cryptophthalmos and syndactyly, are absent from all our affected individuals, this is therefore the first report to define mutations in the FRAS1/ FREM complex which lead to a human phenotype distinct from Fraser syndrome.

## 474/W/Poster Board #132

**Molecular basis of Melnick-Needles syndrome: first cases with confirmation of mutations in filamin A exon 22 in boys with the perinatal-lethal phenotype.** *M.R.S. Carvalho[1], H.H. Santos[2], P.P. Garcia[3], L. Pereira[1], L.L. Leão[2,5], R.A.P.L. Aguiar[4], A.M.A. Lana[3], M.J.B. Aguiar[2,5].* 1) Departamento de Biologia Geral, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil; 2) Serviço Especial de Genética do Hospital das Clínicas da Universidade Federal de Minas Gerais, Brazil; 3) Departamento de Anatomia Patológica e Medicina Legal da Universidade Federal de Minas Gerais, Brazil; 4) Departamento de Ginecologia e Obstetrícia da Faculdade de Medicina da Universidade Federal de Minas Gerais, Brazil; 5) Departamento de Pediatria da Universidade Federal de Minas Gerais, Brazil.

Introduction Melnick-Needles syndrome (MNS) is a rare condition with X-linked dominant inheritance, and lethal in males. MNS is caused by mutations in FLNA, which encodes a protein filamina A. Three mutations have been already described, all of them in FLNA exon 22 (D1184E, A1188T and S1199L). Besides, FLNA mutations heve been identified in patients with Otopalatodigital syndromes type 1 and type 2 and Frontometaphyseal dysplasia. Objectives To acertains mutations in FLNA exon 22 in two stillbirth with post-mortem diagnosis of MNS perinatal-lethal phenotype and their mothers with a clinical diagnosis of MNS. Methodology DNA was extracted from paraffin embedded tissues for children and peripheral blood from their mothers. The analysis of the mothers DNA was performed by PCR and direct sequencing. Sequencing of children DNAs was obtained after heminested PCR and cloning into plasmid TOPO2.1 (Invitrogen). Results We identified in one child the mutation 3845C → T (in NCBI reference sequence NM 001456.3) in exon 22 that replaces an serine by a leucine residue at position 1199. In another boy, a double SNP was identified, 3776G → A and 3777G → T - (in NCBI reference sequence NM 001456.3) which lead to the substitution of a glycine by an aspartic acid at position 1176. Conclusion Through genetic and molecular analysis we confirmed the clinical diagnosis of individuals. The mutation 3845C → T, which had been described in literature only in mothers of affected or in mosaics men, it was identified for the first time in a stillbirth affected, confirming its role in the development of the phenotype. The second mutation (G1176D) was identified for the first time in this study and was present both in the mother (in heterozygosis) and in the stillbirth. The replacement of a small and neutral amino acid by a large and acid one is sugestive of gain of function. This is the first study confirming FLNA mutations in boy affected by the MNS perinatal-lethal phenotype.

## 475/W/Poster Board #133

**Pursuit of additional 'Cohesinopathy' loci involved in human developmental disorders.** *M. Deardorff[1], E. Loy[1], M. Kaur[1], M. Berman[1], L. Conlin[1], X. Gai[2], J. Perin[2], T. Shaikh[1], H. Hakonarson[3], L. Jackson[4], I. Krantz[1].* 1) Genetics, Children's Hosp Philadelphia, Philadelphia, PA; 2) Bioinformatics, Children's Hosp Philadelphia, Philadelphia, PA; 3) Center for Applied Genomics, Children's Hosp Philadelphia, Philadelphia, PA; 4) Drexel School of Medicine, Philadelphia, PA.

The Cornelia de Lange syndrome (CdLS) or Brachmann-de Lange syndrome (BDLS) (OMIM 122470) is a multisystem developmental disorder characterized by facial dysmorphia, hirsutism, growth and cognitive retardation, gastrointestinal abnormalities and limb deficiencies. To date, we have identified mutations in approximately 65% of patients with CdLS. These mutations involve the genes NIPBL, SMC1A and SMC3, all of which are involved in sister chromatid cohesion. We have yet to elucidate a cause of CdLS in 35% of patients with typical features of CdLS and nearly 80% of patients with variant features of CdLS. To facilitate identification of genes that may cause CdLS or these variant phenotypes, we have used genome-wide CNV analysis. To date, 269 individuals submitted to our study for whom causative mutations had not been previously identified were analyzed for potentially pathogenic copy number variations using Illumina HapMap550K SNP arrays. Analysis to the level of 2 consecutive abnormal SNPs has revealed 8932 potential variants ranging from 2bp to 20Mb. Of these CNVs, 5191 were 5 SNPs or less. We have identified deletions in 5 patients that include NIPBL and were validated using MLPA. There were no deletions that included SMC1A and SMC3, consistent with previous findings of only missense mutations in these genes. We have identified a number of large chromosomal abnormalities in individuals with phenotypic overlap with CdLS, but who do not meet full clinical criteria. We are hypothesizing that these overlapping clinical features may be similar to CdLS due to the involvement of additional genes involved in the pathogenesis of the 'Cohesinopathies'. In our cohort, we have identified two patients with overlapping de novo deletions on 19p13 and two additional patients with overlapping de novo duplications on 19q13. We have identified six additional patients with overlapping abnormalities from other sources and are working to collect their detailed clinical and cytogenetic data. We speculate that the underlying basis of the clinical features in these and other patients may be related to Cohesin function during human development.

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## 476/W/Poster Board #134

**Transcriptional and Epigenetic Dys-regulation in *NIPBL* and Cohesin Mutant Human Cells - A Comprehensive Genomic Study in Cornelia de Lange Syndrome.** *J. Liu[1], Z. Zhang[2], M. Bando[3], T. Ito[3], M.A. Deardorff[1,4], J.R. Li[1], D. Clark[1], M. Kaur[1], S. Tandy[1], T. Kondoh[5], E. Rappaport[6], N.B. Spinner[1,4], H. Vega[7], L.G. Jackson[8], K. Shirahige[3], I.D. Krantz[1,4].* 1) Division of Human Genetics, Abramson Research Institute, The Children's Hospital of Philadelphia, Philadelphia, PA 19104; 2) Center for Biomedical Informatics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104; 3) Laboratory of Chromosome Structure and Function, Department of Biological Science, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, B-20, 4259, Nagatsuta, Midori-ku, Yokohama City, Kanagawa 226-8501, Japan; 4) The University of Pennsylvania School of Medicine, Philadelphia, PA 19104; 5) Division of Developmental Disability, Misakaenosono Mutsumi Developmental, Medical, and Welfare Center, Konagai-cho Maki 570-1, Isahaya, 859-0169, Japan; 6) NAPCORE, The Children's Hospital of Philadelphia, Philadelphia, PA 19104; 7) Instituto de Genética, Universidad Nacional de Colombia, Bogotá, Colombia; 8) Department of Obstetrics and Gynecology, Drexel University School of medicine, Philadelphia, PA 19104.

Cohesin regulates sister chromatid cohesion during the mitotic cell cycle with NIPBL facilitating its loading and unloading. In addition to this canonical role, cohesin has also been demonstrated to play a critical role in regulation of gene expression in nondividing cells. Heterozygous mutations in the cohesin regulator, *NIPBL*, or cohesin structural components *SMC1A*, and *SMC3*, result in the multisystem developmental disorder Cornelia de Lange Syndrome (CdLS). Genome-wide assessment of transcription in 16 mutant cell lines from severely affected CdLS probands as well as in 17 matched healthy controls has identified a unique profile of dysregulated gene expression that was validated in an additional 101 samples and correlates with phenotypic severity. This profile could serve as a diagnostic and classification tool. Cohesin binding analysis demonstrates a preference for intergenic regions suggesting a *cis*-regulatory function mimicking that of a boundary/insulator interacting protein. However, the binding sites are enriched within the promoter regions of the dysregulated genes and are significantly decreased in CdLS probands, indicating a potential alternative role of cohesin as a transcription factor. Genome wide DNA methylation assays on 63 CdLS probands with *NIPBL*, *SMC1*, or *SMC3* mutations have revealed significant DNA methylation difference at 375 CpG sites ($p<0.01$) between controls and severely affected individuals, DNA methylation levels of 81% (304) of these sites correlate with disease severity as mildly affected probands have less changes than severe probands. Cohesin preferentially binds to hypomethylated DNA and the binding is prohibited by DNA hypermethylation. However, no evidence has been found that gene mis-expression in CdLS is correlated to the levels of DNA methylation, indicating NIPBL and cohesin may regulate transcription and affect DNA methylation through different pathways. Additionally, skewed X inactivation identified among CdLS probands suggests a novel functional role of NIPBL and cohesin involved in the "choice" step during the X inactivation process.

## 477/W/Poster Board #135

**Identification of *RSK2* mutations in Polish patients with Coffin-Lowry syndrome.** *D. Jurkiewicz, E. Popowska, E. Ciara, D. Piekutowska-Abramczuk, M. Borucka-Mankiewicz, P. Kowalski, A. Tanska, M. Gajdulewicz, M. Kugaudo, K. Chrzanowska, M. Krajewska-Walasek.* Medical Genetics, Children's Memorial Health Institute, Warsaw, Poland.

Coffin-Lowry syndrome (CLS, MIM#303600) is a rare X-linked semidominant disorder. Affected males show severe mental retardation, facial dysmorphism, and various skeletal anomalies. In females the intensity of symptoms is variable. CLS is caused by mutations in the *RSK2* gene (*RPS6KA3*) located in Xp22.2. The gene encodes for a serine/threonine kinase (RSK2) acting in the Ras/MAPK signaling pathway involved in regulating wide range of cellular functions. Mutations in the *RSK2* gene are very heterogenous and lead to premature termination of translation and/or to loss of phosphotransferase activity of RSK2. In this study we present results of the *RSK2* gene mutation analysis in a group of Polish patients with clinically recognized Coffin-Lowry syndrome. Mutation screening of the entire *RSK2* gene was performed in sixteen patients. All twenty-two exons and the intron-exon bounaries of *RSK2* gene were amplified by PCR and subsequently analyzed by SSCP and sequencing technics. The molecular analysis revealed the presence of five different mutations in the *RSK2* gene, including two splice site (c.845+1G>A, c.1842-2A>T), one frameshift (c.896delT, p.Leu299TyrfsX13), one stop codon (c.1672C>T, p.Arg558X) and one missense (c.1610A>G, p.His537Arg) mutation. One splice site mutation was of maternal origin, remaining variants appeared *de novo*. Identified mutations are localized in the regions of the *RSK2* gene coding for N-terminal and C-terminal kinase domains and can be predicted to have a detrimental effect on the RSK2 function. The study was supported by Polish Ministry of Science Project 0624/P01/2006/31 (N40103131/0624).

## 478/W/Poster Board #136

**A Novel Form of Recessive Cerebellar Atrophy Maps To 5q31.1-q33.1.** *Z. Arlier[1,2], A.K. Ozturk[1,2], K. Bilguvar[1,2], K. Yasuno[1,2], L.E. Kolb[1,2], S. Giray[3], R.P. Lifton[4,5,6], M.W. State[2,4,7], M. Gunel[1,2,4,8].* 1) Neurosurgery, Yale University School of Medicine, New Haven, CT; 2) Program on Neurogenetics, Yale University School of Medicine, New Haven, CT; 3) Neurology, Baskent University Faculty of Medicine, Ankara, Turkey; 4) Genetics, Yale University School of Medicine, New Haven, CT; 5) Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT; 6) Internal Medicine, Yale University School of Medicine, New Haven, CT; 7) Child Study Center, Yale University School of Medicine, New Haven, CT; 8) Neurobiology, Yale University School of Medicine, New Haven, CT.

In the absence of associated abnormalities, structural defects of the cerebellum can be non-specific. While certain conditions such as Dandy-Walker syndrome may be readily evident, the majority of cases of structural cerebellar abnormalities pose diagnostic challenges. Broadly, these abnormalities can be divided into those that are unilateral and bilateral. Bilateral abnormalities can further be subdivided into those that are midline (vermian), involve the pons in addition to the cerebellum (pontocerebellar), or mostly affect the cerebellar hemispheres. Genetic causes of cerebellar atrophy tend to be progressive and symmetric. A number of metabolic and genetic causes have now been identified that lead to cerebellar atrophy, and distinguishing between these syndromes clinically can be quite challenging, and if there is an obvious genetic mode of inheritance, then molecular biology and linkage analysis may lead to the most robust results about the etiology of cerebellar atrophy. Here, we present a consanguineous family, a first cousin once-removed union, with three affected and six healthy offspring. All affected demonstrate bilateral cerebellar atrophy in cranial magnetic resonance imaging. They have mental retardation and their gait is ataxic. We performed genome-wide homozygosity mapping and parametric linkage analysis using Affymetrix NSP 250K SNP genotyping chips. Affected only analysis revealed three loci with theoretical maximum LOD score of 2.65. Inclusion of unaffected sibling further refined the linkage, and only one locus on chromosome 5q31.1-q33.1 remained with a LOD score of 3.45. This region spans 14.5 million base pairs and contains 220 genes. There are two entries in OMIM associated with cerebellar ataxia from this genetic locus. Spinocerebellar ataxia 12, which is caused by mutations in PPP2R2B, is inherited in autosomal dominant fashion. Marinesco-Sjorgen Syndrome, a recessive syndrome caused by SIL1 mutations, is characterized by cerebellar ataxia, congenital cataracts, retarded somatic and mental maturation, hypergonadotropic hypogonadism and hypotonia. Although our patients lack the characteristic manifestations of this syndrome, we re-sequenced all the exons, and exon-intron boundaries of SIL1. No variants other than common SNPs were identified. We believe that this family represents a novel recessive form of cerebellar ataxia. Mutation screening is currently underway.

## 479/W/Poster Board #137

**Cerebellar dysfunction and glutamatergic deficits in murine models of spinocerebellar ataxia type 5 (SCA5).** *K.R. Armbrust[1,2], X. Wang[3], T. Obu[1,2], T.J. Ebner[3], L.P.W. Ranum[1,2].* 1) Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN; 2) Institute of Human Genetics, University of Minnesota, Minneapolis, MN; 3) Neuroscience, University of Minnesota, Minneapolis, MN.

Spinocerebellar ataxia type 5 (SCA5) is a slowly progressive neurodegenerative disease of the cerebellum caused by mutations in the *spectrin, beta, non-erythrocytic 2* gene, which encodes the protein β-III spectrin. Here we report the development and characterization of the first transgenic mammalian models of SCA5 which express mutant β-III spectrin in cerebellar Purkinje cells. Behavioral studies with a 3xFLAG-tagged SCA5 murine model and a second conditional murine model that drives expression of untagged β-III spectrin show that overexpressing mutant β-III spectrin in cerebellar Purkinje cells causes cerebellar dysfunction. Histologic analysis of the 3xFLAG-tagged murine model shows that the SCA5 mutation alters the Purkinje cell distribution of the mutant β-III spectrin protein itself. Further studies with the conditional tet-regulated mice show that overexpression of untagged mutant β-III spectrin alters the localization of the glutamate transporter EAAT4 and the metabotropic glutamate receptor mGluR1α. Additionally, *in vivo* optical imaging of flavoprotein autofluorescence shows that the SCA5 mice have a concomitant deficit in mGluR1-mediated long term potentiation (LTP). These findings suggest that abnormalities in perisynaptic glutamatergic transmission contribute to SCA5 pathogenesis.

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## 480/W/Poster Board #138

**Spinocerebellar ataxia type 12 identified in two Italian families.** *A. Brussino[1], C. Graziano[2], D. Giobbe[3], E. Dragone[1], M. Ferrone[1], R. Lodi[4], C. Tonon[4], A.S. Gabellini[5], R. Rinaldi[6], S. Miccoli[2], E. Grosso[1], M.C. Bellati[1], L. Orsi[7], N. Migone[1], A. Brusco[1].* 1) Department of Genetics, Biology and Biochemistry, University of Torino, and S.C.D.U. Medical Genetics, A.O.U. San Giovanni Battista, Torino Italy; 2) U.O. Medical Gentics, S.Orsola-Malpighi Hospital, and University of Bologna, Italy; 3) Neurology III, A.O.U. San Giovanni Battista, and Department of Neurosciences, University of Torino, Italy; 4) Dipartimento di Medicina Interna, dell'Invecchiamento,e delle Malattie Nefrologiche, University of Bologna, Italy; 5) U.O. Neurology, Ospedale Maggiore, Bologna, Italy; 6) Neurologic Unit, Policlinico S. Orsola-Malpighi, AOSP di Bologna, Italy; 7) Neurology I, A.O.U. San Giovanni Battista, and Department of Neurosciences, University of Torino, Italy.

SCA12 is an autosomal dominant cerebellar ataxia (ADCA) with onset in the 4th decade, clinically characterized by the association of action tremor of arms and heads, mild ataxia, dysmetria and hyperreflexia. Bradikinesia, and sensory/motor neuropathy are also present, and older subjects may develop cognitive decline. The genetic mutation underlying SCA12 is a ≥51 CAG expansion in the 5' region of the brain-specific regulatory subunit B of the protein phosphatase 2A gene (*PPP2R2B*). SCA12 is very rare, and no patient has been identified in Europe, except for a single ethnic group in India where it accounts for up to 16% of ADCAs probably due to a founder effect. As part of our diagnostic routine, we screened 181 ataxic patients for expansion in the SCA12 gene between 2003 and 2008. The survey comprised cases with autosomal dominant, probably recessive and unknown transmission. We found two carriers of an expanded SCA12 allele (57 CAG). One patient belonged to a family with a clear autosomal dominant transmission; the other was apparently sporadic. Clinical and genetic analyses in the two families revealed the presence of a symptomatic subject carrying the expansion in one and two asymptomatic carriers, aged 68 and 37 yrs, in the other. The two families originated from the Ferrara province, in north-eastern Italy: haplotype reconstruction showed that both shared a common haplotype of four microsatellites (one of which was internal to the *PPP2R2B* gene) and four intragenic SNPs, different from that described in Indian SCA12 patients. In the three affected patients, the disease onset at 53±7,6 yrs (range 45-60) with either action tremor of head and hands, or fine hands movement impairment, or gait instability. Cognitive impairment and psychiatric disorders were not reported. MRI, performed in symptomatic patients, showed mild cortical atrophy in two, associated with mild cerebellar atrophy in the third. These data indicate that, although rare, SCA12 can be found in Italy: considering the results of a previous survey of 225 ataxic patients, performed by our group (Brusco et al., 2004), SCA12 may count for up to 0.5% (2/406) of Italian SCAs. The age at onset and variability of symptoms are in agreement with those already described. Because of the late age at onset and slow progression, SCA12 subjects may be classified as sporadic ataxia: this suggests that SCA12 testing, due to its simplicity, should be routinely extended both to sporadic and familial cases.

## 481/W/Poster Board #139

**Spinocerebellar ataxia type 15: clinical and molecular-genetic features of two Italian families.** *E. Di Gregorio[1], G. Vaula[2], M. Godani[3], L. Orsi[4], S. Jensen[5], E. Salmon[6], G. Ferrari[7], S. Squadrone[8], M.C. Abete[8], C. Cagnoli[1], A. Brussino[1], A. Brusco[1].* 1) Gen Biol & Biochem, Univ Torino, and SCDU Medical Genetics, San Giovanni Battista Hospital, Torino, Italy; 2) Neurology II, A.O.U. San Giovanni Battista, and Department of Neurosciences, University of Torino, Italy; 3) Neurological Department, S. Andrea Hospital, La Spezia, Italy; 4) Neurology I, A.O.U. San Giovanni Battista, and Department of Neurosciences, University of Torino, Italy; 5) Neurological Department, Civic Hospital, Carrara, Italy; 6) Department of Neurology and Cyclotron Research Centre, University of Liege, Liege, Belgium; 7) UO Complessa di Neurologia, Ospedale di Ivrea, Italy; 8) C.Re.A.A., Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy.

SCA15 is a pure cerebellar ataxia characterized by onset between 10 and 50 yrs, very slowly progressive gait ataxia, dysarthria, titubation, postural or action tremor of hand, neck and trunk, mild hyperreflexia, gaze-evoked nystagmus, and impaired vestibulo-ocular reflex. MRI shows a marked cerebellar atrophy, particularly in the vermis, whereas the brainstem is not involved. Type 1 inositol 1,4,5-triphosphate receptor (*ITPR1*) is reported as the SCA15 causing gene. At present, six families are described worldwide: five carry a partial deletion of *ITPR1* and one a missense mutation. We examined a four generation Italian family segregating an autosomal dominant form of cerebellar ataxia, and found a positive linkage at the SCA15 locus. In this family and in 43 familial SCA index cases, we performed a gene-dosage sensitive quantitative PCR (qPCR) to search for *ITPR1* gene deletion, using a probe mapping in *ITPR1* exon 3. We identified two families with a deletion that was further characterized by a custom array-CGH spanning 3 Mb centred on the *ITPR1* gene. In one family, the deletion extended over ~238 Kb including *ITPR1* exons 1-39, while in the second it spanned ~447 Kb and comprised *SUMF1* exons 1-7 and *ITPR1* exons 1-59. In these two SCA15 families, clinical data were available for 10 patients and brain MRI for six. The disease onset between 25 and 72 yrs (50 ± 17 yrs, mean ± SD); the first symptoms were unsteadiness, gait ataxia and dysarthria, variably associated with nystagmus, hyperreflexia, dysmetria and dysphagia. In all affected subjects MRI showed cerebellar vermis atrophy with a mild involvement of the hemispheres in some individuals. Overall, the phenotype mainly overlapped known SCA15 cases. However, our data show that this disease may have a greater variability in the age at onset (25-72 yrs) than described and that we also noticed facial dyskinesias in four cases, never reported in SCA15 patients. The variable disease expression mimicked anticipation in one family. Our results further support *ITPR1* gene as causative of SCA15, and describe two novel families in Italy. Clinical signs of pure cerebellar ataxia with slow progression, and MRI showing predominant vermis atrophy, are suggestive of SCA15. In these cases, the search for *ITPR1* deletions by qPCR is mandatory and simple.

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## 482/W/Poster Board #140

**Molecular epidemiology of spinocerebellar ataxias in Japanese population.** *Y. Ichikawa, S. Tsuji, J. Goto.* Dept Neurology, Univ Tokyo, Tokyo, Japan.

**Background:** Spinocerebellar ataxias (SCAs) are heterogeneous neurodegenerative diseases characterized by progressive ataxia occasionally accompanied with other features including pyramidal and extrapyramidal signs. At least 26 gene loci associated for autosomal dominant cerebellar atxias (ADCAs) have been mapped. The disease genes have been identified in 15 SCA subtypes : SCA1, 2, Mahdado-Joseph disease (MJD)/SCA3, SCA5-8, SCA10-15, 17, 27 and dentarorubral-pallidoluysian atrophy (DRPLA). A single nucleotide substitution (-16C>T) in the 5'UTR of *PLEKHG4* was identified as the strongly associated with the families linked to chromosome 16q22.1 (16qADCA). **Objectives:** To reveal the frequencies of SCAs in Japanese population. **Patients and Methods:** The subjects include 480 Japanese patients with SCAs during the period 1993 and 2008 on consecutive referral basis for molecular diagnosis. Three hundred and forty subjects from 303 Japanese families had family history, 93 patients were diagnosed as sporadic ataxia, and 47 patient's family history were unclear because of insufficient information. Among the 303 families with hereditary ataxia, 3 families with consanguinity and only siblings in one generation were affected, consistent with an autosomal recessive pattern of inheritance. The gene loci of SCA1-8, MJD/SCA3, SCA12, SCA17 and DRPLA were examined by the fragment analyses of the PCR products containing triplet repeat sequences. Detection of the -16C>T substitution in *PLEKHG4* was accomplished by DHPLC analysis and direct sequencing method. **Results:** Among 303 families and 47 patients with insufficient family history, MJD was the most common inherited ataxias in Japanese population (91 families). The second common was SCA6 (49 families), and followed by DRPLA (48 families), 16qADCA (38 families), SCA1 (17 families), SCA2(10 families), SCA17(7 families) and SCA8(5 families). We found the first Japanese family with SCA12 mutation. SCA7 mutation has not been detected in our cohort. Among 93 patients diagnosed as sporadic ataxia, 17 patients s have expanded triplet repeats. Of these, the SCA6 mutation was the most frequently detected (9 cases, 52.9%). **Conclusion:** The most frequent ADCA in Japanese population was MJD, subsequently SCA6 was ranked at the second. The expanded triplet repeats of ADCAs were detected in sporadic cases. The causative genes of around 17 % of our 303 hereditary ataxias remained to be identified.

## 483/W/Poster Board #141

**SPG11 - clinical heterogeneity and genetic data in a large international cohort.** *A. Rolfs[1,2], U. Goelnitz[2], P. Bauer[3], K. Boycott[4], A. Dionne[5], N. Dupres[5], J. Girouard[6], P. Huan-Kee[7], W. Kristofertisch[8], L. Krogh[9], B. Lindvall[10], W. Meschino[11], K. Metcalfe[12], I. Meijer[5], I. Navarro Vera[13], A. Summers*[14], L. Velsher[14], M. Wittstock[15].* 1) Albrecht-Kossel-Institute, Univ Rostock, Rostock MV, Germany; 2) Centogene GmbH, Rostock, Germany; 3) Department of Human Genetics, University of Tübingen, Germany; 4) Children's Hospital of Eastern Ontario, Dept of Genetics, Ottawa, ON, Canada; 5) Dept of Neurological Science, Faculty of Medicine, Laval University, CHA-Enfant Jesus, Quebec, Canada; 6) CHA, Hopital Enfant-Jesus, Quebec, QC, Canada; 7) Singapore Baby & Child Clinic, Mount Elizabeth Medical Centre, Singapore; 8) Sozialmedizinisches Zentrum Ost, Donauspital, Wien, Austria; 9) Odense University Hospital, Dept of Clinical Genetics, Denmark; 10) Dept of Neurology, Örebro, Sweden; 11) North York Hospital, Genetics Program, Toronto, Canada; 12) Clincal Genetics, St. Mary's Hospital, Manchester, UK; 13) Centro de Analisis Geneticos, c/Santa Teresa 45, Zaragoza, Spain; 14) North York General Hospital, Toronto, Canada; 15) Dept of Neurology, University of Rostock, Germany.

Spatacsin mutations cause an autosomal - recessive (AR) form of hereditary spastic paraplegia (HSP), SPG type 11 (SPG11). Typically SPG11 is been diagnosed in cases with slight ataxia, thin corpus callosum (TCC), mental impairment, pyramidal signs, increased deep tendon reflexes and only rarely neuropathy. We have analysed in a large cohort of patients with HSP the clinical heterogeneity and the frequency of clinical symptoms. In a cohort of 251 patients with AR-HSP we analysed SPG11 gene by sequencing the gene including the exon-intron boundaries and deletion screening by MLPA. We have been able to detect 82 mutated alleles in 44 patients with 55 different mutations. In 7 cases we found homozygous mutations and in 6 patients we have not been able to detect a second mutation. For 36 patients we got detailed clinical data including MRI results (35 cases). Age at onset ranged from 8 to 38 years with a mean of 22.0+/-9.6. Onset was characterized by gait disorders (21/36, 58%), polyneuropathy (7/36, 19%), dementia, dystonia, tremor and dysarthria (each in three patients). Interestingly only in 15/34 (44%) a thin Corpus callosum (TCC) has been demonstrable. This low frequency of TCC is in contrast to other reports and raises the question of the specificity of TCC for SPG11. Larger deletions seem to correlate with a more severe phenotype including earlier manifestation. Our data demonstrate a great phenotypic variability and a high percentage of large deletions within the SPG11 gene.

## 484/W/Poster Board #142

**The genetic basis of EAST (Epilepsy, Ataxia, Sensorineural Deafness, Tubulopathy) syndrome.** *H.C. Stanescu[1], S. Feather[2], D. Bockenhauer[1], A.A. Zdebik[1], G. Landoure[1], O. Al Masri[3], Y. Anikster[4], E. Klootwijk[1], M. Arcos-Burgos[5], A. Dobbie[2], W.A. Gahl[6], R. Warth[7], E. Sheridan[2], R. Kleta[1].* 1) University College London / GOSH, London, United Kingdom; 2) Leeds & Bradford Teaching Hospitals / University of Leeds, Leeds, UK; 3) Sheikh Khalifa Medical City, Abu Dhabi, UAE; 4) Sheba Medical Center, Tel-Hashomer, Israel; 5) University of Miami, Miami, FL, USA; 6) National Institutes of Health, Bethesda, MD, USA; 7) University of Regensburg, Regensburg, Germany.

Five patients from two consanguineous families presented with epilepsy beginning in infancy, severe ataxia, moderate sensorineural deafness, and a renal salt losing tubulopathy with normotensive hypokalemic metabolic alkalosis. We investigated the genetic basis of this hitherto unknown autosomal recessive disease, which we call EAST syndrome. Whole genome linkage analysis was performed in an extended inbred family with four affected children. Newly identified mutations in a potassium channel gene were evaluated using a heterologous expression system. Protein expression and function were further investigated in genetically modified mice. Linkage analysis identified a single relevant locus on chromosome 1q23.2 with a LOD score of 4.98. This region contained KCNJ10, which encodes a potassium channel expressed in brain, inner ear, and the kidney. Sequencing of this candidate gene revealed homozygous missense mutations in affected persons in both families. These mutations, when expressed heterologously in Xenopus oocytes, significantly and specifically decreased potassium currents. Mice with Kcnj10 deletions became dehydrated and exhibited definitive evidence of renal salt wasting. In conclusion, mutations in KCNJ10 cause this specific disorder consisting of epilepsy, ataxia, sensorineural deafness, and tubulopathy. Our findings indicate a major role for KCNJ10 in hearing, brain activity, renal salt handling and, hence, possibly also in blood pressure maintenance and its regulation.

## 485/W/Poster Board #143

**SCA8 Generates from homozygous mothers and coexists with several SCA/FRDA gene expansions.** *V. Volpini[1], H.S. Nicolás[1], J. Corral[1], L. De Jorge[1], J. Infante[2], O. Combarros[2], J. Berciano[2], M. Calopa[3], A. Matilla[4], D. Genís[5].* 1) Molecular Genetics Diagnosis, IDIBELL, L'Hospitalet de LLobregat, Barcelona, Spain; 2) Dep. of Neurology, Hosp. Univ. Marqués de Valdecilla, Santander, Spain; 3) Dep. of Neurology, Hosp. Univ. de Bellvitge, L'Hospitalet de LLobregat, Barcelona, Spain; 4) Health Sciences Research Institute Germans Trías i Pujol, Badalona, Barcelona, Spain; 5) Dep of Neurology, Hosp. Univ. Josep Trueta, Girona, Spain.

Spinocerebellar ataxias (SCA) are a clinically and genetically heterogeneous group of neurodegenerative disorders in which several genes have been cloned: SCAs1-3, SCAs6-7, SCA12 and SCA17; mainly sharing a CAG repeat expansion mutation which generally encodes a polyglutamine tract. In SCA8 the mutation is a CTG/CAG repeat. In our casuistic, over 162 unrelated index cases 6.17% were SCA1; 25.93% SCA2; 33.95% SCA3; 7.41% SCA6; 6.17% SCA7; 15.43% SCA8; 1.85% SCA17; and 3.09% DRPLA. In 25 SCA8 index cases the allele range goes from 85 to 726 repeats (143.27% ±67.47%; Pearson Coef. = 47.09%). Maternal transmissions elongate the CTG combined sequence from +3 to +13 repeats. Paternal transmissions contract from -1 to -17 repeats. We have found several giant SCA8 expansions in two families, with CTG ranging from 375 to 1,126 (N= 12) repeats. All 12 adult carriers remain unaffected until now. All of them have inherited the giant SCA8 CTG expansions from homozygous SCA8 mothers that have genotypes with alleles of moderate size. Studying 90 individuals from general population the distribution of SCA8 results in two major groups: a) 15 to 34 CTGs, with frequency 98%; and b) 77 to 86 CTGs, with frequency 2%. We have found three pedigrees in which SCA8 expansions coexist respectively with SCA2, SCA3 and Friedreich's ataxia (FRDA) in the respective index cases. In all of three families the expansions segregates independently in the rest of relatives. Other family shows SCA8 and SCA1 expansions in several members, but none joint both. The same occurs in a pedigree with FRDA and SCA8 and in other pedigree with SCA6 and SCA8. Our very preliminary data suggest that the familial coexistences could be due to high population prevalence of no penetrant SCA8 alleles. More studies will be necessary to conclude other putative cause-effect relation, if exist, involving SCA8 expansions in the origin of other triplet elongations from a founder common ancestry.

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## 486/W/Poster Board #144

**To Screen or not to Screen: *GJB2* in American Minorities.** *J. Samanich[1], J. Shan[2], J. Chobot-Rodd[3], R. Castellanos[2], M. Babcock[2], A. Shanske[1], S. Parikh[4], B. Morrow[2].* 1) Center for Congenital Disorders, Children's Hospital at Montefiore, Bronx, NY, USA; 2) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY, USA; 3) Children's Evaluation and Rehabilitation Center, Albert Einstein College of Medicine, Bronx, NY, USA; 4) Department of Otolaryngology, Montefiore Medical Center, Bronx, NY, USA.

In many ethnic populations, up to 50% of persons with familial autosomal recessive nonsyndromic sensorineural hearing impairment (NSHI) have biallelic mutations in *GJB2*. We reported the paucity of mutations in *GJB2* and deletions in *GJB6* in Caribbean Hispanic and African admixture populations in 2007. We have now collected 102 additional subjects with NSHI, for a total of 209. Of the 209, 36% are inherited and the rest have sporadic occurrence. The hearing impairment ranged from unilateral mild to bilateral profound, with the majority in the latter category. Combining results we sequenced the single coding exon of the *GJB2* gene in 209 subjects, PCR screening for del(GJB6-D13S1830) in 32 NSHI patients with a heterozygous variation in *GJB2*, and Multiplex Ligation-Dependent Probe Amplification (MLPA) testing of *GJB2*, *GJB3* and *GJB6* exon deletions or amplifications (P163- GJB-WFS1 kit) in 70 subjects. Seven unrelated individuals had biallelic *GJB2* mutations of 209 total in both studies representing 3% of our population. Of 418 chromosomes examined, variations of *GJB2* sequence found and (number of chromosomes) were: 35delG (mutation; 10), 94C>T (mutation, R32C; 3), 139G>T (mutation, E47X; 3), 167delT (mutation; 3), 269T>C (mutation, L90P; 1), 427C>T (mutation, R143W; 1), 101T>C (mutation vs. polymorphism; M34T; 1), 109G>A (mutation vs. polymorphism; V37I; 2), -15C>T (polymorphism; 3), 79G>A (polymorphism, V27I; 10), 380G>A (polymorphism, R127H; 8), 457G>A (polymorphism, V153I, 2), 670A>C (indeterminate, K224Q; 2), -3331C>G (novel; 1), -6T>A (novel; 3), 503A>G (novel, K168R; 5), and 684C>A (novel; 1). Of 158 probands of Hispanic or African descent, five (3%) had biallelic pathogenic mutations, four had monoallelic mutations and 149 had no disease-causing mutations in *GJB2*. At the same time, no major deletions were identified either by PCR screening (del [GJB6-D13S1830]) or by MLPA analysis (*GJB2* or *GJB6*). These results re-confirmed our previous report that *GJB2* is not the major contributor to the genetic basis of NSHI for the Bronx minority admixture populations.

## 487/W/Poster Board #145

**Unique spectrum of MYO15A mutations associated with autosomal recessive deafness in Turkey.** *M. Tekin[1,2], F.B. Cengiz[2], D. Duman[2], A. Sirmaci[1,2], S. Erbek[3], H. Ozturkmen-Akay[4], H. Ozdag[5].* 1) Dept Human Genetics, Univ Miami, Miami, FL; 2) Div Pediatric Genetics, Ankara Univ, Ankara, Turkey; 3) Dept Otorhinolaryngology, Baskent Univ, Ankara, Turkey; 4) Dept Radiology, Dicle Univ, Diyarbakir, Turkey; 5) Biotechnology Institute, Ankara Univ, Ankara, Turkey.

Biallelic mutations in the MYO15A gene at DFNB3 locus have been shown to cause nonsyndromic deafness. In order to find causative mutations, we first screened 104 unrelated multiplex Turkish families with nonsyndromic deafness using homozygosity mapping with Affymetrix microarrays or microsatellite markers. Parents were consanguineous and GJB2 mutations were negative in each family. Screening of linked families for mutations in 66 exons and flanking intronic regions of the MYO15A gene using SSCP and DNA sequencing showed five previously unreported mutations in eight families including c.5807_5813delCCCGTGG (p.Ala1936fs) (3 families), c.10002_10003dupCCGGCCC (p.Ser3335fs) (2 families), c.867C>G (p.Tyr289Ter), c.4198G>A (p.Val1400Met), and c.4439T>C (p.Ser1481Pro). Each homozygous mutation cosegregated with deafness and was absent in Turkish hearing controls. Recurrent mutations were associated with conserved haplotypes in unrelated families, suggesting founder effects. Biallelic GJB2 mutations have been reported to present in 18.9% of comparable families in Turkey. In conclusion, we estimate the prevalence of homozygous MYO15A mutations in autosomal recessive nonsyndromic deafness in Turkey as 0.098 (95% CI is 0.041-0.155), which demonstrates their significant contribution. This study was supported by TUBITAK (105S464).

## 488/W/Poster Board #146

**Large international cohort study in CMT - frequency of subtypes and clinical phenotype.** *U. Goelnitz[1], C. Tanislav[2], S. Baker[3], K. Lagerstedt[4], Y. Mehraein[5], M. Wittstock[6], V. Mering[7], H. Kingston[8], L. Krogh[9], T. Boettcher[10], P. Bauer[11], A. Rolfs[1, 12].* 1) Molecular Genetics, Centogene GmbH, Rostock, Germany; 2) Dept. of Neurology, Universität Giessen, Am Steg 16, 35385 Gieβen, Germany; 3) McMaster University, Physical Medicine and Neurology, 1200 Main Street West, Hamilton, Canada; 4) Dept of Clincial Genetics, Karolinska University Hospital, Solna, Stockholm. Sweden; 5) Institut für Humangenetik, Universitätsklinikum Homburg, Germany; 6) Dept of Neurology, University of Rostock, Gehlsheimerstr. 20, 18147 Rostock, Germany; 7) Dept of Neurology, University of Dresden, Fetscherstrasse 74, Dresden, Germany; 8) St. Mary 's Hospital, Clinical Genetics, Hathersage Road, Manchesgter, England; 9) Dept. of Clinical Genetics, SDR, Boulevard 29, Odense, Denmark; 10) Klinikum Neubrandenburg, Dept. of Neurology, Neubrandenburg, Germany; 11) Dept. of Clinical Genetics, University of Tübingen, Tübingen, Germany; 12) Albrecht-Kossel-Institute for Neuroregeneration, University of Rostock, Germany.

Charcot-Marie-Tooth (CMT) disease or hereditary motor and sensory neuropathy (HMSN) is caused by mutations in several genes expressed in myelinating Schwann cells and the axons they ensheathe. CMT include a wide range of pathological disorders characterized by damage of peripheral nerves. So far, there are five different types of CMT (1, 2, 3, 4, X) with more than 35 subtypes, associated with more than 40 genes. The most frequent types are CMT1, CMT2 and CMTX. CMT1A is associated with tandem duplication of a 1.5 Mb DNA fragment on chromosome 17p11.2-p12 that codifies the peripheral myelin protein PMP22. A straight on genetic analysis of the clinically heterogenous cases is hampered by the large number of genes being involved in the pathophysiology. The products of genes associated with CMT phenotypes are important for the neuronal structure maintenance, axonal transport, nerve signal transduction and functions related to the cellular integrity. Here we describe a large international cohort of 1.246 patients with positive family anamnesis for polyneuropathy and where genetic material has been send to us for detailed genetic analysis. In 478 patients (38,4%) we have been able to demonstrate the genetic basis of the polyneuropathy by complete sequencing of the causative genes: CMT1 (including HNPP): 21,9%; CMT2: 12,8%; CMT4: 3.6%. In the CMT1 group pmp22 and MPZ gene cause nearly 60% of all CMT1 cases, followed by CMT1c (LITAF, 25 pts.); CMT1d (EGRS2, 12 pts.) and CMT1f (NEFL, 8 pts.). In CMT2 group we were able to detect the following frequencies: CMT2A2 (MFN2, 42 pts.; 26.3%); CMT2d (GARS, 39 pts.); CMT2k (GDAP1, 21 pts.) and CMT2A1 (KIF1b, 19 pts.); CMT2b, CMT2b1, CMT2e, CMT2f and CMT2i have been detected in less than 5% of all CMT2 cases. CMT4 cases reveal the largest clinical heterogeneity: CMT4a (GDAP1) describes 40,7% of all CMT4 cases and 2.3% of all genetically clarified cases. This is followed by CMT4c1 (LMNA, 6 pts.), CMT4b2 (SBF2, 4 pts.), and CMT4b1, CMT4c and CMT4c4 with less than 4 cases. The results of detailed molecular genetic investigations have impact on the appropriate diagnosis, genetic counselling and possible new therapeutic options for CMT patients.

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## 489/W/Poster Board #147

**A comprehensive diagnostic system for hereditary spastic paraplegia employing resequencing microarray, Sanger sequencing and comparative genomic hybridization array allows efficient identification of various types of mutations in the causative genes.** *H. Ishiura, Y. Takahashi, J. Goto, S. Tsuji.* Dept Neurology, Univ Tokyo, Tokyo, Japan.

[Objective] To establish a high throughput molecular diagnosis system for hereditary spastic paraplegia (HSP), where various kinds of mutations including point mutations, insertion/deletions and copy number variations have been described. [Background] HSP is a genetically heterogeneous neurodegenerative disorder which is clinically characterized by progressive lower limb spasticity and pyramidal weakness. Up to present, more than 40 (SPGs 1-45) genetic loci and 19 causative genes have been identified. To establish genotype-phenotype correlation and to understand pathophysiology, comprehensive genetic analysis is demanded. [Method] We established a high-throughput DNA microarray resequencing system (Affymetrix) for analyzing complete nucleotide sequences of all the coding exons and splicing junctions of 13 causative genes (*L1CAM*, *PLP1*, *atlastin*, *spastin*, *NIPA1*, *paraplegin*, *KIAA0196*, *KIF5A*, *HSP60*, *BSCL2*, *spartin*, *maspardin*, and *REEP1*). To detect small insertion/deletion mutations frequently found in *spastin* and *REEP1*, Sanger method was employed in these 2 genes. For patients with thin corpus callosum and cognitive dysfunction, *SPG11* was analyzed with Sanger method because SPG11 is frequent in such patients. In addition, to detect large rearrangements, we designed an oligonucleotide CGH array (Agilent, 1 probe / 200bp in average) in which sequences of 16 causative genes (*CYP7B1*, *SPG11*, and *ZFYVE27* in addition to 13 genes above) were tiled. The study enrolled 128 HSP patients: 49 patients with autosomal dominant inheritance (ADHSP), 11 autosomal recessive patients, 6 familial patients with undetermined mode of inheritance, and 62 apparently sporadic patients. [Results] In 49 ADHSP patients, we detected 27 *spastin*, 1 *atlastin*, 2 *KIAA0196*, and 2 *REEP1* mutations, in whom 65% (32/49) were diagnosed in total. *Spastin* mutations were found in familial (1/6) and sporadic (4/62) patients. In addition, 4 SPG11 were found in 12 patients with thin corpus callosum and cognitive dysfunction. Collectively, 33% (42/128) of patients were given diagnosis with various kinds of mutations; 22 point mutations, 14 small insertion/deletions, and 8 large insertion/deletions. [Conclusion] The combination of three methods found nucleotide substitutions, small insertion/deletion mutations, and large rearrangements effectively. The system is suitable for the comprehensive mutational analysis of HSP.

## 490/W/Poster Board #148

**Adult-onset spastic paraplegia type 2 with a novel mutation in the exon 7 of *PLP1*: An autopsy case.** *A. Iwaki[1], S.O. Suzuki[2], K. Arakawa[3], K. Furuya[3], N. Fujii[3], Y. Fukumaki[1], T. Iwaki[2].* 1) Div. Hum. Mole. Genet., Res. Ctr. Genet. Info., Med. Inst. Bioreg., Kyushu Univ., Fukuoka, Japan; 2) Dep. Neuropath., Neurol. Inst., Grad. School of Med. Sci., Kyushu Univ., Fukuoka, Japan; 3) Dep. Neurol., Neuro-Muscular Cent., Omuta Hosp., Fukuoka, Japan.

Spastic paraplegia type 2 (SPG2) and Pelizaeus-Merzbacher disease (PMD) are allelic disorders caused by mutations in the X-linked gene for myelin proteolipid protein 1 (*PLP1*). Different point mutations of *PLP1* lead to a variety of clinical phenotypes from mild form (SPG2) to the most severe dysmyelinating form (connatal PMD). The onset of SPG2 is usually in the first decade of life, and adult-onset is rare. We experienced an autopsy case of adult-onset spastic paraplegia. The patient was a 66-year-old male whose elder brother had died of a similar disease. At the age of 33, he noticed difficulty in walking. He gradually became abasic in 7 years. He also developed progressive dementia and eventually became bed-ridden by 5 years before death. He died of pneumonia. At autopsy, the brain weight was 1275g. Diffuse, moderate atrophy of the cerebrum with the dilated ventricular system and thin corpus callosum was noted. Histopathologically, the central nervous system showed widespread myelin paler in the white matter. By contrast, the gray matter and the peripheral nerves were well preserved. Direct sequencing of the seven exons of the *PLP1* gene revealed a point mutation, Tyr262Cys, located at the C-terminal end of PLP1, which is composed of 276 amino acids. The clinical course suggests that this novel mutation may affect maintenance of myelin rather than maturation of oligodendrocytes.

## 491/W/Poster Board #149

**Loci for human cortical malformations revealed by copy number analysis on high-density SNP array.** *G.M. Mancini[1], R. Schot[1], B.C. Dumee[2], M.C.Y. de Wit[3], I.F.M. de Coo[3], M.H. Lequin[4], W.B. Dobyns[5], P.J. van der Spek[2], J.M.H. Verkerk[2].* 1) Clinical Genetics, Erasmus Univ MC, Rotterdam, Netherlands; 2) Bioinformatics, Erasmus Univ MC, Rotterdam, Netherlands; 3) Child neurology, Erasmus Univ MC, Rotterdam, Netherlands; 4) Radiology, Erasmus Univ MC, Rotterdam, Netherlands; 5) Human Genetics, Univ Chicago, Illinois.

Approximately 220 patients with malformations of cortical brain development (MCD), like microcephaly with simplified gyration, lissencephaly, heterotopia, (non-TSC) focal dysplasia and polymicrogyria, were recruited in 15 years at our academic hospital. An etiological diagnosis was possible in 40 percent of the cases after systematic neuro-radiologic, clinical genetic, routine cytogenetic/FISH and molecular genetic tests (de Wit et al., Archives of Neurology, 2008). In DNA of the remaining circa 140 patients and parents, if available, analysis of copy number variations (CNV) was performed on Affymetrix 250K SNP arrays (CNAG program). Miller-Dieker microdeletions were excluded a priori. CNVs were considered pathogenic if 1) not reported in the Toronto database (DGV) as polymorphic, 2) confirmed by independent techniques (FISH, Q-PCR), 3) included known microdeletion/duplication syndromes or 4) were de novo and included (candidate) genes, and 5) parents were available for testing. We identified pathogenic changes in about 13 percent of the patient samples, mostly from the polymicrogyria and heterotopia group. Additionally, an interesting group was represented by about 10 samples regarding unclassified variants (UVs) and containing 1) inherited homozygous deletions/duplications from heterozygous parents, 2) inherited homozygous small intron deletions in candidate genes, 3) deletions/duplications reported for rare and different clinical phenotypes, 4) de novo deletions in “gene desert” areas linked to disease phenotype, 5) microdeletions smaller than 1Mb including miR loci. Each of these concerned unique patients and MCD phenotypes. Most microarray data concern patients with MR/MCA without brain imaging data. Exchanging results and experience from array data with rare UVs, in properly classifed cohorts, will greatly speed up the process of identifying new genes and disease mechanisms for cortical malformations.

## 492/W/Poster Board #150

**The spectrum of collagen IV alpha-1 and -2 mutations in humans.** *F.W. Verheijen[1], E. Verbeek[1], M.W. Wessels[1], D.J. Licht[2], P. Govaert[3], R. Hintzen[4], M.H. Lequin[5], J. Dudink[3,5], I.F.M. de Coo[4], M. Meuwissen[1], G.M. Mancini[1].* 1) Clinical Genetics, Erasmus Univ MC, Rotterdam, Netherlands; 2) Division of neurology, Children’s Hospital, Philadelphia, Pennsylvania; 3) Neonatology, Erasmus Univ MC, Rotterdam, Netherlands; 4) Neurology, Erasmus Univ MC, Rotterdam, Netherlands; 5) Radiology, Erasmus Univ MC, Rotterdam, Netherlands.

Collagen IV abnormalities have been related to a variety of renal problems and stroke-like events in animal models. Collagen IV is the main non-fibrillary collagen forming a mesh network around vascular endothelia and contributing to the normal resistance of the vascular wall. Six genes (three couples, each couple in tandem on the same chromosome) encode for collagen alpha-IV chains. Col4a1 and col4a2 are mapped to the synthenic area of human chromosome13q34, sharing the same promoter. Procollagen-4alpha1 and -4alpha2 molecules together contribute to the assembly of the collagen IV triple helix, both together with a 2:1 ratio, or associated with procollagen-4alpha-5 chains. Mutations in both col4a1 and col4a2 essentially induce prenatal bleeding in brain and eyes of mouse mutants, documented by weakness and abnormal structure of the extracellular basement membrane of vascular endothelia. In humans, mutations in COL4A1 have been associated with the occurrence of several phenotypes including familial porencephaly, prenatal intraparenchymal hemorrhage, recurrent intraparenchymal stroke, cataract, retinal microbleeds, or with hereditary angiopathy, nephropathy, aneurysms and muscle cramps (HANAC syndrome). Although in mice similar phenotypes have been described for both col4a1 and col4a2 mutations, until now no COL4A2 mutations have been reported. We previously reported pathogenic COL4A1 mutations in four families with intraparenchymal cerebral stroke ranging from documented prenatal bleeding to adult onset stroke. We now report new variants of COL4A1 mutation phenotypes with severe brain and ocular abnormalities and, to our knowledge, for the first time human familial COL4A2 mutations.

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## 493/W/Poster Board #151

**Spontaneously active glycine receptors: a novel pathogenic mechanism in hyperekplexia.** *S. Chung[1], J-F. Vanbellinghen[2], J. Mullins[1], D.F. Gilbert[3], J. Hantke[4], R.J. Harvey[4], J.W. Lynch[3], M.I. Rees[1,5].* 1) Dept Neuroscience, Univ Wales, Swansea, Swansea, United Kingdom; 2) Laboratoire de Génétique Moléculaire, University of Liège, Liège, Belgium; 3) Queensland Brain Institute, University of Queensland, Brisbane, Australia; 4) Department of Pharmacology, School of Pharmacy, London, United Kingdom; 5) Institute of Medical Genetics, Cardiff University, United Kingdom.

Hereditary hyperekplexia, or startle disease, is caused by genetic defects in glycinergic inhibitory system. This rare but treatable neurological disorder is characterized by an exaggerated startle reflex and extreme muscle stiffness in response to sudden, unexpected auditory or tactile stimuli. To date, hyperekplexia mutations have been identified in several genes involved in the function of glycinergic synapses. Mutations in GLRA1 encoding the glycine receptor (GlyR) $\alpha$1 subunit are the most common cause, accounting for ~70% of previously reported hyperekplexia mutations, followed by defects in SLC6A5 encoding the glycine transporter GlyT2 accounting for ~20% of cases. Functional analysis of GLRA1 mutations indicated that glycinergic transmission is disrupted by defective allosteric coupling of agonist binding to chloride ion channel gating and/or impaired cell membrane trafficking of GlyRs. In this study, we describe a novel dominant GLRA1 mutation that results in the substitution of a tyrosine to a cysteine at amino acid position 128 (Y128C). Whilst the majority of dominant mutations are located in the pore-forming M2 domain or flanking regions of the GlyR $\alpha$1 subunit, the new dominant mutation Y128C is located in the N-terminal extracellular domain. When transiently expressed in HEK293 cells, either as homomeric $\alpha$1 GlyRs or heteromeric $\alpha$1 $\beta$ GlyRs, the Y128C substitution resulted in spontaneously-opening channels. Although, tonic channel opening has been recognized as one of the common mechanisms underlying channelopathies, this is the first report of a hyperekplexia mutation associated with leaky current. The discovery of mutations associated with hyperekplexia coupled with a detailed understanding of the molecular basis of GlyR impairment provides invaluable insights into the pathophysiology of this disorder.

## 494/W/Poster Board #152

**Severe orthopedic and gastrointestinal complications in a child with generalized myokymia and a *KCNA1* mutation.** *L. Seaver[1,2], K. Nagamori[2], G. Yim[2], E. Raney[3], C. Wang[4], D. Sureka[5], G. Enns[5].* 1) Kapiolani Medical Specialists, Honolulu, HI; 2) Department of Pediatrics, John A. Burns School of Medicine Honolulu, HI; 3) Shriners Hospital, Honolulu, HI; 4) Division of Neurology, Stanford University School of Medicine, Stanford, CA; 5) Division of Medical Genetics, Stanford University School of Medicine, Stanford, CA.

*KCNA1* mutations have been associated with Episodic Ataxia type 1, which can present with symptoms of dizziness, ataxia, seizures and myokymia. We report a case with early onset neuromuscular disease causing significant orthopedic and gastrointestinal complications.

The now 8 year old boy presented with firm abdomen since infancy. He required umbilical and inguinal herniorrhaphy at age 4. Short stature, gross motor delay, joint contractures and a crouched gait were evaluated at age 2-3 yr including normal serum CK and MRI of brain and spine. Muscle biopsy showed fiber type disproportion with type I predominance. Sural nerve biopsy was normal. EMG and NCV showed no evidence of myopathy, but a neuropathic process involving motor nerves with significant axonal loss. Between ages 5-8 yr he has had progressive deformation of his ribs, pectus carinatum, hip dislocation and loss of ambulation due to contractures. He has required soft tissue releases of knees and ankles. Gastroesophageal reflux, episodic vomiting, hypoglycemia and failure to thrive led to the diagnosis of a large hiatal hernia which required surgical repair.

His examination at age 5 yr showed short stature (HA 30 months), weight 50th centile for HA, OFC 25-50th centile, a rigid abdomen, fixed flexion contractures of hips, knees and ankles, finger contractures with full passive range of motion, visible and palpable repetitive muscle contractions of arms and fingers. The diagnosis of hyperekplexia was considered. Family history revealed a father and aunt with hand tremor. *GLRA1* sequencing was negative. A heterozygous mutation (Phe184Cys, c.551T>G) in *KCNA1* was recently identified, confirming the diagnosis of myokymia associated with Episodic Ataxia type 1.

Kinali et al. reported a boy with infantile onset of symptoms including muscle stiffness and motor delay with progressive short stature, kyphoscoliosis, hiatal and inguinal hernias. Spells of dizziness began at age 10 (Neuromuscul Disord 14:689, 2004). Our case has similar but more rapid and severe progression of symptoms. Both cases emphasize significant skeletal and GI complications and extreme variability in familial symptoms suggesting that this diagnosis should be considered in the differential diagnosis of hyperekplexia and neuromyotonia in children.

## 495/W/Poster Board #153

**TOPORS, mutated in retinal degeneration, is a novel centrosomal and ciliary protein.** *A.Z. Shah[1], H. Khanna[2], T. Sedmak[3], K. Nagel-Wolfrum[3], C.A. Murga-Zamalloa[2], I. Lopez[4], M. Papaioannou[1], P. Munro[1], M. Cheetham[1], R.M. Rios[5], R. Koenekoop[4], P. Beales[6], K. Matter[1], U. Wolfrum[3], A. Swaroop[7], S.S. Bhattacharya[1], C. Chakarova[1].* 1) Institute of Ophthalmology, UCL, London EC1V 9EL, UK; 2) Department of Ophthalmology, University of Michigan, Ann Arbor, Michigan-48105, USA; 3) Johannes Gutenberg University of Mainz, Institute of Zoology, D-55099 Mainz, Germany; 4) The McGill Ocular Genetics Laboratory, McGill University Health Centre, Montreal, Quebec H3H 1P3, Canada; 5) CSIC-CABIMER, Seville-41092, Spain; 6) Molecular Medicine Unit, Institute of Child Health, University College London, London, UK; 7) 7Neurobiology-Neurodegeneration and Repair Laboratory (N-NRL), National Eye Institute, NIH, Bethesda, Mariland-20892, USA.

**Purpose:** After identifying mutations in TOPORS which cause autosomal dominant retinitis pigmentosa we undertook this study to better understand the localization and the role of this ubiquitously expressed protein in photoreceptors, dividing and quiescent cells. **Methods:** Subcellular localization of TOPORS was examined in human and mouse retina and in cultured cell lines by confocal immunofluorescence. Light- and dark-adapted mice were used to assess potential light-evoked differential localization of TOPORS in photoreceptors. The effect of TOPORS knockdown was assessed by RNAi. Yeast two-hybrid was used to determine whether TOPORS interacts directly with proteins from retrograde transport that have previously shown to co-IP. **Results:** Here we report for the first time that TOPORS, which is mutated in patients with autosomal dominant retinal degeneration, is a novel component of the centrosome in dividing cells, ciliary basal body in quiescent cells and connecting cilium in photoreceptors. Immunocytochemical studies demonstrate that endogenous TOPORS exhibits cell cycle dependent localization in the nucleus, centrosome and midbody. TOPORS was localized to the inner centriolar cylinder using ninein and $\beta$-arrestin-2 which is suggestive of a role in centriole biogenesis and stability. However, knockdown of expression of TOPORS does not affect the number of centrioles, or microtubule organisation. TOPORS remained associated with the centrosome even when retrograde transport was disrupted by overexpressing p50-dynamitin. TOPORS localizes only to the connecting cilia in light adapted mice and distributes diffusely throughout the whole photoreceptor cell when the mice are dark adapted. Moreover, TOPORS exhibits differential association with visual arrestin and transducin in light-adapted condition. We observed no co-localization of TOPORS with PNA (marker for cone photoreceptors) demonstrating its absence from the cone photoreceptor cells in human and mouse retinas. Yeast two-hybrid results indicated TOPORS does not interact with retrograde transport proteins directly. **Conclusions:** TOPORS is a novel component of the cilia in rod photoreceptor cells and is also localized in the basal body of the cilia in quiescent cells and in the centrosome of dividing cells. Our work highlights the increasing importance of ciliary function and adds to the growing list of ciliopathies.

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## 496/W/Poster Board #154

**NOVEL VLDLR MICRODELETION IDENTIFIED IN TWO TURKISH SIBLINGS WITH PACHYGYRIA AND PONTOCEREBELLAR ATROPHY.** *L.E. Kolb[1,2], Z. Arlier[1,2], C. Yalcinkaya[3], A.K. Ozturk[1,2], O. Erturk[3], F. Bayrakli[1,2], K. Bilguvar[1,2], K. Yasuno[1,2], T. Ozcelik[4,5], B. Tuysuz[6], M.W. State[2,7,8], M. Gunel[1,2,7,9]*. 1) Neurosurgery, Yale University School of Medicine, New Haven, CT; 2) Program on Neurogenetics, Yale University School of Medicine, New Haven, CT; 3) Neurology, Division of Child Neurology, Istanbul University Cerrahpasa Faculty of Medicine, Istanbul, Turkey; 4) Department of Molecular Biology and Genetics, Faculty of Science, Bilkent University, Ankara, Turkey; 5) Institute of Materials Science and Nanotechnology, Bilkent University, Ankara, Turkey; 6) Pediatrics, Division of Genetics, Istanbul University Cerrahpasa Faculty of Medicine, Istanbul, Turkey; 7) Genetics, Yale University School of Medicine, New Haven, CT; 8) Child Study Center, Yale University School of Medicine, New Haven, CT; 9) Neurobiology, Yale University School of Medicine, New Haven, CT.

Congenital ataxia with cerebellar hypoplasia is a heterogeneous group of disorders that presents with motor disability, hypotonia, incoordination, and impaired motor development. These disorders present initially with general symptoms such as delayed motor milestones and hypotonia during the post natal period and early childhood, followed by the gradual onset of ataxic gait during the first few years of life. Some cases improve as motor functions develop, while others worsen early during infancy as motor demands increase on coordination. This heterogeneous group of disorders has been associated with multiple other diseases including brain malformations, genetic syndromes, and congenital infections. It is thought that 4% of cases are due to a perinatal cause, while 45% of the cases are due to prenatal causes. The remaining half is speculated to be of unknown etiology. Of the associated brain malformations, cerebellar hyoplasia seems to be the most common, occurring in slightly less than 50% of cases. The most severe cases have been noted to have marked hyoplasia of the vermis, and mild to moderate involvement of the neocerebellum, but in other cases the imaging findings have not correlated with the severity of the disease. Recently mutations in the Very Low Density Lipoprotein Receptor (VLDLR) have been identified in patients in the Turkish, Iranian and Hutterite populations with cerebellar atrophy and congenital ataxia following an autosomal recessive type of inheritance. The Hutterite and Iranian patients presented with Dysequillibrium syndrome, while the Turkish patients exhibited quadrupedal gait. We present a new Turkish family with two siblings affected with cerebellar atrophy and pachygyria with a novel homozygous deletion in the VLDLR gene identified by using high density single nucleotide polymorphism (SNP) chips for homozygosity mapping and identification of copy number variations (CNV) within these regions. The patients are offspring of a first cousin mating and suffer from ataxia, dysarthria, dysmetria, and dysdiadochokinesis. Magnetic resonance scans of affected siblings show pachygyria and cerebellar atrophy. Mapping and crossing of the breakpoint was performed which revealed a 21,194 bp deletion including exons 2, 3, 4, and part of exons 1 and 5 that begins on 2,612,144 bps and ends on 2,633,338 bps on Chromosome 9. Our findings confirm that VLDLR is an integral part of cerebellogenesis.

## 497/W/Poster Board #155

**Mutations in PCDH19 (protocadherin 19) are a frequent cause of isolated infantile epileptic encephalopathy in females.** *C. Depienne[1, 2, 3], D. Bouteiller[1], B. Keren[2], E. Cheuret[4], K. Poirier[5], O. Trouillard[2], A. Brice[1, 2, 3], R. Nabbout[6], E. LeGuern[1, 2, 3]*. 1) INSERM UMR_S975, Paris, France; 2) AP-HP, Département de génétique et cytogénétique, Fédération de Génétique, Hôpital de la Salpêtrière, F-75013, Paris, France; 3) Université Pierre et Marie Curie-Paris6, CNRS, UMR-S975, Paris, F-75013, France; 4) Service de neurologie pédiatrique, Hôpital des enfants, Centre Hospitalier Universitaire de Toulouse, Toulouse, France; 5) Institut Cochin, Inserm U567, UMR 8104, Université René Descartes, Paris 5, France; 6) Département de Neuropédiatrie, AP-HP, Hôpital Necker-Enfants malades, Paris-Descartes, Paris, France.

Dravet syndrome (DS) is a sporadic, early-onset epileptic encephalopathy characterized by the association of febrile and afebrile seizures of multiple types and cognitive and language impairment; 75% of the patients with DS have de novo mutations in the SCN1A gene. To identify new genes responsible for DS, we screened 41 SCN1A-negative patients for microrearrangements using high-density SNP microarrays. Since a de novo hemizygous deletion encompassing only PCDH19 (Xq22.1) was found in a male patient, we sequenced the coding region of this gene in 73 other SCN1A-negative patients. Nine different point mutations (four missense and five truncating mutations) were identified in 11 unrelated female patients. Patients with PCDH19 and SCN1A mutations had very similar clinical features although there were slight but constant differences in the evolution of the patients, including fewer polymorphic seizures, in particular rare myoclonia and atypical absences, in those with PCDH19 mutations. Mutations in PCDH19 were recently reported to cause EFMR (epilepsy and mental retardation limited to females), a remarkable X-linked disorder with male-sparing. To determine the clinical spectrum associated with PCDH19 mutations, we screened 95 additional patients (56 females and 39 males) who presented various epileptic encephalopathies, most of which had no family history. Five additional point mutations, 2 whole gene deletions, one partial gene deletion and 4 partial gene duplications were found in 12 unrelated female patients. So far, all the patients with PCDH19 mutations but one were females. A possible pathogenic mechanism associated with PCDH19 mutations is cellular interference, postulating that the co-existence of PCDH19-positive and PCDH19-negative cells (due to random X-inactivation in females) is pathogenic. According to this hypothesis, mosaic males could also be affected. Using FISH, we demonstrated that the male patient in whom the gene was deleted was indeed mosaic for the deletion. Together, these results suggest that PCDH19 plays a major role in epileptic encephalopathies, with a wide clinical spectrum encompassing DS and EFMR. This disorder mainly affects females. The identification of an affected mosaic male strongly supports the hypothesis that cellular interference is the pathogenic mechanism.

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## 498/W/Poster Board #156

**Epilepsy and mental retardation limited to females with PCDH19 mutations can present de novo or in single generation families.** *K.E. Hynes[1,2], P. Tarpey[3], L.M. Dibbens[1,4], M.A. Bayly[1], S.F. Berkovic[5], L. Vandeleur[1], S.J. Turner[5], N.J. Brown[6,7], T.D. Desai[5], E. Haan[1,4], G. Turner[8], J. Christodoulou[9], H. Leonard[10], D. Gill[11], Z. Afawi[12], T. Lerman-Sagie[13], D. Lev[13], M. Stratton[3], I.E. Scheffer[5,6], J. Gecz[1,2,4].* 1) Genetic & Molecular Pathology, SA Pathology (Women's and Children's Hospital), Adelaide, SA , Australia; 2) School of Molecular and Biomedical Science, University of Adelaide, Adelaide, Australia; 3) Wellcome Trust Sanger Institute, Hinxton UK; 4) School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, Australia; 5) Epilepsy Research Centre and Department of Medicine, University of Melbourne, Austin Health, Heidelberg, Victoria, Australia; 6) Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Melbourne, Australia; 7) Barwon Health, Geelong, Victoria; 8) GOLD Service, Hunter Genetics, Waratah, New South Wales 2298, Australia; 9) Western Sydney Genetics Program, The Children's Hospital at Westmead and Disciplines of Paediatrics and Child Health & Genetic Medicine, University of Sydney, Sydney Australia; 10) Telethon Institute for Child Health Research and Centre for Child Health Research, University of Western Australia, Perth; 11) TY Nelson Department of Neurology, The Children's Hospital at Westmead, Westmead, New South Wales, Australia; 12) Department of Neurology, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel; 13) Metabolic Neurogenetic Unit, Wolfson Medical Centre, Holon, Israel.

Epilepsy and Mental Retardation Limited to Females (EFMR) is an intriguing X-linked disorder with paradoxical sex reversal in phenotype, such that females are affected whilst transmitting males are unaffected. Mutations in the protocadherin 19 (PCDH19) gene have been identified in seven unrelated families with EFMR. Here, we assessed the frequency of PCDH19 mutations in individuals with clinical features which overlap those of EFMR. We analysed 185 females from three cohorts: 42 with Rett syndrome (RS) who were negative for MECP2 and CDKL5 mutations, 57 with autism spectrum disorders alone (ASD) and 86 with epilepsy with or without intellectual disability (ID). No mutations were identified in the RS and ASD cohorts. Among the 86 females with epilepsy (of whom 51 had seizure onset before 3 years), with or without ID, we identified two (2.3%) missense changes. One (c.1671C>G, p.N557K), reported previously without clinical data, was found in two affected sisters, and is the first EFMR family identified without a multigenerational family history of affected females. The second (c.826T>C, p.S276P), is a novel de novo missense change identified in a sporadic female. The change S276P is predicted to result in functional disturbance of PCDH19 as it affects a highly conserved residue adjacent to the adhesion interface of EC3 of PCDH19. Two of the 3 girls identified with a PCDH19 mutation had features of ASD in addition to seizures and learning concerns. Whilst we did not identify any mutations in PCDH19 in the ASD-alone cohort, gene expression profiling of EFMR female fibroblasts identified differential expression of Autism associated genes (LAMB1 and MET) when compared to control females, suggesting a convergence between ASD and EFMR at a functional level with probable defects in neuronal migration and synaptogenesis in both disorders. The identification of a De novo PCDH19 mutation in a sporadic female highlights that mutation analysis should be considered in isolated instances of girls with infantile onset seizures and developmental delay, in addition to those with the characteristic family history of EFMR.

## 499/W/Poster Board #157

**Mutation screening of the MAGI2 gene in idiopathic infantile spasms.** *A. Muir[1,2], E. Tam[2], T. Babatz[3], W.B. Dobyns[3], F. Zara[4], P. Striano[4], J. Sa[5], B.A. Minassian[6], L.R. Osborne[1,2].* 1) Molecular Genetics, University of Toronto, Toronto, Canada; 2) Medicine, University of Toronto, Toronto, Canada; 3) Department of Human Genetics, University of Chicago, IL; 4) Muscular and Neurodegenerative Disease Unit, G. Gaslini Institute, Genoa, Italy; 5) Serviço de Genética Médica, Hospital pediátrico de Coimbra, Coimbra, Portugal; 6) Program in Genetics & Genomic Biology, Hospital for Sick Children, Toronto, Ontario, Canada.

Infantile Spasms (IS) is a disorder of the developing nervous system that begins in the first year of life. It is characterized by jerks of the neck, trunk and extremities lasting 1-2 seconds, occurring in numerous clusters throughout the day. The spasms must be abolished if the prognosis is to be improved, otherwise the immature brain appears to remain hyperexcitable and proper neurodevelopment is impeded, however current medications are variably effective and have severe side effects. In around half of IS cases, the spasms are merely one part of a much wider spectrum of neurological symptoms, however in the other half of cases IS occurs in the absence of any brain malformation or neurological insult and is classed as idiopathic.

We recently identified a new locus for IS on chromosome 7q11.23-q21.1 through deletion mapping of Williams-Beuren syndrome individuals with or without IS. A single gene, *MAGI2*, was located within the critical interval. MAGI2 is a synaptic scaffold protein previously shown to interact with Stargazin, a protein that is disrupted in an epileptic mouse model, making it an excellent candidate for idiopathic IS. We have now initiated a study to determine whether *MAGI2* mutations can in fact cause idiopathic IS. Sequence analysis was performed on all 22 exons of *MAGI2* in cohorts of children with IS from the US, Italy and Portugal. A total of 82 DNA samples were fully analyzed and five potentially deleterious sequence changes were identified, none of which were found in a panel of 300 control chromosomes. All five changes result in amino acid substitutions, and all were predicted to significantly affect protein function using the SIFT (Sorting Intolerant From Tolerant) program. In two cases the sequence change was inherited from a parent, and in one of these cases the parent also had a positive history of seizures. We hypothesize that since these sequence changes are amino acid substitutions rather than changes that would result in a truncated protein product, the phenotype may not be fully penetrant. In summary, we have identified potentially pathogenic *MAGI2* mutations in 6% of our symptomatic IS cohort through a sequence-based screen of the coding region. We have recently developed an MLPA assay to detect intragenic deletions and this may increase the detection rate of *MAGI2* mutations in this population.

## 500/W/Poster Board #158

**RESCUE OF FMR1 KNOCKOUT PHENOTYPES IN ADULT MICE AFTER RESTORATION OF FMRP.** *R. Zong[1], E.J. Mientjes[2], C.M. Spencer[1], R. Paylor[1], B.A. Oostra[2], D.L. Nelson[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA; 2) Department of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands.

Absence of FMRP causes fragile X syndrome, the most common inherited form of mental retardation. Fragile X syndrome is widely considered to be a developmental disorder; yet direct evidence to support this argument is limited. We have generated mouse models that allow expression of FMRP to be manipulated in a time-dependent manner to determine FMRP requirement during development. One approach was used to allow restoration of full Fmr1 expression from a gene that has an interfering neomycin cassette in intron 1 that can be removed with Cre recombination. Another approach is the temporal deletion of Fmr1 in mice during postnatal or embryonic development, achieved by in vivo expression of a Tamoxifen-inducible Cre recombinase to ablate the Fmr1 promoter and first exon flanked by lox P sites. With these inducible mouse models, we were able to effectively restore or delete FMRP in adult animals, and to examine a number of known abnormal phenotypes present in Fmr1 KO animals. Our results indicate that restoration of FMRP in adult (4-6 week old) mice resulted in the rescue of several phenotypes including abnormal dendritic spines, deficiency on the accelerating rotarod, and altered expression levels of the FMRP target PSD-95 in the hippocampus. Our findings suggest that defective neurons in fragile X syndrome can be repaired after restoration of FMRP, and that the damage to neurons may not be incurred during development, providing important data in support of the potential for therapies in patients. We also find that adult deletion of FMRP in mice results in the development of abnormal dendritic spines, and abnormal rotarod performance, demonstrating that both of these phenotypes are sensitive to the acute presence of FMRP. Further studies including additional behavioral tests are in progress.

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## 501/W/Poster Board #159

**Next Generation Technologies for Gene Discovery in Autosomal Dominant Retinitis Pigmentosa.** *S.P. Daiger[1,2], L.S. Sullivan[1], S.J. Bowne[1], D.G. Birch[3], J.R. Heckenlively[4], S.H. Blanton[5], E.A. Pierce[6], L. Ding[7], E.J. Sodergren[7], R.S. Fulton[7], G.M. Weinstock[7].* 1) Human Genetics Center, School of Public Health, The Univ. of Texas HSC, Houston, TX; 2) Dept. of Ophthalmology and Visual Science, Medical School, The Univ. of Texas HSC, Houston, TX; 3) Retina Foundation of the Southwest, Dallas, TX; 4) Kellogg Eye Center, Univ. of Michigan, Ann Arbor, MI; 5) Miami Inst. for Human Genomics, Univ. of Miami, Miami, FL; 6) Scheie Eye Institute, Univ. of Pennsylvania, Philadelphia, PA; 7) Genome Sequencing Center, Washington Univ., St. Louis, MO.

Autosomal dominant retinitis pigmentosa (adRP) is a heterogeneous set of inherited diseases characterized by night blindness in childhood, followed by progressive retinal degeneration, often culminating in legal or complete blindness by midlife. Although adRP is a single-gene disorder in affected individuals and families, many different genes and mutations are involved; for example, mutations in 19 genes are known to cause adRP and these account for a minor fraction of cases in many populations. AdRP represents a model system for gene discovery and mutation detection using “next generation” methods such as targeted, ultra-high-throughput sequencing and micro-array based detection of deletions.

Over the past decade we have tested a cohort of over 220 adRP families by a variety of methods and detected disease-causing mutations in 60%. The remaining 88 families are likely to harbor non-coding mutations or mutations in novel genes. We selected 21 families without known mutations for the first phase of advanced testing. DNAs from a pair of affected individuals from each family, plus positive controls, were amplified by PCR using 1,000 primer pairs covering 42 adRP genes and candidate genes. The PCR products were sequenced by 454 Titanium pyrosequencing (50X depth) and by Illumina Solexa reversible-dye-terminator sequencing. In addition, selected samples were tested using Affymetrix 6.0 SNP/CNV arrays to detect deletions and validate SNPs found by sequencing. Variants in the resulting data were compared to dbSNP and known mutations, and analyzed for pathogenicity by several methods. Approximately 100 rare, potentially-pathogenic variants were found in affected pairs; of these, 5 segregate with disease in one family each and meet multiple criteria for pathogenicity. Thus we detected mutations in 24% of the test group or 2% of the entire adRP cohort.

In the second phase of the study, we are using capture arrays and targeted enrichment to isolate DNA from over 600 candidate genes covering more than 10 MB of sequence in each affected individual, and sequencing the targeted fragments using ultra-high-throughput methods. DNAs are also being tested using Affymetrix arrays. By this combination of approaches we expect to identify novel adRP genes and mutations, and prepare for the day when inexpensive whole-genome sequencing becomes available.

## 502/W/Poster Board #160

**Genetic spectrum and mitochondrial assessment in Korean patients with early-onset Parkinson disease.** *SY. Kim[1], SY. Kim[2], HS. Ko[2], BS. Jeon[3], MW. Seong[4], JY. Kim[2], SS. Park[1,2].* 1) Department of Laboratory Medicine, Seoul National University Hospital, Seoul, Korea; 2) Clinical Research Institute, Seoul National University Hospital, Seoul, Korea; 3) Department of Neurology, Seoul National University Hospital, Seoul, Korea; 4) Department of Laboratory Medicine, National Cancer Center, Goyang, Korea.

Parkinson disease (PD) is the most important cause of Parkinsonism defined as common clinical features of tremor, rigidity, bradykinesia, and postural instability. Monogenic mutations have been identified in familial, early-onset PD (EOPD) patients, and evidences indicate that these genes are related with mitochondria. In this study, we aimed to characterize the mutational spectrum of Korean EOPD patients, and to investigate molecular deteriorations in consequence of genetic abnormalities. We enrolled 114 Korean patients clinically diagnosed with PD and with symptomatic onsets younger than 41 years (onset age, median 36, range 12-40). Four causative genes, *PARK2*, *PINK1*, *SNCA*, and *PARK7* (*DJ-1*), were analyzed by direct sequencing, multiplex ligation-dependent probe amplification (MLPA), and RT-PCR. In the mutation-positive individuals, mitochondrial DNA was quantified from leukocyte by real-time PCR, and mitochondrial respiratory chain complex I activity was measured in the lymphoblastoid cell line by automated spectrophotometric method. Genetic analyses revealed that 18.4% (21/114) of Korean EOPD patients had mutations in one of these four genes. The *PARK2* mutations were identified in 19 patients. Fourteen patients were homozygote or compound heterozygote of two causative mutations. Exon deletion was the major type of *PARK2* mutation, occurred within 24 alleles. Two alleles were exon duplications, and two were missense mutations. Two patients had heterozygous duplication or missense mutation in each. Three patients harbored exon deletions, but their heterozygosity could not be confirmed. A heterozygous *PINK1* missense mutation was found in a patient, and the *SNCA* missense mutation was in other patient. No *PARK7* mutation was found in this study. There was no difference in mitochondrial DNA quantity between 14 EOPD patients with *PARK2* mutation, 19 normal controls, and 20 late-onset PD patients (Kruskal-Wallis test, p=0.678). Mitochondrial complex I activity showed no difference between groups of 12 EOPD patients with *PARK2* mutation and eight normal controls (Mann-Whitney test, p=0.624). This study indicated that *PARK2* is the most important gene in Korean EOPD patients. No significant difference was observed in mitochondrial DNA quantity or complex I activity in patients with mutations. The molecular genetic characteristics identified in this study will be helpful for diagnosis and understanding of EOPD.

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## 503/W/Poster Board #161

**Novel Progressive Myoclonus Epilepsy Disease Gene or Genetic Conundrum?** *J. Turnbull[1,2], J.M. Girard[1], H. Lohi[1], E.M. Chan[1], S. Omer[3], C. Bennett[4], A. Chakrabarty[5], N. Pencea[1], X.C. Zhao[1], P.X. Wang[1], S.W. Scherer[1,2], C.A. Ackerley[6], B.A. Minassian[1].* 1) Genetics & Genome Biology, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 3) Department of Neurology, St George's Hospital, London, England; 4) Department of Clinical Genetics, Leeds General Infirmary, Leeds, United Kingdom; 5) Department of Neuropathology, Leeds General Infirmary, Leeds, United Kingdom; 6) Department of Pathology, Hospital for Sick Children, Toronto, Ontario, Canada.

The progressive myoclonus epilepsies (PMEs) are a group of diseases with myoclonic seizures and progressive neurodegeneration. Lafora disease is an autosomal recessive teenage-onset fatal PME with pathognomonic periodic acid-Schiff (PAS) positive diastase resistant accumulations of abnormally branched glycogen known as Lafora bodies. The disease is caused by mutations in either the EPM2A (laforin) or EPM2B (malin) genes. We identified a consanguineous Pakistani family with three children with classic clinical Lafora disease and no mutations in or linkage to either EPM2A or EPM2B. Muscle biopsy was strongly positive for Lafora bodies, but skin biopsy was equivocal, with extremely small PAS positive diastase resistant structures that could not definitively be called abnormal. In contrast, in common forms of Lafora disease, Lafora bodies are most striking in skin. We performed genome-wide linkage using the Affymetrix 10K SNP chip and found a 7MB region (containing 54 genes) on chromosome 4 that gave the maximum predicted LOD score (2.56) for this family. There are two strong functional candidate genes in this region. STBD1 is one of only three mammalian proteins, including laforin, containing a CBM20 type of carbohydrate binding domain. SCARB2 mutations cause the action myoclonus-renal failure syndrome, a PME associated with renal failure and no Lafora bodies. Extensive mutation screening of both genes and quantitative and functional analyses of STBD1 and SCARB2 in this family revealed no differences between affected and unaffected members. We next sequenced 35 additional genes in the region and identified a Phe to Leu sequence variant in the PRDM8 gene. This variant was not found in 500 ethnic-matched controls including 200 from the same region. The affected residue and the exact nucleotide are conserved across species. In cell culture experiments we found colocalization in the nucleus for both PRDM8/EPM2A and PRDM8/EPM2B. Co-immunoprecipitation experiments showed exogenous interactions between each of the three proteins. It is possible that this sequence change is an extremely rare innocuous variant in the PRDM8 gene and the actual disease gene remains to be uncovered, or an actual disease-causing mutation. While further functional experiments are underway, with this report we seek additional non-EPM2A, non-EPM2B families with Lafora disease and illustrate an example of the difficulties in genetic analysis of extremely rare forms of very rare diseases.

## 504/W/Poster Board #162

**New CMT2C families map to 12q23-24 and narrow the previously published linkage region.** *G. Landoure[1,2,3], H. Stanescu[2,4], Y. Shi[1], A. Taye[1], A. La Pean[1], C. Ludlow[5], C. Sumner[1,6], K. Fischbeck[1], R. Kleta[2,4].* 1) Neurogenetics Branch, NINDS/NIH, Bethesda, MD. 20892; 2) University College London, London UK; 3) Department of Neurology, Hospital of Point "G", Bamako Mali; 4) NHGRI, NIH, Bethesda, MD 20892; 5) Laryngeal and Speech Section, NINDS/NIH, Bethesda, MD 20892; 6) Neurology department, The Johns Hopkins University, Baltimore, MD USA.

Charcot-Marie-Tooth disease type 2C (CMT2C) is a rare autosomal dominant axonal neuropathy with weakness and atrophy in distal limb muscles and diaphragm, vocal fold, and intercostal muscle involvement. Sensory symptoms are usually mild. The disease usually starts in the first decade, but the age of onset and the clinical severity are variable. The locus for CMT2C has been mapped to 12q23-24 with a physical distance of 5.7 Mb between markers D12S105 and D12S1330, and a LOD score of about 5. Subsequently, two other CMT2C families were studied, and narrowed the region to 3.9 Mb between D12S105 and D12S1340 with a LOD score of 2.1. Here we report two families with CMT2C. In addition to the disease manifestations reported earlier, several of our patients had bilateral sensorineural hearing loss and impairment of bladder voiding, and one, the most affected, reported recurrent diarrhea. Because the clinical presentation and the electrophysiological studies were similar to the previous reported CMT2C families, we performed fine mapping with 12 polymorphic markers within the previously defined interval. We found linkage in both families with a total LOD score of about 10. Haplotype reconstruction showed that all affected individuals had the disease allele and narrowed the region from 3.9 Mb to about 2.6 Mb between markers D12S105 and D12S1343. This new region contains 38 protein-coding genes. Three unaffected individuals from one family (the mother of the proband and her 71 year old sister and sister's son) carried the disease-associated haplotype. Although electrophysiological studies were not done, their clinical examinations were completely normal. This may be due to an incomplete penetrance or a new mutation. These studies further refine the location of the gene for CMT2C. Candidate gene sequencing is currently under way to identify the disease gene.

## 505/W/Poster Board #163

**Two novel mutations of the Aristaless Related Homeobox gene *(ARX)*.** *S. Rujirabanjerd[1], U. Intusoma[2], T. Hansakunachai[3], K. Tongsippunyoo[1], O. Plong-on[1], E. Thammacharoen[1], T. Sripo[1], P. Limprasert[1].* 1) Department of Pathology, Faculty of Medicine, Prince of Songkla University, Hat Yai, Thailand; 2) Department of Pediatrics, Faculty of Medicine, Prince of Songkla University, Hat Yai, Thailand; 3) Department of Pediatrics, Faculty of Medicine, Thammasat University, Pratumthanee, Thailand.

Aristaless Related Homeobox gene *(ARX)* is an X-linked mental retardation (XLMR) gene located on the short arm of the X chromosome (Xp22). Mutations in this gene can cause a vast phenotypic spectrum ranging from non-syndromic to syndromic forms of mental retardation. The most common mutation is the c.428_451 dup mutation in exon 2. Since the gene was initially identified, it has been screened in several patients with XLMR. Here we report two novel nucleotide changes in *ARX* coding region, which we speculate to be causative in these XLMR families. The first mutation is a single nucleotide substitution in exon 2 (c.430G>T p.A114S) in a patient diagnosed with autism following DSM IV criteria. This patient also has some degree of intellectual disability (IQ=64). This change occurs at a highly conserved amino acid and could not be detected in 500 normal X chromosomes. Until present, only c.428_451 dup mutation has been reported in autistic patients. Therefore, this is the first missense change in the *ARX* gene detected in patients with autistic features. The second mutation is also a nucleotide substitution in the same exon (c.769C>T p.R257C). This change also occurs at a highly conserved amino acid and could not be detected in normal controls. The patient is a Thai boy with seizure and spastic cerebral palsy. Interestingly, he also has a family history of sensory neural hearing loss which never been reported in families with *ARX* mutations. We conclude that these two novel nucleotide changes are likely to be disease-causing mutations in these families.

## 506/W/Poster Board #164

**A novel *SLC9A6* gene mutation in a Japanese patient resembling Angelman syndrome.** *Y. Takahashi[1], M. Funatsuka[2], K. Hosoki[1], Y. Ueda[1], T. Itoh[1], H. Shiraishi[1], K. Saito[2], S. Saitoh[1].* 1) Department of Pediatrics, Hokkaido Univ Grad Sch Med, Sapporo, Japan; 2) Institute of Medical Genetics, Tokyo Women's Med Univ, Tokyo, Japan.

*SLC9A6* mutations were first reported in families exhibiting an X-linked mental retardation syndrome mimicking Angelman syndrome (AS) that is caused by loss of function of the *UBE3A* gene. The patients have cardinal features similar to those of AS including severe developmental delay, mental retardation with absent or minimal use of words, ataxia, easily provoked laughter, epilepsy, nystagmus and microcephaly. Distinct clinical features of the patients with *SLC9A6* mutations included cerebellar atrophy, and slow progression of symptoms. We examined 22 affected males who were initially suspected to have AS but genetic investigations including the *SNURF-SNRPN* DNA methylation test(identifies deletion, UPD, and imprinting defect classes of AS), and *UBE3A* mutation screening excluded known causes of AS. PCR-amplification and sequencing of coding sequences of the *SLC9A6* gene used genomic DNA derived from peripheral blood of patients. A novel *SLC9A6* gene mutation (c.441delG, p.S147fs)was identified in one patient. The mutation is predicted to introduce a frameshift and subsequently generate a premature stop codon. Our patient has the typical AS phenotype with cerebellar atrophy as seen in a subset of patients with *SLC9A6* mutations. Further investigations of other family members are in progress.

*SLC9A6* has been reported to play a role in the growth of dendritic spines. The function of the product of *UBE3A*, encoding an E3 ubiquitin ligase, is also associated with dendritic spine formation. Therefore, *UBE3A* may regulate the turnover of SLC9A6 polypeptide levels, or localization of the polypeptide, in dendritic spines during formation or function of the developing brain. An involvement in a common biological pathway may thus explain the phenotypic resemblance between patients with AS and those with *SLC9A6* mutations.In conclusion, *SLC9A6* gene mutation screening should be performed in male patients who are suspected to have AS but not confirmed by genetic examination. Neverthless, most male AS-like patiens without known causes of AS do not have *SLC9A6* gene mutations(21 of 22 in our study) and could be explained by small intragenic deletions in *UBE3A*, non-coding regulatory mutations in *UBE3A* or *SLC9A6*, or other genetic or epigenetic abnormalities affecting *UBE3A* or unknown genes.

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## 507/W/Poster Board #165

**Two-percent of patients suspected of having Angelman syndrome have *TCF4* mutations.** *K. Takano[1], C. Moyes[2], M.J. Lyons[1], C. Skinner[1], J.R. Jones[1], C.E. Schwartz[1].* 1) Greenwood Genetic Center, Greenwood, SC; 2) Whakatane Hospital, Whakatane, New Zealand.

Haploinsufficiency as well as various mutations in the *TCF4* gene have been found to be associated with the Pitt-Hopkins syndrome (PTHS) [Amiel J, AJHG 80: 988, 2007, Zweier C, AJHG 80: 994, 2007]. PTHS (OMIM 610954) is characterized by severe mental retardation, a wide mouth plus other distinctive facial features (fleshy lips, beaked nose, broad nasal bridge) and breathing abnormalities. Because of some phenotypical overlap with Angelman syndrome, some have suggested that PTHS be considered in its differential diagnosis.

To explore this possibility, we screened 86 patients who were suspected of having Angelman syndrome (AS). All the patients were negative for UBE3A testing and 53 were negative for methylation analysis. We identified 2 mutations in this cohort: one frame shift mutation, c.1151delG (p.S384Tfsx7) and a missense mutation, c. 1745G>C (p.R582P).

Patient 1 (p.S384Tfsx7) is a 7 year old male with global developmental delay, microcephaly, broad nasal bridge, open and wide mouth, prominent lower lip, short stature and staring spells. Patient 2 (p.R582P) is a 16 year old male with severe mental retardation, blond hair, bright red lips and an abnormal gait. He also has frequent cyanotic episodes that have associated abnormal limb movements characterized as ballismus and dystonia. Mild hyperventilation occurs prior to the occurrence of these episodes. The patient also has cryptorchidism, convergent strabismus and planovalgus deformities of his feet.

The *TCF4* gene encodes a basic helix-loop-helix (bHLH) transcription factor which belongs the family of E proteins. The bHLH domain binds to the common DNA sequence called E-box and forms homo-and heterodimers with other classes of HLH proteins. The p.S384Tfsx7 mutation lacks this domain. The p.R582P mutation lies within the bHLH domain in which seven other missense mutations have been reported. Both mutations affect the critical function of the bHLH domain of the TCF4 protein.

In summary we found 2 *TCF4* mutations in 86 patients (2%) suspected to have AS. Screening for mutations in this gene should be considered in patients who present with findings of AS but who have been negative for methylation and UBE3A testing.

## 508/W/Poster Board #166

**Dysfunctional Estrogen Signaling: A Novel Cause of Intellectual Disability**. *C.D. van Karnebeek[1,2], P. Atkins[1,2], C. du Souich[1,2], R. Rupps[1,2], T. Woodward[3], M. Demos[4], C.F. Boerkoel[1,2].* 1) Medical Genetics, B.C. Children's & Women's Health Centre, Vancouver, British Columbia, Canada; 2) Child & Family Research Institute, B.C. Children's & Women's Health Centre, Vancouver, British Columbia, Canada; 3) Department of Psychiatry UBC, Mental Health & Addictions Research Institute, Vancouver, British Columbia, Canada; 4) Department of Neurology, B.C. Children's & Women's Health Centre, Vancouver, British Columbia, Canada.

**Background:** Identification of the underlying cause of intellectual disability (ID) is of fundamental importance for optimizing patient care, but is often forbiddingly complex. New insight in etiology reduces the burden of extensive medical work-up for the affected child and society as a whole. In a non-consanguineous 3-generation family with ID, we identified disruption of the estrogen receptor beta (*ER*β) gene as such a novel cause. **Aim:** To precisely define the molecular defect in the ERβ gene in this family and its role in the pathogenesis of ID. **Methods & Results:** Detailed physical and neurologic examination of the affected family members showed mild dysmorphic features and a moderate learning disability with reduced memory and normal insight. Routine karyotyping (550 bands) identified a reciprocal translocation (14;18) co-segregating with ID. Breakpoints have been mapped with FISH analysis and PCR to a region on chromosome 18 not containing any annotated genes, and to a region on chromosome 14 that only contains the *ER*β gene. Using RNA isolated from their lymphoblastoid cell lines, it will be determined whether the gene disruption results in loss of *ER*β expression, in expression of a truncated ERβ, or expression of an ERβ fusion protein. A customized test will be developed to distinguish the specific neurocognitive profile of the three affected individuals in this family. Using the results of this test and of their fMRI scans, specific regions of the brain affected in the family members with ID, and thus by ERβ deficiency, will be identified. These results will be related to published data on distribution of ERβ in the mouse brain and results of our immunohistochemistry studies of expression in human brain. **Conclusions:** To our knowledge this is the first report of human neurologic disease arising from an ERβ abnormality. A causal relation with ID is highly likely, as knock-out mice display morphologic brain abnormalities and cognitive defects, including impairment of spatial learning in females. Insight in the molecular and physiologic basis of the disease in this family increases our understanding of the genetic causes of ID and the role of estrogen in human physiology. Delineation of the neuropsychiatric profile associated with this ERβ mutation facilitates targeted molecular testing for other patients fitting this profile. Ultimately, therapies improving cognitive functioning in this group may be developed based on this model for disease.

## 509/W/Poster Board #167

**High resolution melt (HRM) analysis of DMD gene variants using whole genome amplified (WGA) samples.** *PS. Lai[1], OS. Yim[1], ZS. Chong[1], PS. Low[1], SKH. Tay[1], EPH. Yap[2,3].* 1) Dept of Pediatrics, Yong Loo Lin School of Medicine, National Univ Singapore, National Univ Hospital, Singapore 119074; 2) Defence Research and Technology Office, Ministry of Defence, Singapore 669645; 3) DSO National Laboratories, Defence Medical and Environmental Institute, Singapore 117510.

Whole-Genome Amplification (WGA) provides a way to generate large amounts of genome-equivalent templates from low starting input DNA. While WGA templates have been used for applications such as genotyping, CGH, DHLC analysis, etc., there are few reports of its application in high resolution melt (HRM) analysis. The latter is a simple, rapid method for mutation scanning of sequence variants based on differentiation of PCR products during thermal denaturation. HRM analysis routinely uses 10-50 ng DNA while WGA allows amplification of DNA from 1-10 pg of starting DNA material. This study investigates the amenability of using WGA samples for HRM to genotype mutations in the DMD (Duchenne muscular dystrophy) gene. Four variants were analyzed; c.77A>G and c.79G>C mutations in exon 2, and two SNPs, rs1800263 (c.4131A>T) and rs1801187 (c.5234G>A), in exons 30 and 37 respectively. Wild-type and mutant homozygotes, and heterozygotes were generated from lymphocyte samples from patients, family members and normal controls. To generate WGA products for use as melt analysis templates, aliquots containing 1.0, 0.1 and 0.01 ng of genomic DNA were amplified by multiple displacement amplification (MDA) technique. Paired genomic DNA and WGA products were then PCR amplified, and the amplicons spanning 141-184 bp were analyzed on the LightScanner (Idaho Technology) in a blind study. The results show that WGA templates exhibited variability and genotype profile overlaps with initially high discordance rate of more than 50%. However, discordant results were eliminated with $MgCl_2$ optimization, and after Exo-SAP1 treatment of WGA products prior to melt analysis. All homozygotes and heterozygotes could be detected with high sensitivity and specificity. Use of WGA samples for DMD mutation screening by HRM can be beneficial for future applications in clinical settings when sources of sampling (eg. invasive tissue sampling or biopsies of single embryos, oral buccal swabs, Guthrie cards, etc.) yield DNA of low quantity or quality.

## 510/W/Poster Board #168

**NDUFS4 mutations cause Leigh syndrome with predominant brainstem involvement.** *D. Lev[1,2], A.S. Lebre[3], L. Minai[3], A. Saada[4], J. Stephan[3], S. Cohen[5], A. Rotig[3], A. Munnich[3], T. Lerman-Sagie[2,6], E. Leshinsky-Silver[2,5].* 1) Inst Medical Genetics, Wolfson Medical Ctr, Holon, Israel; 2) Mitochondrial Disease Center, Wolfson Medical Center, Holon, Israel; 3) Service de Génétique, Hôpital Necker Enfants Malades, Paris, France; 4) Metabolic Unit, Hadassah Medical Center, Jerusalem, Israel; 5) Molecular Genetics Laboratory, Wolfson Medical Center, Holon, Israel; 6) Pediatric Neurology Unit, Wolfson Medical Center, Holon, Israel.

Complex I deficiency is a frequent cause of Leigh syndrome. We describe a non-consanguineous Ashkenazi-Sephardic Jewish patient with Leigh syndrome due to complex I deficiency. The clinical and neuroradiological presentation showed predominant brainstem involvement. Blue Native PAGE analysis revealed a markedly decrease of fully assembled complex I. The patient was found to be compound heterozygous of two mutations in the NDUFS4 gene: p.Asp119His (a novel mutation) and p.Lys154fs (recently described in an Ashkenazi Jewish family). These findings support the suggestion that the p.Lys154fs mutation in NDUFS4 should be evaluated in Ashkenazi Jewish patients presenting with early onset Leigh syndrome even before enzymatic studies. Our results further demonstrated that NDUFS4 presents a hotspot of mutations in the genetic apparatus of oxidative phosphorylation and the subunit it encodes is essential for the assembly of complex I.

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## 511/W/Poster Board #169

**Mouse Models for Myotonic Dystrophy Type 2 (DM2) Lacking Aberrant Splicing Implicate Novel Cytoplasmic Pathomechanisms.** *R. Krahe[1], M. Sirito[1], M. Wojciechowska[1], L.L. Bachinski[1], L.T. Timchenko[2], C.S. Van Pelt[1], D.R. Mosier[2], K.A. Sheikh[3], G. Zhang[3], P. Mancias[3], B. Udd[4].* 1) University of Texas M. D. Anderson Cancer Center, Houston, TX; 2) Baylor College of Medicine, Houston, TX; 3) University of Texas Health Sciences Center at Houston, Houston, TX; 4) Tampere University Hospital, Finland.

DM is caused by unstable microsatellite expansions -- in DM1 a $(CTG)_n$ expansion in the 3' UTR of *DMPK*, in DM2 a $(CCTG)_n$ expansion in intron 1 of *ZNF9*. In spite of similar mutations, DM1 and DM2 are clinically distinct diseases. The prevailing paradigm is that DM1 and DM2 are toxic RNA diseases: transcription of these repeats into mutant $(CUG)_n/(CCUG)_n$-containing RNAs is both necessary and sufficient to cause disease by formation of nuclear foci and interference with the splicing of downstream "effector" genes. Consequently, efforts so far to identify therapeutic targets have focused on the disruption of the insoluble nuclear foci and the correction of aberrant splicing. We have generated transgenic mice expressing a mutant DM2 expansions of $(CCTG)_{121}$ in intron 1 of the *human skeletal actin* (*HSA*) gene and knock-in mice expressing $(CCTG)_{189}$ in intron 1 of *Znf9*. Both mouse models show muscle weakness and an overall muscle pathology associated with human DM, including aberrant type 2 muscle fibers, sarcoplasmic masses and central nuclei. The transgenic mice also have myotonia by EMG. Other phenotypic features include cardiomyopathy and sudden cardiac death, suggestive of arrhythmia, abnormalities of lens development and retinal atrophy, and azoospermia in males. Recapitulating pathological features that have been shown for DM patients, both of our transgenic and knock-in DM2 mice show nuclear foci that sequester MBNL1, elevated steady-state levels of CUGBP1, mediated by phosphorylation through phospho-PKC, and dysregulation of CUGBP1 target genes. However, both DM2 mouse models lack aberrant splicing for *Clcn1, Atpa2a1, Ryr1, Tnnt3, Tnnt2, Ank2, Capzb* and *Fxr1*, genes that have been identified as misspliced in DM patients. In addition, our DM2 mice show cytoplasmic foci and other cytoplasmic abnormalities, including activation of the ER stress response, which is predicted to globally attenuate translation. Our mouse models indicate that DM-like progressive myopathy can result in the absence of aberrant splicing. They complement existing mouse models for DM and are uniquely suitable for the dissection of alternative cytoplasmic pathomechanisms in DM.

## 512/W/Poster Board #170

**Genetic heteroneneity in Karak syndrome demonstrated by Linkage analysis and mutation screening in PANK2 and PLA2G6 genes in 8 casanguineous Saudi Arabian families.** *H. Azzedine[1], M.A.M. Salih[2], A. Khan[3], E. Mundwiller[1], A. Aldriss[4], S.A. Elmalik[4], M.M. Kabiraj[5], G. Stevanin[1].* 1) INSERM/UPMC UMRS 975, Hosp de la Salpetriere, Paris, France; 2) Division of Pediatric Neurology , College of Medicine, King Saud University, Riyadh, Saudi Arabia; 3) King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia; 4) Department of Physiology, College of Medicine, King Saud University, Riyadh, Saudi Arabia; 5) Division of Clinical Neurophysiology, Department of Neuroscience, military Hospital, Riyadh, Saudi Arabia.

A heterereogeneous group of severe neurological disorders like Aceruloplasminaemia, Neuroferritinopathy, Hallervorden-Spatz syndrome, HARP syndrome, and Friedreich ataxia involve excess brain iron accumulation. The "eye of the tiger" sign is a common neuroradiological finding in neurodegeneration with iron brain accumulation type 1 and 2 (NBIA1, 2), infantile neuroaxonal dystrophy (INAD) as well as in Karak syndrome (KS). Mutations in PANK2 and PLA2G6 genes were implicated in NBIA, INAD and KS disorders. We describe 8 consanguineous Saudi families with Karak syndrome. These consisted of 13 affected individuals (6 males and 7 females, aged 19 months-24 years). Onset ranged between about 8 months and 7 years with difficulty in walking or progressive cerebellar ataxia and spasticity associated, later, with extrapyramidal signs, intellectual decline and axonal form of Charcot-Marie- Tooth disease (CMT2). Ambulation was lost between 4 1/2 and 15 years. One male patient died at 24 years. Ophthalmic evaluations revealed abnormal vertical saccades and pursuit. Brain MRI showed iron deposition in the putamen in all patients aged more than 4 years. Five of these families were genotyped for PANK2 and PLA2G6 loci using 10 microsatellite markers. PANK2 locus was excluded in all the families while assignment of the families to the PLA2G6 locus was established by homozygocity mapping in 4 of them. Using direct sequencing of the PLA2G6 gene we identified, at a homozygous state, 3 novel mutations and one other already reported in the 4 linked families. One of these 5 families was neither linked to PLA2G6 nor to PANK2 genes demonstrating further genetic heterogeneity. Three new other consanguineous families with the same phenotype and belonging to the same country are under investigation. The final results will be presented during the meeting.

## 513/W/Poster Board #171

**Identification and functional characterization of Lrrk2 N1437H as a novel pathogenic cause of familial Parkinson disease.** *M. Farrer[1], C. Viarino-Guell[1], J.C. Dachsel[1], K. Haugarvoll[1,2], M. Toft[1,2], L. White[2], A. Felic[1,2], J. Kachergus[2], A. Ortolaza[1], S.A. Cobb[1], S. Lincoln[1], O.A. Ross[1], J.A. Aasly[2].* 1) Dept Neuroscience, Mayo Clinic , Jacksonville, Jacksonville, FL; 2) Dept of Neurology, St. Olav's Hospital, Norwegian University of Science and Technology, Trondheim, Norway.

Genealogical investigation of a large Norwegian family (F04) with autosomal dominant parkinsonism (PD) has revealed 15 affected family members over 4 generations. Affected subjects present with typical levodopa-responsive PD, albeit with early onset disease (mean 49 years). Genetic investigation identified a novel *LRRK2* mutation c.4309 C>A (p.Asn1437His) that co-segregates with disease (LOD=3.15). The variant was absent in 623 Norwegian control subjects. One additional Norwegian patient (1/692) was identified as a carrier and shares the same ancestral haplotype as F04. The mutation is located within the ROC domain of the protein, and functional characterization demonstrates it pathogenic effects on Lrrk2 dimerization, GTPase and subsequent kinase activities.

## 514/W/Poster Board #172

**Different origins and demographic histories of the LRRK2 G2019S mutation in Parkinson's disease.** *S. Lesage[1,2], E. Patin[3], C. Condroyer[1,2], A.L. Leutenegger[4,5], E. Lohmann[1,2], S. Belarbi[6], P. Pollak[7], A.M. Ouvrard-Hernandez[7], S. Bardien-Kruger[8], H. Tomiyama[9], C. Pirkevi[10], N. Basak[10], A. Dürr[1,2,11], N. Hattori[9], A. Orr-Urtreger[12,13], M. Tazir[6], L. Quintana-Murci[3], A. Brice[1,2,11], the French Parkinson's Disease Genetics Study Group (FPDGSG).* 1) INSERM, UMR_S975 (Formerly UMR_S679), Paris, France; 2) Pierre et Marie Curie-Paris6 University, UMR_S975 CRicm, Pitié-Salpêtrière, Paris, France; 3) Institut Pasteur, Human Evolutionary Genetics, CNRS URA3012, Paris, France; 4) Inserm, U535, Villejuif, France; 5) Paris-Sud University, IFR69, UMR_S535, 94817 Villejuif, France; 6) Service de Neurologie, CHU Mustapha, Alger, Algeria; 7) Service de Neurologie, CHU de Grenoble, Grenoble, France; 8) University of Stellenbosch, Cape Town, South Africa; 9) Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan; 10) BoÄŸaziÃ§i University, Molecular Biology and Genetics Department, Neurodegeneration Research Laboratory, Istanbul, Turkey; 11) AP-HP, Pitié-Salpêtrière Hospital, Department of Genetics and Cytogenetics, Paris, France; 12) Genetic Institute, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel; 13) Sackler Faculty of Medecine, Tel-Aviv University, Tel-Aviv, Israel.

BACKGROUND: Mutations in the Leucine-Rich-Repeat Kinase 2 (LRRK2) gene have been identified in families with autosomal dominant Parkinson's disease (PD) and in sporadic cases, with G2019S, as the most common mutation. This mutation greatly varies according to ethnicity and geographic origins: it is rare in Asia (<0.1% in Japan), accounts for 1-7% in Europe and 20-40% in PD Ashkenazi Jews and North Africa. Previous studies reported that patients from these populations shared at least three haplotypes. AIM: To assess natural histories of the G2019S mutation in a large multi-ethnic population panel. PATIENTS AND METHODS: We genotyped 74 markers [20 short tandem repeat (STR) and 54 single nucleotide polymorphisms (SNP)] spanning a 16 Mb- genomic region around G2019S in 191 individuals from 126 families with G2019S (121 heterozygous and 5 homozygous). Of 126 families, 70 were from North Africa, 13 from France, 7 from other European countries, 1 from Turkey, 1 from Japan, 2 from South Africa, and 32 were of Jewish origin, mostly from Eastern Europe. The haplotypes were reconstructed using Phase v.2.1.1. The age of G2019S was estimated by 2 different maximum-likelihood methods. The network analyses were performed using NETWORK v.4.5. RESULTS: - We identified 3 different haplotypes: Haplotype 1, which was shared by 95% of mutation carriers, Haplotype 2, which was initially identified in 3 European-American families, was also found in 2 French families; and Haplotype 3, which has been primarily observed in Japanese PD patients, was also shared by a Turkish family. - NETWORK analyses showed that Haplotype 1 and Haplotypes 2 and 3 were placed at each edge of the network, suggesting that G2019S arose independently at least 2 times in humans, on Haplotype 1 and on the revolutionary-related Haplotypes 2 and 3. - Population distribution of the intra-allelic diversity of the most widespread Haplotype 1 showed 25%, 33% and 67% of recombining haplotypes in North-African Arabs, Europeans and Ashkenazi Jews, respectively. - Our G2019S age estimations indicated that the LRRK2 mutation initially arose among Near-Easterners ~6,000 years ago, much earlier than in previous estimations. CONCLUSION: We have shown using a large multi-ethnic population panel that PD-related G2019S mutation has independently appeared in humans at least twice, one of these events having occurred most likely in the Near-East, at least 6000 years ago.

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## 515/W/Poster Board #173

**Transgenic mouse model of CUG RNA toxicity in brain.** *D.D. Rudnicki[1], A.I. Seixa[1], K.A. Metcalf Pate[2], D.W. Chung[1], D.A. Swing[3], L. Tessarollo[3], R.L. Margolis[1].* 1) Psychiatry, CMSC 8-121, Johns Hopkins School of Medicine, Baltimore, MD; 2) Molecular and Comparative Pathobiology , Ross 459, Johns Hopkins School of Medicine, Baltimore, MD; 3) Mouse Cancer Genetics Program, Building 560, Room 32-31D, NCI, Frederick.

Our group has previously identified an autosomal dominant disorder, Huntington's disease-like 2 (HDL2), with clinical and pathological features nearly indistinguishable from Huntington's disease (HD). Like HD, HDL2 is a relentlessly progressive adult onset neurodegenerative disorder, and like HD, HDL2 is caused by a CAG/CTG expansion mutation. However, the HDL2 locus is on chromosome 16q24.3, and the repeat falls within a variably spliced exon of junctophilin-3 (JPH3) in the CTG orientation. We have recently discovered that HDL2 brain contains intranuclear RNA foci, detectable by riboprobes against either CUG repeats or against JPH3 sequence flanking the repeat. These foci closely resemble the RNA foci detected in DM1 brain. We have also shown that overexpression of an untranslatable JPH3 construct containing an expanded CUG repeat is toxic to cultured cells. We therefore hypothesized that transcripts with an expanded CUG repeat may be toxic to the mammalian brain. To directly test this hypothesis, we have generated transgenic mice expressing an untranslatable fragment of JPH3 exon 2A, with either 10 or 100 CTG triplets, driven by the mouse prion promoter (PrP) vector. Preliminary data indicate that (CTG)100, but not (CTG)10, mice develop RNA foci by 7 months of age. Unlike HDL2 human brain tissue, we have so far not detected ubiqutin-positive protein inclusions in cerebral cortex of the (CTG)100 mice. Early behavioral analysis suggests impaired motoric function of the (CTG)100 mice by 5 months of age (abnormal performance on the inverted platform cling test). These initial findings suggest that the mouse model may be a useful tool in studying the relationship between expanded CUG repeats and neurotoxicity.

## 516/W/Poster Board #174

**The impact of *Hsp70i* over expression on the CMT peripheral neuropathic phenotype.** *J. Saliba[1], M. Khajavi[1], D. Pehlivan[1], J.R. Lupski[1,2,3].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Children's Hospital, Houston TX.

Hereditary peripheral neuropathies encompass a large collection of clinically and genetically heterogeneous disorders. These disorders lead to the deterioration of the peripheral nerves along with secondary muscle wasting. Charcot Marie Tooth disease (CMT) is the most common hereditary peripheral neuropathy with a prevalence of 1 in 2,500. CMT is most often the result of a 1.4 Mb duplication of the 17p11.2-p12 region of chromosome 17 that includes the dosage sensitive peripheral myelin protein 22 gene (*PMP22*). In addition, a number of point mutations in over two dozen genes including *PMP22* have been discovered to be associated with CMT. Current treatments for subjects with CMT are not preventive, but rather are based on mitigation of symptoms to allow the patient to better function with the disease. Therapeutic methods attempting to regulate *PMP22* expression have shown promise, however these methods will not be effective in the alleviation of cellular stress and ultimately Schwann cell apoptosis caused by the aggregation of PMP22 point mutated proteins in the ER. The oral administration of curcumin, a small molecule derived from the curry spice tumeric, has been shown to significantly decrease the percentage of apoptotic Schwann cells and partially alleviates the severe neuropathic phenotype of *Trembler-J* (*Tr-J*) mice in a dose dependent manner. Comparative expression profiling of the sciatic nerves of curcumin treated and untreated *Tr-J* mice revealed the potential specific molecular and cellular pathways affected by curcumin. Curcumin treatment leads to a significant increase in the expression of the heat shock protein, Hsp70i. Reduction of *Hsp70i* dosage in Tr-J mice results in a more severe peripheral neuropathy and upon curcumin treatment a rise in mortality. To investigate if the induction of *Hsp70i* expression can alleviate symptoms of peripheral neuropathies, we have produced *Tr-J* mice that over express *Hsp70i*. The *Tr-J* mice with one copy of transgenic *Hsp70i* present with a visibly healthier clinical phenotype and also display improved neuromotor function as assessed by rotarod testing versus *Tr-J* mice with wild type *Hsp70* expression. These results implicate Hsp70i as a primary component involved in the effectiveness of curcumin treatment in the partial mitigation of peripheral neuropathy.

## 517/W/Poster Board #175

**A novel insertion in GDAP1 gene in a Charcot-Marie-Tooth disease patient.** *A. Abbasi[1], M. Sadeghizadeh[1], M. Behmanesh[1], M. Houshmand[2,3].* 1) Department of Genetics, Faculty of Basic Sciences, Tarbiat Modares University, Tehran, Iran; 2) Diagnostic Molecular Genetics Lab, Special Medical Center, Tehran, Iran; 3) National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran.

Hereditary motor and sensory neuropathies (HMSN) in genetic literature called also Charcot-Marie-Tooth disease (CMT) are the most common heterogenous disorders of the peripheral nervous system. CMT disease caused by mutations in the ganglioside-induced differentiation-associated protein 1 (GDAP1) gene is a severe autosomal recessive neuropathy originally reported in families with either demyelinating CMT4A neuropathy or axonal neuropathy with vocal cord paresis which maps to the CMT4A locus on chromosome 8q21.1. GDAP1 is a 358 amino acid protein expressed in both the central and peripheral nervous system and a putative role as glutathione S-transferases has been postulated for it. In this study, blood samples from 32 Iranian families and more than 150 members of their families with diagnosis of CMT disease, either axonal or demyelinating, were obtained for genetic analysis of GDAP1 gene. Total genomic DNA was extracted from all family members using standard procedures. The 1.5 Mb CMT1A duplication and point mutations in PMP22 gene were first excluded using restriction enzymes and then MPZ (CMT1B) and GJB1 (CMTX1) genes were excluded using direct sequencing. The GDAP1 gene was then investigated using linkage analysis with three markers (D8S551, D8S286 and D8S164) linked to the GDAP1 locus in the families. PCR amplification and direct sequencing of whole coding regions and exon-intron boundaries of the gene showed a novel insertion in the first exon of the GDAP1 gene in an Iranian patient (c.98-99 insT) leading to a truncated protein. This mutation was not detected in normal subjects which supports the hypothesis that it is responsible for the CMT4A phenotype.

## 518/W/Poster Board #176

**Screening Aminoacyl-tRNA Synthetase Genes for Mutations Causing Peripheral Neuropathies.** *A. Antonellis[1,2], H.M. Harville[1], V. Ionasescu[3], J.R. Lupski[4,5,6,7], G. Nicholson[8,9], K. Talbot[10], J. Vance[11], S. Zuchner[11], E.D. Green[12,13], NISC Comparative Sequencing Program.* 1) Human Genetics, University of Michigan, Ann Arbor, MI; 2) Neurology, University of Michigan, Ann Arbor, MI; 3) Division of Medical Genetics, Department of Pediatrics, University of Iowa, Iowa City, IA; 4) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 5) Neurology, Baylor College of Medicine, Houston, TX; 6) Pediatrics, Baylor College of Medicine, Houston, TX; 7) Texas Children's Hospital, Houston, TX; 8) Northcott Neuroscience Laboratory, ANZAC Research Institute and Molecular Medicine Laboratory, Concord Hospital, Concord, New South Wales, Australia; 9) Faculty of Medicine, University of Sydney, Camperdown, New South Wales, Australia; 10) Department of Clinical Neurology, University of Oxford, Oxford, UK; 11) Miami Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 12) Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 13) NIH Intramural Sequencing Center (NISC), National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Aminoacyl-tRNA synthetases (ARSs) are a ubiquitously expressed, essential class of enzymes responsible for charging tRNA molecules with cognate amino acids. The human nuclear genome encodes all 37 ARSs, which include 17 enzymes responsible for charging tRNA in the mitochondria, 17 enzymes responsible for charging tRNA in the cytoplasm, and 3 bi-functional enzymes that charge tRNA molecules in both locations. Mutations in five ARS-encoding genes have been implicated in inherited neurological diseases in human and mouse, and two (those encoding glycyl- and tyrosyl-tRNA synthetase) are associated with distinct human peripheral neuropathies characterized by either a primary axonopathy or an intermediate form of Charcot-Marie-Tooth disease. These data suggest that all ARS genes can be considered excellent candidates for harboring mutations in patients with peripheral neuropathy and no known mutation. To study this further, we compiled a cohort of 364 such patients, the majority of which have been diagnosed with axonal peripheral neuropathy by a collaborating physician. We screened this cohort for mutations in the 37 human ARS genes using a high-throughput PCR- and DNA sequencing-based approach. These efforts revealed 146 novel predicted missense variants in 25 different ARS genes. Importantly, missense mutations have been shown to be a hallmark of known disease-causing glycyl- and tyrosyl-tRNA synthetase mutations, making our new findings particularly relevant for follow-up analyses. A major effort is now underway to evaluate these variants for disease association and functional consequences. The patient cohort and sequencing strategy will be presented along with interesting vignettes that are emerging from our genetic and functional characterization studies.

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## 519/W/Poster Board #177

**Molecular analysis of spastin gene (SPG4) mutations in patients with familial and sporadic spastic paraplegia.** *D. Di Bella[1], S. Baratta[1], C. Mariotti[1], R. Fancellu[1], N. Nardocci[2], D. Pareyson[3], S. Di Donato[1], C. Gellera[1], F. Taroni[1].* 1) Unit of Genetics of Neurodegenerative & Metabolic Diseases, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy; 2) Division of Child Neurology, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy; 3) Unit of Neurology VIII, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy.

Hereditary spastic paraplegias (HSP) are a clinically and genetically heterogeneous group of neurodegenerative disorders. Mutations in the SPG4 gene are responsible for ~15-40% of autosomal dominant (AD) HSP cases; HSP due to SPG4 mutations is generally described as a pure form of the disease, but has also occasionally been described in association with additional clinical features. This gene encodes spastin, a member of the AAA family of ATPases. The spastin gene was analysed by dHPLC and/or direct sequencing in 238 unrelated index cases, including 117 AD cases, 19 cases compatible with an autosomal recessive pattern of inheritance and 102 sporadic cases. Moreover, a subgroup of patients was screened for micro-rearrangements in the SPG4 gene by multiplex ligation-dependent probe amplification (MLPA) to assess the frequency of deletions/duplications. Thirty-three different pathogenic mutations (15 missense, 8 frameshift, 5 nonsense, 5 splicing) were found, 21 of which are novel. The mutations were not found in a large control population. Moreover, we identified 7 different heterozygous SPG4 deletions in 13 patients while, interestingly, 3 patients carried 3 different partial duplications of the SPG4 gene, setting at 17.3% the overall frequency of SPG4 rearrangements in the AD group. Overall, in our study, SPG4 point mutations account for 13.9% of spastic paraparesis patients, a frequency which raises to 23.1% if only AD families are considered, consistent with data from other studies. MLPA analysis demonstrates that a relevant proportion (17.3%) of mutation-negative HSP patients in fact carry SPG4 rearrangements, confirming that haploinsufficiency is a major mechanism of disease in SPG4 HSP and indicating that gene dosage analysis should always be performed when screening SPG4 in HSP patients. In conclusion, the data indicate that SPG4 accounts for ~40% of HSP families in the Italian population. [Supported by an Italian Ministry of Health grant (RF Neurodegenerazione ex art 56) to FT].

## 520/W/Poster Board #178

**Copy number variation analysis in patients with malformations of cortical development.** *A.K. Ozturk[1,2], K. Bilguvar[1,2], L.E. Kolb[1,2], M.L. Diluna[1,2], Y. Bayri[1,2], W. Ho[1,2], Z. Arlier[1,2], F. Bayrakli[1,2], K. Yasuno[1,2], C.E. Mason[2,3,4], S. Sanders[2,3,4], R.P. Lifton[3,5,6], M.W. State[2,3,4], M. Gunel[1,2,3,7].* 1) Neurosurgery, Yale University School of Medicine, New Haven, CT; 2) Program on Neurogenetics, Yale University School of Medicine, New Haven, CT; 3) Genetics, Yale University School of Medicine, New Haven, CT; 4) Child Study Center, Yale University School of Medicine, New Haven, CT; 5) Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT; 6) Internal Medicine, Yale University School of Medicine, New Haven, CT; 7) Neurobiology, Yale University School of Medicine, New Haven, CT.

The molecular mechanisms of brain development and function are largely unknown. Despite its fundamental importance, a mechanistic understanding of brain development at the level of the gene is generally lacking and only recently has begun to be elaborated in a broad fashion. Structural brain abnormalities represent disruptions of normal cerebral or cerebellar cytoarchitecture and often lead to severe neurological disorders including epilepsy, mental retardation and global developmental delays, as well as cognitive deficits and other motor and sensory neurological deficits. Recent studies have linked abnormalities in cerebral development to common neuropsychiatric disorders, including, for example, recent compelling evidence that abnormalities of cortical organization and neuronal migration are involved in some forms of Autism Spectrum Disorders, a constellation of syndromes affecting as many as 1 in 150 individuals. Furthermore, recent publications also demonstrated significant contribution of copy number variations (CNV) to neuropsychiatric disorders. In order to search for CNVs underlying structural brain abnormalities, we collected 250 patients from consanguineous families mainly from Eastern Turkey, where the consanguineous marriages are common. Those patients consisted of malformations of cortical development due to either stage of brain development such as cell proliferation or apoptosis, or neuronal migration or late cortical organization. We performed whole-genome genotyping using Illumina Human CNV370 or Human610 beadchips and then conducted CNV analysis by using PennCNV software as well as in-house algorithms. We then annotated these regions in terms of the genes affected and overlap with previously reported CNV loci in database. We eliminated CNVs based on size, overlap with previous reports and whether or not a coding exon is affected. We then confirmed the homozygous CNVs using quantitative PCR. Mutation screening of additional patients for coding homozygous deletions previously not reported is currently underway.

## 521/W/Poster Board #179

**Multiplexed resequencing analysis to identify rare variants in pooled DNA of barcode-indexed DNAs.** *J. Mitsui[1], K. Azuma[2], H. Tozaki[2], H. Ishiura[1], Y. Takahashi[1], J. Goto[1], S. Tsuji[1].* 1) Neurology, University of Tokyo, Tokyo, Japan; 2) Applied BioSystems Japan, Tokyo, Japan.

Genome-wide association studies employing common single nucleotide polymorphisms (SNPs) have been intensively conducted based on the "common disease-common variants hypothesis". However, little is known about disease susceptible rare variants, and efficient methods of detecting multiple rare variants in a large number of samples need to be established. We performed multiplexed resequencing with next-generation sequencer (SOLiD2) employing pooled DNA of barcode-indexed DNAs. We made several sets of pooled DNA of 96 patients with Parkinson disease and 96 normal control subjects as follows; #1: 96 samples of DNA were mixed into 1 set of pooled DNA followed by amplification of the target gene (GBA); #2: 16 samples in each group were mixed as 8 sets of pooled DNA. Each pooled DNA was subjected to PCR amplification of the target gene (GBA) and pooled in 1 tube with barcode-indexing; #3. 6 samples in each group were pooled as 16 sets of pooled DNA. Each pooled DNA was subjected to PCR amplification of the target gene (GBA) and pooled in 1 tube with barcode-indexing. Libraries of these pooled DNAs were prepared and subjected to target resequencing employing SOLiD2 system. To evaluate the results, individual samples were also subjected to direct nucleotide sequence analysis of the coding exons and the flanking splice sites of GBA to identify all variants. Obtained sequence reads were aligned to the reference sequences allowing mismatched color spaces less than 5. The frequencies of variants in the aligned sequences (total reads of 412,303,305) were determined at each nucleotide position (average read of 71,518). In the case of a set of pooled DNA of 6 samples, all the variants were detected with 100.0% of sensitivity. On the other hand, a "false positive" variant appeared in 0.27 kb on the average. Employing barcode-indexing, we could detect a heterozygous rare variant of 1 sample in the 96 samples. The bar coding-based multiplexing with pooled DNA is a suitable method for resequencing of a large number of samples. However, we should be cautious of the false positive reads if multiplicity of pooling is increased. We need to further optimize the protocols and algorithms to extract rare variants efficiently from a large scale of samples.

## 522/W/Poster Board #180

**Polymorphisms within *ataxin-3* and its interaction partners as genetic modifiers of Spinocerebellar ataxia type 3.** *T. Schmidt[1], V. Schmauke[1], N. Schlipf[1], J. Weber[1], T. Schwab[1], D. Wilkens[1], V. Häfele[1], C. Funke[1], C. Richter[1], A. Pohl[1], D. Lenz[1], I. Mäckle-Jentsch[1], P. Bauer[1], L. Schöls[2], O. Riess[1].* 1) Medical Genetics, Univ Tuebingen, Tuebingen, Germany; 2) Neurology, Univ Tuebingen, Tuebingen, Germany.

Spinocerebellar Ataxia Type 3 (SCA3) or Machado-Joseph disease (MJD) is an autosomal dominantly inherited, neurodegenerative disorder which is caused by the expansion of a CAG repeat in the *MJD1 gene* resulting in an expanded polyglutamine repeat in the encoded ataxin-3 protein. SCA3 therefore belongs to the group of the so called polyglutamine diseases. Statistically, a correlation between the number of CAG repeats and the age at onset exists and patients with a higher number of CAG repeats have first symptoms at earlier ages. However, this correlation is not perfect and the number of CAG repeats contributes only about 55 % to the age at onset. Therefore, the remaining 45 % are influenced by other factors, which we try to identify in this study. The actual function of ataxin-3 is not fully understood. However, interaction partners of ataxin-3 have been identified. In this project, we analyzed the coding genes of ataxin-3 and its interaction partners HHR23A, one human homologue of yeast RAD23, and p97/VCP for single nucleotide polymorphisms (SNP) and their influence on the age at onset. We first analyzed already known polymorphisms published in relevant databases in our cohort of SCA3 patients and later performed own screenings for novel SNP. For these screenings, we first generated PCR conditions for the amplification of all coding exons as well as the 5' and 3' untranslated regions (UTR) and the putative promoter and then established for these 51 PCR products conditions for a mutation screen using dHPLC. After screening of a collection of SCA3 patients, putative polymorphisms were identified via sequencing. Finally, an appropriate assay was generated for the genotyping of all SCA3 patient samples collected within the EUROSCA consortium (more than 500 samples). These results were then correlated with the age at onset in order to analyze its impact on the disease. In our analyses, p97/VCP turned out to be highly conserved and the few polymorphisms within this gene seem not to contribute to the age at onset. Interestingly, in the other analyzed genes some of the SNP turned out to have an impact on the age at onset in SCA3 patients. We will present the statistical correlation between these known and novel polymorphisms and the age at onset. We hope that our results will improve the prediction of clinical symptoms and contribute to the understanding of pathogenic processes in SCA3.

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## 523/W/Poster Board #181

**A Novel X-linked 4-Repeat Tauopathy with Parkinsonism and Spasticity.** *P. Navas[1,2], W.H. Raskind[1,2,3], J.B. Leverenz[1,3,5,6], M. Matsushita[2], C.P. Zabetian[3,4,6], A. Samii[3,6], S. Kim[1], N. Gazi[1], L.M. Tisch[2], J.G. Nutt[7], J. Wolff[2], D. Yearout[3,4], J.L. Greenup[1], E.J. Steinbart[3], T.D. Bird[2,3,4].* 1) Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA; 2) Department of Medicine, University of Washington, Seattle, WA; 3) Department of Neurology, University of Washington, Seattle, WA; 4) Geriatric Research Education Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA; 5) VISN 20 Mental Illness Research Education Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA; 6) Parkinson's Disease Research Education Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA; 7) Department of Neurology, Oregon Health Sciences University, Portland, OR.

The parkinsonian syndromes comprise a highly heterogeneous group of disorders. Although 14 loci are linked to predominantly familial Parkinson's disease (PD) and disease-causing mutations have been identified in eleven genes, additional PD loci are likely to exist. We recently identified a multigenerational family of Danish and German descent with a unique syndrome characterized by parkinsonian features and variably penetrant spasticity (XPDS), in which X-linked disease transmission was suggested. XPDS is unlike idiopathic PD because of prominent spasticity and a lack of LB pathology and is unlike classic hereditary spastic paraplegia because of the co-occurrence of parkinsonian features. Autopsy in one individual failed to reveal synucleinopathy; however, there was a significant 4-repeat tauopathy in the striatum. Our objective was to identify the locus responsible for this unique X-linked parkinsonian disorder. Members of the XPDS family were genotyped for markers spanning the X chromosome. Two-point and multipoint linkage analyses were performed and the candidate region refined by analyzing additional polymorphic markers. A multipoint LODmax score of 2.068 was obtained between markers DXS991 and DXS993. Haplotype examination revealed an approximately 20 cM region bounded by markers DXS8042 and DXS1216 that segregated with disease in all affected males and obligate carrier females and was not carried by unaffected at-risk males. The XPDS locus does not overlap with any known X-chromosome PD or spasticity locus and the 28 Mb critical region contains ~200 RefSeq candidate genes and 118 hypothetical protein genes. Candidate genes within the critical region have been prioritized for sequence analysis based on high levels of CNS expression and protein function. For an X-linked disorder, a LOD score of >2 provides significant evidence ($p \leq .05$) in favor of linkage; however, although unlikely, it remains possible that an autosomal locus is responsible for the disease. With this in mind, we used exclusion mapping or direct sequencing to rule out mutations in several autosomal dominant disease genes known to cause syndromes or phenotypes similar to XPDS including SPG3A, SPG4, FBXO7, PLA2G6, DSC3, PARK2, MAPT, SNCA and LRRK2. In conclusion, we have identified a unique X-linked parkinsonian syndrome with variable spasticity and 4-repeat tau pathology, and defined a novel candidate gene locus spanning approximately 28 Mb from Xp11.2-Xq13.3.

## 524/W/Poster Board #182

**The Polycystic Kidney Disease Mutation Database (PKDB): a locus specific database of genetic variants at the *PKD1* and *PKD2* genes.** *S. Rossetti[1], B. Wilson[2], J.P. Kocher[2], V.E. Torres[1], P.C. Harris[1].* 1) Division of Nephrology, Mayo Clinic College of Medicine, Rochester, MN; 2) Bioinformatics Core, Mayo Clinic College of Medicine, Rochester, MN.

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a frequent inherited nephropathy characterized by kidney cysts leading to kidney failure. ADPKD is caused by mutations at two genes, *PKD1* and *PKD2*. Screening for mutations in large populations has revealed that ADPKD is characterized by high allelic heterogeneity. Through literature mining, we have collected all the published *PKD1* and *PKD2* mutations in a locus specific database, PKDB [http://pkdb.mayo.edu]. Data were extracted from the original reports, corrected to the standard reference sequences, and organized into a database. To date the PKDB includes 875 variants for *PKD1* and 139 variants for *PKD2*. This large dataset has allowed for the first time analysis of the mutational pattern of both *PKD* genes. Of the 875 *PKD1* variants, 306, in 386 families (135 frameshifts, 17 large deletions, 109 nonsense and 45 splicing changes) are truncating (~35%), while 263 are missense (~30%) and 37 are in-frame deletions, with the remaining variants being polymorphisms. Of the 139 *PKD2* variants, the largest portion is truncating changes, 99 total, in 177 families (50 frameshifts, 1 large deletion, 30 nonsense, and 18 splicing) (71%), while only 23 are missense mutations (16.5%), 6 are in-frame deletions, with the remaining being polymorphisms. The high level of allelic heterogeneity and lack of any appreciably recurrent mutation illustrates the challenge of molecular diagnostics. The high number of missense mutations (~1/4 of the total) and the high level of non disease-associated polymorphisms (~10 polymorphism per patient in PKD, including many non synonymous) underline the challenge to determine the pathogenicity of amino acid substitutions. As part of PKDB version 3, we have developed a scoring system for the evaluation of amino acid substitutions at both genes, using *in silico* predictors (the biochemical/biophysical nature of the substitution (Grantham Matrix), the evolutionary conservation, the overall domain conservation when available, the predicted secondary structure propensity and solvent accessibility, calculated using bioinformatics tools. Contextual issues, such as other detected variants in the patient, segregation data and previous description of the variant, have been taken into consideration. This has allowed a substantial re-classification of missense mutations at these genes (90 *PKD1* and 9 *PKD2* with 99 new classified as likely pathogenic), aiding diagnostic and research applications.

## 525/W/Poster Board #183

**Analysis of patients with renal hypouricemia in Czech population.** *B. Stiburkova[1], I. Sebesta[1,2], M. Hosoyamada[3], K. Ichida[4].* 1) Charles University in Prague, First Faculty of Medicine, Institute of Inherited Metabolic Disorders; 2) Institute of Clinical Biochemistry and Laboratory Medicine; 3) Division of Pharmacotherapeutics, Faculty of Pharmacy, Keio University, Tokyo; 4) Department of Pathophysiology, Tokyo University of Pharmacy and Life Sciences, Japan.

Hypouricemia, defined as a serum urate levels < 2 mg/dl, may results from decreased uric acid production, but is more commonly due to decreased renal tubular urate reabsorption. Renal hypouricemia is heterogeneous inherited disorder characterized by impaired tubular uric acid (UA) transport. Most patients are clinically asymptomatic, but acute renal failure and/or nephrolithiasis may develop. Major causative genes are SLC22A12 and SLC2A9. hURAT1 (encoded by SLC22A12) is the major urate-anion exchanger in the kidney, which regulates blood urate levels and plays the central role in reabsorption of urate from glomerular filtrate on apical membrane of the proximal tubules. Genetic variations of the SLC22A12 are associated with renal hypouricemia (OMIM*607096). We have selected patients for analysis of the SLC22A12 (with repetitive serum UA < 1 mg/dl and $EF_{UA}$ > 43%) from the group of 569 Czech hypouricemic cases. Sequence analysis in the 17 subjects revealed 3 sequence variations in the promotor and 5'-UTR region and 5 in exonic regions. 3 transition and 4 deletions, yet unpublished, were found in 6 patients. The function and immunohistochemistry analysis in *Xenopus laevis* oocytes showed significantly decreased urate transport activities of these variants. Recently, transporter GLUT9 (encoded by SLC2A9) was identified, member of the glucose transporter family, which plays a key role in urate reabsorption on both sides of the proximal tubules. Genetic variations of the SLC2A9 were revealed in renal hypouricemic patients (OMIM*606142). We have diagnosed Czech family suffering from renal hypouricemia with one nucleotide insertion (nonsense seq. variant) in SLC2A9 gene and no sequence variants in urate transporters SLC22A12, SLC2A9, SLC17A3, ABCC4, ABCG2 and MRP4. This finding gives further evidence that SLC2A9 is causative gene of renal hypouricemia and supports the important role of SLC2A9 in regulation of serum urate levels in humans. Our finding supports the prediction that intact function of both SLC22A12 and SLC2A9 is necessary for normal urate reabsorption. In patients with unexplained hypouricemia and elevated urinary excretion of urate the mutational analysis of several genes coding urate transporters should be performed for confirmation of diagnosis. Detection of more patients with hereditary renal hypouricemia is needed for better understanding of renal urate handling. Supported by grant MSM0021620806 Czech Republic.

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## 526/W/Poster Board #184

**Identification of the second North American family with *REN*-associated medullary cystic kidney disease.** *J.N. Adams[1], A.J. Bleyer[2], T.C. Hart[4], M. Zivna[3], S. Kmoch[3], P.S. Hart[1].* 1) Office of the Clinical Director, NIH, Bethesda, MD; 2) Section on Nephrology, Wake Forest University School of Medicine, Winston-Salem, NC; 3) Center for Applied Genomics and Institute for Inherited Metabolic Disorders, Charles University in Prague, First Faculty of Medicine, Prague; 4) Human Craniofacial Genetics Section, NIDCR, NIH, Bethesda, MD.

Recently, a Renin (*REN*) mutation was identified in a family of Belgian descent segregating autosomal dominant chronic interstitial kidney disease, anemia, and hyperuricemia. The mutation caused deletion of a single leucine residue in the signal peptide (p.L16del), causing reduced renin biosynthesis. We subsequently identified a North American family with an independent origin of the p.L16del mutation. We now present a North American family with a different mutation in *REN* resulting in a more severe clinical phenotype. The proband presented with acute kidney failure after a viral illness resulting in volume depletion at the age of 3 yr. At 6 yr, she suffered from polyuria, nocturia, and anemia. The blood pressure at that time was 82/52 mm Hg with an unremarkable physical examination. A 24 hr urine collection revealed minimal protein, an elevated urine sodium at 5.2 mEq/kg/day, and an elevated urinary volume at 57 ml/kg/day. Her serum uric acid levels were as high as 7.7 mg/dl (normal <6 mg/dl), with a fractional excretion of uric acid of 3.0% (normal 6-10%). She also suffered from anemia responsive to erythropoietin. At age 10, she underwent a 24 hr urine collection for aldosterone revealing 2 ug/24 hr (normal 2-16 ug/24hr). Her father suffered from anemia since childhood. At age 29, he first suffered from gout. At age 33 he underwent a biopsy for renal insufficiency revealing tubulointerstitial scarring with global glomerulosclerosis. A 24 hr urine collection revealed 3450 ml, urinary sodium elevated at 283 mEq/24 hr, and fractional excretion urate 3% (nl >5%). A 24 hr urinary aldosterone was 9 ug/24 hr (nl 17-44 on a low sodium diet). *REN* was sequenced in the affected proband and father, the unaffected mother and sibling as well as the father's parents and unaffected siblings. A c.58T>C change was identified in the affected individuals, predicting substitution of the terminal cysteine residue of the signal peptide with an arginine residue (p.C20R), but not in the unaffecteds. Thus, the mutation appears to have arisen de novo in the father who then transmitted it to the proband. Haplotype analysis is consistent with the mutation have arisen on the maternal chromosome. To date all *REN* mutations causing hereditary interstitial kidney disease have occurred in the signal peptide. Patients with anemia, hyperuricemia and chronic progressive kidney failure should be tested for *REN* mutations, even in the absence of a positive family history.

## 527/W/Poster Board #185

**Novel uromodulin mutations identified in 8 out of the 21 probands with uromodulin associated kidney disease (UAKD).** *K. Hodanova[1], M. Zivna[1], P. Vyletal[1], M. Votruba[1], G. Venkat-Raman[2], T. Ring[3], S. Kmoch[1].* 1) Inst Inher Metab Dis, Charles Univ, Prague, Czech Republic; 2) Renal Unit, Queen Alexandra Hospital, Portsmouth, United Kingdom; 3) Department of Nephrology - Aalborg Hospital , Denmark.

The term of the uromodulin associated kidney diseases (UAKD) emerged recently for a group of genetically heterogeneous autosomal-dominant tubulointerstitial nephropathies characterized by the variable combination of hyperuricemia, gouty arthritis, decreased urinary uromodulin (UMOD) excretion and abnormal UMOD expression in the kidney. UAKD disease complex comprises phenotypes known as a familial juvenile hyperuricemic nephropathy (FJHN) (OMIM 162000), medullary cystic kidney diseases type 1 (MCKD1) (OMIM 174000) and type 2 (MCKD2) (OMIM 603860) and glomerulocystic kidney disease (GCKD). Following our original report of 19 UAKD families and 6 novel UMOD mutations (Vyletal et al, Kidney Int. 2006), we investigated a further 282 probands showing some of the clinical and biochemical hallmarks of the UAKD phenotype. At first, we performed semi-quantitative and qualitative analysis of UMOD in random urine samples by Western blot. Next, based on the reduction in UMOD excretion amount and/or presence of abnormal UMOD immunopositive forms we selected 21 probands from United Kingdom (9) Czech Republic(9), Denmark (1), Netherlands (1) and Israel (1) for UMOD sequence analysis. In these probands we identified 8 UMOD mutations, 6 of which are novel (C112G, R185C, G210D, W230R, P236R, C306Y) and 2 were previously published (C148Y and 383del12/ins9). In one additional case with otherwise normal UMOD sequence we identified cSNP variation in exon 7 resulting in V458L amino acid exchange. Our work further demonstrates the genetic heterogeneity of UAKD, confirming our previous estimates, that in about 38% of the families the disease is associated with UMOD mutation and stress the utility and importance of urinary UMOD analysis in probands with clinical and biochemical signs of UAKD.

## 528/W/Poster Board #186

**Bladder dysfunction in cystinuria male mice is associated with decreased uroplakin expression.** *A. Sahota[1], D.-Y. Cha[2], M. Yang[1], L. Serrano[1], R.M. Levin[3], J.G. Barone[2], J.A. Tischfield[1].* 1) Dept Gen, Rutgers Univ, Piscataway, NJ; 2) Dept Surgery, UMDNJ-RWJMS, New Brunswick, NJ; 3) Dept Pharmaceutical Sci, Albany Coll Pharmacy and Health Sci, Albany, NY.

**Background:** Cystinuria is the most common inherited cause of urinary tract stones in children, with males more severely affected than females. Slc3a1 knockout male mice from age 4 months develop cystine stones in the bladder, but only a small fraction of females (age >20 months) form stones. Cystinuria male mice have partial bladder outlet obstruction, but bladder function in female mice is only mildly affected. The apical layer of the bladder urothelium contains uroplakins that form rigid membrane plaques on the cell surface. These proteins provide a permeability barrier that prevents the leakage of urine into the underlying tissue. The four major uroplakins are UPI(a,b), II, and IIIa and they occur as UPIa/UPII and UPIb/UPIIIa pairs. **Methods:** We examined uroplakin expression in bladders from cystinuria and control mice of different age groups (3-6 animals per group). Total RNA was isolated from fresh bladders after removal of any stone material. Real time PCR was carried out using Syber Green dye and GAPDH was used as an internal control. **Results:** UP1a and UPII were expressed at similar levels in 4-5 and 10-12 months old control male mice. There was a significant, age-dependent reduction in expression of these two transcripts in bladders from cystinuria male mice. The UPIa/GAPDH ratio for the younger age group was 0.55 (p = 0.04) and for the older age group the ratio was 0.07 (p = 0.0002). The corresponding UPII/GAPDH ratios were 0.40 (p = 0.01) and 0.21 (p = 0.0001), respectively. In females, there was no difference in UP1a or UPII expression between control and cystinuria mice among the three age groups tested (4-5, 10-12, and 20-22 months). Expression levels of UPIb and UPIIIa were consistently lower in cystinuria males compared with controls, whereas in cystinuria females the expression of these transcripts was unaffected by phenotype. **Conclusions:** These results demonstrate that the presence of cystine stones in the male bladder is associated with decreased expression of uroplakins, possibly through exfoliation of cells from the urothelial surface expressing these proteins. Breaching of the urothelial barrier may lead to the release of mediators that disrupt bladder function in cystinuria male mice. The lack of a significant effect on bladder function in cystinuria female mice suggests the presence of inhibitors of stone formation. These observations may in part account for the gender differences in disease severity seen in cystinuria patients.

## 529/W/Poster Board #187

**Molecular basis of autosomal polycystic kidney disease (*PKD1, PKD2*): identification of 72 new mutations and two large rearrangements.** *E. CORNEC LE GALL[1,2], MP. AUDREZET[1], B. MERCIER[1], J. CREFF[1], I. QUERE[1], Y. LE MEUR[2], C. FEREC[1].* 1) Inserm U613, Molecular Genetic Laboratory, CHU, Brest, France; 2) Nephrology, Dialysis and Transplantation Unit, CHU, Brest, France.

Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic disorder, with an estimated prevalence ranging from 1/500 to 1/1000. Clinical features are highly variable. The disease is characterized by the progressive development of bilateral fluid-filled cysts in the kidney and others organs and it accounts for 5 to 10 % of the causes of end stage renal disease, roughly in the sixth decade of age, but neonatal occurrence are also reported. In addition, non-cystic manifestations are described, such as intracranial aneurysms in 8 % of the patients or mitral valve prolapse. Mutation-based diagnosis in ADPKD is complicated by genetic and allelic heterogeneity, with 2 genes involved, *PKD1* with 314 pathogenic variants described and *PKD2* with 91 mutations reported. Moreover, *PKD1* is duplicated over 2/3 of its length and is extremely rich in GC nucleotides. Besides, diagnosis of ADPKD is based upon the detection of kidney cysts by abdominal ultrasonography, according to age-dependent criteria. All these aspects have contributed to consider mutation screening time-consuming, expansive and irrelevant. However, tremendous progress have been made in the past decade in understanding molecular pathogenesis, and targeted drugs are on the way, which makes crucial the identification of predictive factors of clinical evolution and thus the identification of causing mutation to define genotype-phenotype correlations. We undertaken a comprehensive study of *PKD1* and *PKD2* in a large cohort of 162 unrelated patients, using newly home-designed conditions for specific amplification followed by sequencing analysis, and we proposed for the first time a new approach for the study of large rearrangments by quantitative PCR. This new strategy enabled us to identify 71 new mutations for *PKD1* and 3 new mutations in *PKD2*. The majority of changes in *PKD1* were predicted to truncate the protein, with 51% of frameshift mutations, 31% of nonsense mutations and 12% of splicing changes. We successfully characterized two large rearrangements, one of them erasing almost all the *PKD1* gene. Beyond our cohort, mutation-based diagnosis turned to be relevant for assessment of ADPKD in some particular situations, such as atypical forms or absence of a familial history. Moreover, it's likely to become a very useful tool for clinicians as new therapies are now emerging.

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## 530/W/Poster Board #188

**Characterization of a broad spectrum of mutations in cystic fibrosis transmembrane conductance regulatory gene (CFTR) gene: An Indian Scenario.** *R. Prasad[1], N. Sharma[1], M. Singh[1], S.K. Singh[1], G. Kaur[2], B.R. Thapa[1].* 1) Department of Biochemistry, Postgraduate Institute of Medical Education and Research, Chandigarh - 160012; 2) Department of Physiology, Government Medical College and Hospital, Chandigarh - 160032.

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. This study was performed on Indian CF patients (n=50) to investigate the spectrum of mutations in the CFTR gene and their association with intragenic and extragenic marker haplotypes. We report identification of 14 previously known and eight novel mutations, namely 3986-3987delC, 876-6del4, 1792InsA, L69H, S158N, Q493L, I530L and E1329Q. The frequency of delta F508 was found to be 27%. Absolute linkage between delta F508 and the KM.19-GATT-TUB9-M470V-T854T haplotype (2-2-1-1-1) predicts a relatively recent appearance of delta F508 in Indian CF patients. Low frequency of delta F508 mutation and detection of eight novel and thirteen rare mutations reflect a heterogeneous spectrum of mutations in Indian CF patients. Failure to detect mutations in 34% of alleles indicates the possible presence of gross deletions involving one or more exons or may indicate the location of the molecular defects in either the noncoding parts of the gene or in the promoter region, which warrants analysis of those regions. Additionally, we studied the mutations in the CFTR gene in CAVD patients. The spectrum and frequency of CFTR mutations in Indian males with congenital absence of vas deferens (CAVD) is unknown. We investigated 50 Indian males, diagnosed with unilateral or bilateral absence of vas deferens at the PGIMER, Chandigarh. This study led to the identification of 12 CFTR gene mutations on 48% of 100 Indian CAVD chromosomes. This study confirms the molecular heterogeneity of CFTR mutations in CAVD.

## 531/W/Poster Board #189

**Endothelial nitric oxide synthase gene VNTR polymorphism and Respiratory Distress in premature infants.** *H. Chen[1], J.G. Aryama[1], K. Yanamandra[1], D. Napper[1], S.A. Ursin[1], A. Pramanik[1], J.A. Bocchini Jr.[1], R. Dhanireddy[2].* 1) Dept Pediatrics, Perinatal Gen, LSUHSC- Shreveport, Shreveport, LA; 2) Dpt Pediatrics, UT Health Science Center, Memphis, TN.

Premature infants frequently experience delayed cardiopulmonary transition from fetal to neonatal stage compared to full term and normal weight newborn infants, due to their immature vasculature, especially in the pulmonary system. This transition is mediated by endogenous nitric oxide (NO) from vascular endothelial milieu. As a result of this delayed transition, premature infants experience a great deal of respiratory distress. Pharmacological inhaled nitric oxide along with supplemental oxygen is instituted frequently to reduce the distress and to facilitate the improved vasculature in those infants. Endothelial nitric oxide (eNO) serves as a vasodilator, relaxes smooth muscle, prevents platelet aggregation, and facilitates improved blood flow, leading to vascular homeostasis. Reduced nitric oxide levels result in vasoconstriction and weak tone leading to decreased blood flow and hypoxia. Mutant eNO synthase (eNOS) genotypes result in reduced nitric oxide levels by decreasing the enzyme activity. Although several studies have been carried out with NO, polymorphic studies at gene level were limited at best. Our earlier studies have revealed a strong association of eNOS promoter polymorphism with delayed cardiopulmonary transition in premature infants. Presently we are studying the association of eNOS variable number of tandem repeats (VNTR) polymorphism and to address the role of eNOS genotypes during respiratory distress in premature infants. In the present investigation we have studied the association of eNOS VNTR genotypes of 27bp repeats in intron 4 region of the gene. We have collected peripheral blood specimens from a total of 281premature infants consecutively from our NICU facility and genotyped by PCR. Our earlier findings in the study of eNOS genotypes in the promoter region have shown an elevation of -786C mutant alleles resulting in lower nitric oxide levels in infants with respiratory distress as compared to controls. However, in the present study the frequency of rear allele *a* consisting of 4 repeats was elevated though not as much as promoter genotypes in patients as compared to controls (0.18 vs. 0.14, odds ratio 1.4). Thus we speculate that the mutant genotype in promoter region of eNOS gene may have a stronger role in the etiology of respiratory distress compared to the VNTR genotypes. Clinical data of patients, ethnic stratification, distribution of genotypes and their significance will be presented.

## 532/W/Poster Board #190

**The role of galectin-3 in the development of pulmonary fibrosis in Hermansky-Pudlak syndrome.** *C. Yeager[1,2], H. Dorward[1], T. Markello[1], B. Gomez[1], K. O'Brien[3], J. Salas[3], B.R. Gochuico[1], W.A. Gahl[1].* 1) Section on Human Biochemical Genetics, Medical Genetics Branch, NHGRI, Bethesda, MD; 2) HHMI-NIH Research Scholar, Howard Hughes Medical Institute, Bethesda, MD; 3) Intramural Program of the Office of Rare Diseases, OD, NIH, Bethesda, MD.

Hermansky-Pudlak syndrome (HPS) is a rare autosomal recessive disorder characterized by abnormal biogenesis of lysosome-related organelles, such as melanosomes and platelet delta granules; defects in the biogenesis of these two organelles result in the oculocutaneous albinism and bleeding diathesis seen in HPS. There are 8 known HPS-causing genes, each encoding a protein that, in combination with other proteins, functions to form or traffic lysosome-related organelles. HPS-1 and HPS-4, subtypes of HPS with defective Biogenesis of Lysosome-related Organelles Complex-3 (BLOC-3), are notable for development of pulmonary fibrosis, which is the leading cause of mortality in HPS-1. All HPS-1 patients show evidence of pulmonary fibrosis by sixty years of age. Currently, the only treatments available for pulmonary fibrosis are investigational drugs and lung transplantation. The etiology of pulmonary fibrosis in HPS-1 and HPS-4 is unknown. Two pneumocytes of interest are the type II epithelial cell and the alveolar macrophage. In HPS-1 pulmonary fibrosis, type II cells are engorged with giant lamellar bodies, which are lysosome-related organelles that contain surfactant. In addition, alveolar macrophages in HPS-1 pulmonary fibrosis contain aggregates of ceroid lipofuscin and are activated. We hypothesized that galectin-3, an oncoprotein expressed by type II cells, contributes to the pathogenesis of HPS-1 pulmonary fibrosis. Our data demonstrate that galectin-3 immunostaining of type II cells and alveolar macrophages is more intense in HPS-1 pulmonary fibrosis lung tissue compared to that in idiopathic pulmonary fibrosis (IPF) or normal lung tissue. In addition, a cell surface predominance of galectin-3 is observed in alveolar macrophages in HPS-1 pulmonary fibrosis, but not IPF, lung tissue. Consistent with these data, bronchoalveolar lavage fluid concentrations of galectin-3 in HPS-1 pulmonary fibrosis are significantly higher than in IPF or normal volunteers (P=0.014 and =0.024, respectively). High alveolar concentrations of galectin-3 may be a consequence of aberrant trafficking of galectin-3 or its binding partners in HPS-1. Dysregulation of galectin-3 may contribute to abnormal modulation of inflammation and repair in the lung, which may lead to pulmonary fibrosis.

## 533/W/Poster Board #191

***COL1* C-propeptide Cleavage Site Mutations Cause OI with Increased Bone Mineralization.** *A.M. Barnes[1], K. Lindahl[2], M. Whyte[3], T. Hefferan[4], M.J. Yaszemski[4], C-J. Rubin[2], A. Kindmark[2], W. McAlister[3], S. Mumm[3], A. Boskey[5], O. Ljunggren[2], J.C. Marini[1].* 1) Bone and Extracellular Matrix Branch, NICHD/NIH, Bethesda, MD, USA; 2) Department of Endocrinology, Medical Sciences, Uppsala University, Uppsala, Sweden; 3) Shriners Hospital for Children, St. Louis, MO, USA; 4) Department of Orthopedics, Mayo Clinic, Rochester, MN, USA; 5) Hospital for Special Surgery, Weill Medical College at Cornell University, New York, NY, USA.

Osteogenesis imperfecta (OI), or brittle bone disease, is most often caused by mutations in the type I collagen genes. Mutations in the type I procollagen C-propeptide cleavage site are of particular interest because they disrupt a unique processing step, but their clinical and biochemical consequences have not been reported. We investigated two children with mild OI who had cleavage site mutations in COL1A1 (P1: $\alpha$1(I)Asp1041Asn) or COL1A2 (P2: $\alpha$2(I)Ala1029Thr). P1 is an 11 yr old girl whose L1-L4 DEXA and pQCT vBMD z-scores were +3. Her elevated BMD contrasted with radiographs demonstrating osteopenia and os-in-os vertebrae. P2 is an 11 yr old boy with an L1-L4 DEXA z-score of 0 and pQCT vBMD of -1.8; his radiographs revealed gracile long bones with osteopenia. Both P1 and P2 are at the 75th percentile for height. Iliac crest histomorphometry was characteristic of mild OI: P1 had increased bone remodeling while P2 had decreased BV/TV; neither had a mineralization defect or signs of osteosclerosis. FTIR imaging analysis in both cortical and trabecular bone revealed elevated mineral/matrix in P1 and P2, compared to age-matched controls or classical OI. P1 and P2 have significantly increased collagen crosslinks in trabecular bone and slightly increased crosslinks in cortical bone. The dermal fibrils of P1 had a normal diameter with slight surface irregularities, while P2 had small fibrils with greater irregularities, including some with blebs. Steady-state collagen electrophoresis showed slight backstreaking of $\alpha$1(I) and $\alpha$2(I) in cell layers of both patients. Chain incorporation was normal in P1 and slightly delayed in P2. Pericellular processing of P1 and P2 was delayed, with increases in pC-$\alpha$1, pro-$\alpha$1 and pro-$\alpha$2, and less mature collagen formation. Pro-$\alpha$1(I) cleavage appears crucial to C-propeptide processing, while defective pro-$\alpha$2(I) specific or non-specific cleavage occurs after $\alpha$1(I) processing. Hence, these mutations define a novel phenotype among type I collagen defects which reveals the importance of the type I procollagen C-propeptide to bone mineralization. In combination with recently reported adult siblings with an $\alpha$1(I)Ala1040Thr substitution and high bone mass on radiographs and histomorphometry (Calcif Tissue Int 82S1: CC01), our cases suggest that defects in pro-$\alpha$1(I) processing lead to high BMD in childhood, with signs of osteopetrosis occurring subsequently.

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## 534/W/Poster Board #192

**A specific interaction between a novel variant in the cis-acting sonic hedgehog regulatory sequence (ZRS) and transcription factor and its association with preaxial polydactyly with triphalangeal thumb.** *M. Farooq[1], L. Hansen[2], H. Eiberg[3], J.T. Troelsen[3], M. Boyd[3], M. Aslam[1], M.S. Hussain[1], M. Tariq[1], A. Ali[1], I. Ahmad[1], N. Tommerip[2], K.W. Kjaer[2], S.M. Baig[1].* 1) Human Molecular Genetics, National Inst for Biotech and Genet Eng (NIBGE), Faisalabad, Punjab, Pakistan; 2) Wilhelm Johannsen Centre for Functional Genome Research, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark; 3) Department of Cellular and Molecular Medicne, University of Copenhagen, Copenhagen, Denmark.

Intact Sonic Hedgehog cis-regulatory sequence also known as 'ZRS' located in intron 5 of LMBR1 is important for proper spatiotemporal expression of Sonic hedgehog (SHH) in the developing limb bud. Many point mutations have been identified in ZRS leading to disruption of SHH expression during limb development leading to preaxial duplication in upper limbs. In this study, preaxial polydactyly and triphalangeal thumb in Pakistani family with autosomal dominant inheritance was analyzed molecularly. The phenotype was confirmed by radiographic examination of three affected members. Linkage analysis using microsatellite markers D7S550, D7S559 and D7S2423 was performed and maximum multipoint LOD score of 1.93 at an allele frequency of $\theta$= 0.1 was obtained. This region spans SHH and its cis-acting regulatory element (ZRS), which is well conserved among various species lying in intron 5 of LMBR1. Direct Sequencing of ZRS identified a novel point mutation (T>G) in ZRS element at base position 4976 in intron 5 of LMBR1. Restriction enzyme analysis confirmed that the mutation co-segregated with the phenotype. The mutation was absent in 280 unrelated normal chromosomes from the same ethnic group. We analyzed the effect on transcription factor binding affinity of identified point mutation. Electrophoretical mobility shift assay (EMSA) demonstrated a marked difference between wt and the mutant probe which uniquely bound a specific subset of nuclear transcription factors extracted from Caco-2 cells. We suggest that altered transcription factor affinity may be important for our understanding of how single nucleotide substitutions in long distance regulatory elements changes cis-regulation of its target gene.

## 535/W/Poster Board #193

**A distinct type II collagen phenotype: premature arthritis.** *P. Kannu[1,2,3], J. Bateman[2,3], S. Randle[5], S. Cowie[2], D. du Sart[2,3], S. McGrath[6], M. Edwards[4], R. Savarirayan[2,3].* 1) Medical Genetics Unit, Queen's University, Kingston, Ontario, Canada; 2) Murdoch Childrens Research Institute Flemington Road, Melbourne 3052 Australia; 3) University of Melbourne Parkville 3052 Australia; 4) Hunter Genetics, Hunter-New England Area Health Service, Newcastle, AUSTRALIA; 5) Glenroy Specialist Centre, 842 Pascoe Vale Rod, Melbourne, AUSTRALIA; 6) Princeton Medical Centre, 60 Lindsay Street, Hamilton 2303, AUSTRALIA.

Mutations in the gene encoding type II collagen (COL2A1) give rise to a spectrum of phenotypes predominantly affecting cartilage and bone. These chondrodysplasias are typically characterized by disproportionate short stature, eye abnormalities, cleft palate, and hearing loss. Less recognized is that mutations in COL2A1 can also present with degenerative joint disease in the absence of any other phenotypic clues. We report two Australian families presenting with an isolated arthritic phenotype, segregating as a dominant trait affecting both large and small joints, prior to age 30 years. Sequencing of the COL2A1 gene in the propositi revealed two novel sequence changes resulting in glycine substitutions in the triple helical domain of type II collagen. We review the increasing evidence implicating COL2A1 mutations in individuals presenting with isolated degenerative joint disease, aiming to alert physicians assessing these patients to this possibility. The importance of finding a COL2A1 mutation in such patients lies in the subsequent ability to accurately assess recurrence risks, offer early (including prenatal) diagnosis, and provide information regarding natural history of the condition. Most importantly, it enables at-risk individuals to be identified for implementation of preventative strategies (i.e. weight loss, joint-friendly exercise programs) and early ameliorative management of their condition.

## 536/W/Poster Board #194

**Brachydactyly Caused By a Frameshift Mutation in CHSY1.** *B. Merriman[1], H. Lee[1], B. O'connor[1], M. Shboul[2], C. Bonnard[2], J. Tian[2], A. Al-Hadidy[4], A. Masri[4], H. Hamamy[3], S. Nelson[1], B. Reversade[2].* 1) Dept Human Genetics, Univ of California, Los Angeles, Los Angeles, CA; 2) Institute of Medical Biology, A*Star, Singapore; 3) Geneva Foundation for Medical Education and Research, Geneva, Switzerland; 4) Department of Pediatrics and Radiology, Jordan University Hospital, Jordan.

We studied a consanguineous Jordanian family with two affected children exhibiting Brachydactyly Type A4 (OMIM 605282), and one unaffected child. A novel high-throughput strategy comprised of SNP-chip based homozygosity mapping, array-based genomic pulldown of exonic regions, and massively parallel resequencing on the Illumina GAII platform, was used to efficiently screen the coding regions of all 177 known genes in the 16.8 Mb candidate locus and identify a candidate causal mutation as a delG frameshift nonsense mutation at base 92 of exon 1 of CHSY1, which results in a severely truncated protein. CHSY1 is an enzyme involved in the synthesis of chondroitin sulfate, which is involved in extracellular matrix deposition and morphogenesis. This gene has not been previously been implicated in Brachydactyly or other Mendelian disorders, but gene co-expression analysis shows it is involved in NOTCH signaling, which has been linked to Brachydactyly via mouse knockout models of the Jagged2 and y-Secretase genes. We perform studies of protein expression in primary patient fibroblast cells to demonstrate greatly reduced CHSY1 levels and altered NOTCH signaling, thereby supporting the sequence-based evidence that this is the causal mutation in this family.

## 537/W/Poster Board #195

**Absence of FGF23 or DMP1 Mutations in 30 Patients Diagnosed Clinically/Biochemically With Sporadic X-Linked Hypophosphatemia.** *S. Mumm[1,2], D. Wenkert[2], M. Huskey[1], V.A. Wollberg[2], M.P. Whyte[1,2].* 1) Div Bone and Mineral Diseases, Washington University School of Medicine, St Louis, MO; 2) Ctr Metabolic Bone Disease, Shriners Hosp Children, St Louis, MO.

Heritable forms of hypophosphatemic rickets (HR) include: X-linked dominant HR (XLH, caused by de-activating mutations in the PHEX gene), autosomal recessive HR (ARHR, caused by de-activating mutations in the DMP1 gene), and autosomal dominant HR (ADHR, caused by activating mutations in the FGF23 gene). Over the past 26 years, we have cared for 259 pediatric HR patients from throughout the United States. After excluding 12 patients with HR from McCune-Albright syndrome, epidermal nevus syndrome, tumor-induced rickets, or cystinosis, etc., there were 73 sporadic cases diagnosed clinically/biochemically with XLH. Here, we investigated whether 30 of these 73 sporadic patients (those for whom DNA was available) have mutations in either the DMP1 or FGF23 genes. We noted that routine biochemical studies and pedigree review of our dominant HR cases have never shown male-to-male transmission indicating autosomal dominant inheritance. Genomic DNA was isolated from blood leukocytes. All five coding exons and adjacent mRNA splice sites of the DMP1 gene, and exon 3 (containing all known ADHR mutations) of the FGF23 gene, were amplified by PCR and sequenced in both directions. The patients' DNA sequences were analyzed by alignment to a control sequence using Vector NTI AlignX software, and by visually examining each DNA sequence electropherogram. No mutations in either the DMP1 or FGF23 genes that might cause either ARHR or ADHR, respectively, were identified among these 30 sporadic HR cases. Mutation studies of exons and mRNA splice sites of the larger and more complex PHEX gene should reveal specific defects that cause HR in most of this population. Accordingly, our cumulative experience with 259 hypophosphatemic rickets patients indicates that both ARHR and ADHR are exceedingly rare in the United States. Exclusion of defects in the DMP1 and FGF23 genes in our sporadic HR cases helps guide the care of these patients.

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## 538/W/Poster Board #196

**MOdb, a new mutation database for the Multiple Osteochondromas causing genes EXT1 and EXT2.** *W. Wuyts[1], I. Jennes[1], E. Pedrini[2], M. Zuntini[2], M. Mordenti[2], S. Balkassmi[3], C. Asteggiano[4], B. Casey[5], E. Bakker[3], L. Sangiorgi[2].* 1) Dept Medical Genetics, Univ Antwerp, Antwerp, Belgium; 2) Department of Medical Genetics, Rizzoli Orthopaedic Institute, Bologna, Italy; 3) Center for Human and Clinical Genetics, Leiden University Medical Centre, The Netherlands; 4) Center for the Study of Congenital Metabolophaties (CEMECO), Children's Hospital of Santísima Trinidad, National University of Cordoba, Argentina; 5) Molecular Genetics Laboratory, British Columbia Children's Hospital, Vancouver, BC, Canada.

Multiple osteochondromas (MO) is an autosomal dominant disease characterized by the formation of multiple cartilage-capped bone tumors growing outward from the metaphyses of the long bones. MO is genetically heterogeneous and is associated with mutations in Exostosin-1 (EXT1) or Exostosin-2 (EXT2), both tumor-suppressor genes of the EXT gene family. All members of this multigene family encode glycosyltransferases involved in the adhesion and/or polymerization of heparin sulphate (HS) chains at HS proteoglycans (HSPG's) with EXT1 and EXT2 implicated in the regulation of chondrocyte proliferation and differentiation. EXT1 is located at 8q24.11-q24.13 and comprises 11 exons, while EXT2 is located at 11p12-p11 and contains 16 exons. To date, an EXT1 or EXT2 mutation is detected in 70-95% of affected individuals. We have created the Multiple Osteochondromas Mutation Database (MOdb) based on the Leiden Open source Variation Database (LOVD) software system to provide an overview of known mutations and polymorphisms in both EXT genes. The variants currently described in the database were identified in 5 laboratories from MO reference centers in Europe, Canada and Argentina. Supplementary, data from published reports were included. If available, additional information, such as de novo origin, mode of inheritance, ethnic origin and functional consequences of mutations investigated, was included in the database. Furthermore, general information on the EXT1 and EXT2 genes is provided, accessible through links to other resources (Genbank, Entrez, OMIM). Currently, the MOdb lists over 900 variant entries of which more than 550 are unique. It provides a global overview of mutation distribution, frequency and nature of the identified mutations for both EXT genes. This database is a central resource of MO sequence variant data for investigators that will facilitate the interpretation of new mutations, variants and polymorphisms in patients. It is available online (http://medgen.ua.ac.be/LOVD/home.php) and open to future submissions.

## 539/W/Poster Board #197

**PCNT mutations discriminate MOPDII from SECKEL Syndrome.** *L. Chessa[1], M. Della Monica[2], M. Piane[1], G. Piatelli[3], P. Lulli[1], F. Lonardo[2], F. Ferrari[1], G. Scarano[2].* 1) II Faculty of Medicine, Sapienza University, Roma, Italy; 2) Medical Genetics Department, Gaetano Rummo General Hospital, Benevento, Italy; 3) Neurosurgery Unit, Istituto Giannina Gaslini, Genova, Italy.

A 3 years old boy with proportionate dwarfism and severe microcephaly, high forehead with high hairline, sparse scalp hair, beaked nose, mild retrognathia and hypotonia was diagnosed at birth as Seckel sindrome on the basis of reduced weight, length and cranial circumference, not taking into account the delivery at 33 weeks. At 3 years of age the patient showed all the measurements under the 3rd centile, dysmorphic face with beaked nose and small teeth, hypotonia and mild psychomotor delay, delayed bone age with metaphyseal widening, no appearance of the ossification nuclei in the femoral head, and irregular distal femoral epiphyses. Moreover, he suffered a paresis due to a cerebrovascular malformation. Western blot analysis of the patient's lymphoblastoid cell line lysate showed the absence of the protein pericentrin. Subsequent molecular analysis identified a novel homozygous single base insertion (c.1527_1528insA) in exon 10 of the PCNT gene, which leads to a frameshift (Treo510fs) and to premature protein truncation. Based on the clinical and radiological features as well on the molecular results a diagnosis of MOPD II syndrome was finally established. This case, together with the literature reports, confirms that PCNT mutations are diagnostic of MOPDII syndrome.

## 540/W/Poster Board #198

**A missense mutation in the aggrecan C-type lectin domain disrupts extracellular matrix interactions and causes dominant familial osteochondritis dissecans with short stature and early osteoarthritis.** *B.-A. Jonsson[1], E.-L. Stattin[1], F. Wiklund[2], K. Lindblom[3], P. Önnerfjord[3], Y. Tegner[4], T. Sasaki[5], A. Struglics[6], S. Lohmander[6], N. Dahl[7], D. Heinegård[3], A. Aspberg[8].* 1) Department of Medical Biosciences, Medical and Clinical Genetics, Umeå University Hospital, Umeå, Sweden; 2) Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden; 3) Department of Experimental Medical Sciences, Lund University, Lund, Sweden; 4) Department of Health Sciences, Technical University Luleå, Sweden; 5) Department of Experimental Medicin I, University of Erlangen-Nuernberg, Erlangen, Germany; 6) Department of Clinical Sciences, Lund University, Lund, Sweden; 7) Department of Genetics and Pathology, The Rudbeck Laboratory, Uppsala University, Uppsala, Sweden; 8) Department of Biology, University of Copenhagen, Copenhagen, Denmark.

Osteochondritis dissecans is a disorder where fragments of articular cartilage and subchondral bone dislodge from the joint surface. We analyzed a five generation family segregating autosomal dominant familial osteochondritis dissecans. Affected family members presented with OCD in knees, hips and elbows, short stature, and early osteoarthritis. A genome wide scan and a multipoint linkage analysis identified aggrecan (ACAN) as a prime candidate gene for the disorder. DNA sequence analysis of the ACAN-gene revealed heterozygosity for a missense mutation (c.6907G>A) in affected individuals, resulting in a p.V2303M amino acid substitution in the aggrecan G3 domain C-type lectin. This domain is important for the interaction with other proteins in the cartilage extracellular matrix. To determine the effect of the V2303M substitution on secretion and interaction, we performed binding studies with recombinant mutated and wild type G3 proteins. We found decreased affinity or complete loss of interaction between V2303M aggrecan and fibulin1, fibulin2 and tenacinR. Analysis of articular cartilage from an affected family member confirmed that V2303M aggrecan is produced and present. The results verified that V2303M results provide a molecular mechanism for the etiology of familial osteochondritis dissecans and confirm the importance of the aggrecan C-type lectin interaction for cartilage function in vivo.

## 541/W/Poster Board #199

**Screening the synemin gene for disease causative mutations in patients with myopathic and neurological diseases.** *Y. MIZUNO[1], J.R. GUYON[2], K. OKAMOTO[1], L.M. KUNKEL[2].* 1) Neurology, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan; 2) Howard Hughes Medical Institute/Division of Genetics, Children's Hospital Boston and Harvard Medical School, 300 Longwood Avenue, Boston, Massachusetts 02115, USA.

Purpose: Synemin is a novel intermediate filament protein that was originally identified as an $\alpha$-dystrobrevin-binding protein through a yeast two-hybrid screen. There are two isoforms and the smaller, $\beta$-synemin, is predominantly expressed in skeletal muscle whereas $\alpha$-synemin is expressed in both muscle and brain. The $\beta$-synemin gene consists of 5 exons and encodes a 1,253 amino acid protein. $\alpha$-Synemin is encoded by the same 5 exons, although exon 4 is not spliced away, resulting in C-terminus extended by an additional 312 amino acids. Based on its association with $\alpha$-dystrobrevin, synemin is thought to be a structural protein in muscle. Investigations are underway to understand the structural and genetic characteristics of synemin and its potential role in disease. Methods: The intracellular expression patterns of synemin were assayed using confocal microscopy in skeletal muscle and in situ hybridization and immunohistochemical analyses in brain. Its potential role in human disease was investigated by sequencing the genomic $\beta$-synemin sequence in 156 normal controls and 71 myopathic patients with unknown etiology. Summary of results: (1) Synemin expression was localized in skeletal muscle cells to the costamere and muscle Z-lines. (2) In the brain, synemin expression was localized in neurons between the midbrain and pons. (3) Sequencing results identified a potential heterozygous nonsense mutation (C598T: CAG to TAG) in exon 1 in three individuals including a myopathic patient, the patient's unaffected father, and one unaffected control. In other individuals, 12 amino acid altering single-nucleotide polymorphisms were also identified. (4) Histological analyses confirmed that synemin-expressing neurons also express tryptophan hydroxylase-1, a marker of serotoninergic nerve fibers. Conclusions: (1) Synemin is hypothesized to function in the stabilization of muscle by linking the myofibrillar Z-lines with the dystrophin-associated protein complex at the costameres. Its function in brain is still unknown. (2) The C598T mutation was found to be present in 0.44% (2/454) of the analyzed population suggesting that only a very small percentage of population (estimated 0.00002%) would be homozygous carriers. (3) Future studies of synemin-expressing neurons will be required to elucidate synemin's function in brain and continued sequence analyses will help ascertain whether mutations in synemin could contribute to muscle or psychiatric diseases.

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### 542/W/Poster Board #200

**Decrease of Sarcolemmal nNOS as a Molecular Marker in Disorders Associated with Decreased Mobility and Muscle Atrophy.** *J.L. Simmers[1], E.F. Hedderick[3], E. Andres-Mateos[1], E.M. MacDonald[1], T.N. Burks[1], R. Marx[1], R.D. Cohn[1,2,3].* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD; 3) Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD.

Muscle fatigue and atrophy are common clinical findings shared by a variety of hereditary and acquired conditions. Maintaining mobility and muscle integrity is crucial for the management and quality of life of these patients. Previous research connected neuronal nitric oxide synthase (nNOS) to essential roles in regulating muscle blood flow and muscle mass. In healthy muscle, nNOS is associated with the dystrophin-glycoprotein complex (DGC) at the sarcolemma. In dystrophin-deficient muscular dystrophies, such as Duchenne muscular dystrophy, a disruption of the DGC is accompanied by loss of sarcolemmal nNOS which in turn causes a decrease in muscle blood flow and the development of significant fatigue. This phenomenon is attenuated by administration of the PDE5 inhibitor, Sildenafil. To assess the expression pattern of nNOS in a variety of genetic disorders, human muscle biopsies were screened by immunofluorescence. We find that patients presenting with congenital hypotonia, including structural myopathies, mitochondrial disorders, and metabolic myopathies or with uncharacterized hypotonia syndromes exhibit loss or decrease of sarcolemmal nNOS. Moreover we find that patients with acquired forms of myopathy such as immobilization atrophy, denervation, and steroid-induced myopathy show decreased or loss of nNOS expression from the sacrolemma. Interestingly, all of these patients experience significant muscle fatigue associated with varying degrees of immobility. Similarly, in mouse models for steroid induced atrophy, we find a decrease in sarcolemmal nNOS accompanied by activation of well-characterized muscle atrophy pathways. Our results demonstrate that the decrease of sarcolemmal nNOS represents a molecular marker of muscle atrophy, often related to impaired mobility. These findings are of substantial clinical significance as the loss of sarcolemmal nNOS increases proteolysis and potentially causes a decrease in muscle blood flow in response to exertion in a variety of patients with inherited genetic disorders or acquired forms of muscle atrophy. Therefore, the physiological consequences of mislocalized nNOS may exacerbate any underlying muscle pathology. Given previous observations from muscular dystrophy mouse models, administration of Sildenafil may provide significant therapeutic benefit to a many of these patients.

### 543/W/Poster Board #201

**Evaluation of the JNK Signalling Cascade in Muscular Dystrophies.** *C. van Erp[1], R. Marx-Rattner[1], H. Dietz[1,2], R. Cohn[1].* 1) School of Medicine, Johns Hopkins Med Inst, Baltimore, MD; 2) Howard Hughes Medical Institute.

Muscular dystrophies are a heterogeneous group of disorders which are most often associated with significant skeletal muscle wasting and weakness. It has previously been demonstrated that levels of the cytokine TGFβ are significantly increased in skeletal muscle of both patients with Duchenne muscular dystrophy (DMD) and the dystrophin-negative mdx mouse. We have recently shown that antagonism of TGFβ via administration of TGFβ neutralizing antibody (TGFβNab) or the angiotensin II type 1 receptor blocker losartan attenuates the disease progression in mdx mice by improving steady-state muscle architecture, muscle regeneration and in vivo physiological function. TGFβ is known to activate the c-Jun N-terminal kinase pathways (JNK's), via crosstalk with the MAPK pathways. We therefore evaluated the expression pattern of JNK signalling in various animal models of muscular dystrophy. Interestingly, we find that JNK signalling is significantly increased in the skeletal muscle of mdx and laminin alpha2 deficient mice (a model for congenital muscular dystrophy type 1A) compared to wild-type mice, while levels of ERK and P38 remain unchanged. Preliminary results indicate that treatment of these mouse models with TGFβNab and/or losartan causes decrease of JNK signalling to a level comparable to wild-type mice supporting the notion of cross-talk between these pathways. Given the critical role that the JNK signalling pathway plays in the tissue homeostasis of various organs, we investigated whether administration of a JNK inhibitor would be able to alter activity of the JNK signalling cascade in skeletal muscle. Indeed, our data demonstrate that treatment with a JNK inhibitor can significantly decrease the activity of JNK in skeletal muscle of mdx mice to levels equal to and below the JNK levels in wild-type mice. We are currently exploring different pharmacological manipulations of JNK signaling in various mouse models of muscular dystrophies to investigate whether these treatments will result in therapeutic benefits for various forms of muscular dystrophies.

### 544/W/Poster Board #202

**Germline and somatic mosaicism for an *FGFR2* mutation in the mother of a child with Crouzon syndrome.** *J. Lim[1], A. Goriely[1], T. Lester[2], H. Lord[2], D. Johnson[3], H.V. Firth[4], A.O.M. Wilkie[1,3].* 1) Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom; 2) Genetics Laboratory, Oxford, United Kingdom; 3) Craniofacial Unit, Oxford Radcliffe Hospitals NHS Trust, United Kingdom; 4) Clinical Genetics, Addenbrookes Hospital, Cambridge, United Kingdom.

Crouzon syndrome is a disorder characterised by craniosysnostosis and facial dysostosis involving over 50 different missense mutations in the Fibroblast Growth Factor Receptor-2 (*FGFR2*) gene. Although Crouzon syndrome is inherited as an autosomal dominant trait, most cases present as *de novo* mutations arising from unaffected parents. It was previously shown that, like other dominant disorders involving mutations in *FGFR2* or *FGFR3* (including Apert, Pfeiffer and Muenke syndromes and achondroplasia), Crouzon syndrome mutations show an exclusive paternal origin and a paternal age effect, meaning that the unaffected fathers tend to be older than the population average. Although the occurrence of germline mosaicism was previously suggested in Crouzon syndrome, these reports predated the identification of causative *FGFR2* mutations and none has been molecularly confirmed. The female proband presented at the age of 12 years with visual disturbance and was diagnosed clinically as having Crouzon syndrome. This was confirmed by identification of a heterozygous *FGFR2* c.1007A>G (p.D336G) mutation that locates in the IgIII domain mutation hotspot. There was no significant family history and both parents were clinically normal, but routine mutation analysis of DNA obtained from the peripheral blood of the mother (aged 28 years at the time of her daughter's birth) identified a low level of the *FGFR2* c.1007A>G mutation by direct sequencing. This finding was further confirmed by demonstrating mosaicism of the mutation in 3 different tissues (an independent blood sample, saliva and hair root) on direct sequencing. The mutation level was estimated in blood as 18% by *HhaI* restriction digest of a fluorescently labelled PCR product. The results of quantification using Pyrosequencing will be presented. To our knowledge, this is the first case of proven germinal and somatic mosaicism in the parent of a child with an apparently *de novo FGFR2* mutation. Whilst this is an unusual situation, it underlies the importance of always checking for parental mosaicism in apparent 'paternal age effect' *FGFR2* and *FGFR3* mutations, as its presence substantially increases the recurrence risk which is otherwise counselled as being less than 1%.

### 545/W/Poster Board #203

**Development and functional analysis of wildtype and R266Q GNE expression plasmids.** *Y. Valles-Ayoub[1,2], C. Saechao[1,4], A. Haghighatgoo[1,2], Z. Khokher[1,3], D. No[1,3], S.L. Martinez[1], I. Shah[1,4], S. Esfandiarifard[1], A.C. Riley-Portuges[1], C. Jay[5], M. Pietruszka[1], D. Darvish[1,2].* 1) HIBM Research Group (HRG), Inc., Encino, CA; 2) VA Greater Los Angeles (VA-GLA/UCLA), Los Angeles, CA; 3) Los Angeles Mission College, Sylmar, CA; 4) California State University, Northridge (CSUN), Northridge, CA; 5) Gradalis, Inc., Dallas, TX.

Hereditary Inclusion Body Myopathy (HIBM) is an adult onset progressive muscle wasting disorder affecting proximal and distal muscle, ultimately leading to severe disability. The etiology of HIBM is associated with mutations in the UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE) gene, which encodes a bifunctional epimerase/kinase enzyme involved in the first two steps of sialic acid biosynthesis. Disruption of the sialic acid pathway leads to decreased sialylation of glycoproteins. Hyposialylation of alpha-dystroglycan, a muscle glycoprotein, jeopardizes the structural integrity of muscle and leads to myopathy. The functionality of a mutated UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE/MNK) enzyme, and thus sialic acid production, can be recovered by the introduction and expression of a wildtype GNE gene. In order to demonstrate this, we used the lectin-resistant Chinese hamster ovary (CHO) cell line, which lacks UDP-N-acetylglucosamine 2-epimerase activity. The CHO cells were transfected with a pUMVC3-GNE recombinant vector expressing either a wildtype GNE or an R266Q mutant insert. The R266Q mutation occurs in the allosteric domain of GNE/MNK and is associated with sialuria, a metabolic disorder in which feedback inhibition of the GNE gene is defective and sialic acid is overproduced. CHO cells transfected with wildtype or R266Q GNE expression vectors displayed approximately an 8.5-fold and 11.5-fold increase in sialic acid, respectively, in comparison to CHO cells transfected with the pUMVC3 bone vector. We intend to utilize this data to initiate a gene therapy model in mice and investigate its safety and effectiveness in the alleviation of muscle atrophy and dysfunction manifested by HIBM.

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## 546/W/Poster Board #204

**Mutation analysis of limb enhancers in individuals with isolated limb malformations.** *J.E. VanderMeer[1,2], L.S.L. Clarke[3], G. Bejerano[4,5], N. Ahituv[1].* 1) Department of Bioengineering and Therapeutic Sciences and Institute for Human Genetics. University of California, San Francisco, San Francisco, CA; 2) Biomedical Sciences Graduate Program, UCSF; 3) Department of Genetics, Stanford University, Stanford CA; 4) Department of Developmental Biology, Stanford University; 5) Department of Computer Science, Stanford University.

The discovery of mutations leading to human disease in coding sequences has been extremely successful. However, the identification of mutations that lead to disease in the remaining 98% of the genome is challenging. Regulatory elements, such as enhancers, make prime candidates for harboring these disease-causing mutations due to their vital role in regulating gene promoters. The majority of characterized human enhancers show tissue-specific expression, suggesting that mutations within them will lead to an isolated disease phenotype. Previous studies have identified over 90 limb-specific enhancers where mutations could lead to isolated limb malformations. In addition, two recent examples in the literature illustrate cases where mutations or copy number variation of a limb enhancer leads to isolated limb malformations in humans. In order to identify additional mutations, we are currently collecting DNA from patients with isolated limb malformations and have enrolled over 100 patients as well as their immediate family members. Where large pedigrees are available, we are using linkage analysis to further pinpoint the genomic position where a potential deleterious mutation could reside. However, most isolated limb malformations occur as sporadic cases without useful pedigree information. For these patients, we use a candidate approach by sequencing limb enhancers in the vicinity of genes involved in specific limb patterning processes whose disruption could lead to the morphological characteristics observed in these patients. Sequence variants in these enhancers are screened for their prevalence in cases compared to controls, potential transcription factor binding changes, and the degree of evolutionary conservation at that site to determine which are more likely to be causative. Selected variants will be further characterized for differential enhancer activity compared to the reference allele using a transgenic mouse enhancer assay. Combined, these assays will result in an increased knowledge about the pathogenesis of human limb malformations and limb development and may lead to improved patient counseling and the development of molecular testing including prenatal genetic screens. In addition, this work will provide a model for studying the role of enhancers in other isolated disease phenotypes.

## 547/W/Poster Board #205

**MMP20 Hemopexin Domain Mutation in Amelogenesis Imperfecta.** *S.-K. Lee[1], F. Seymen[2], H.-Y. Kang[1], K.-E Lee[1], K. Gencay[2], B. Tuna[2], J.-W. Kim[1,3].* 1) Department of Cell and Developmental Biology, Dental Research Institute and BK21 Program, Seoul National University, Seoul, Korea; 2) Department of Pedodontics, Istanbul University, Istanbul, Turkey; 3) Department of Pediatric Dentistry, Dental Research Institute and BK21 Program, Seoul National University, Seoul, Korea.

Proteolytic enzymes serve important functions during dental enamel formation, and mutations in the kallikrein 4 (KLK4) and enamelysin (MMP20) genes cause autosomal recessive amelogenesis imperfecta (ARAI). So far only one KLK4 and three MMP20 mutations have been reported in ARAI kindreds. To determine whether ARAI in a family with a hypomaturation type enamel defect is caused by mutations in the genes encoding enamel proteolytic enzymes, we performed mutational analysis on candidate genes. Mutational and haplotype analyses revealed an ARAI-causing point mutation (c.910G>A, p.A304T) in exon 6 of MMP20 that results in a single amino acid substitution in the hemopexin domain. Western blot analysis showed decreased expression of the mutant protein, but zymogram analysis demonstrated this mutant was a functional protein. The proband and an affected brother are homozygous for the mutation, and both unaffected parents are carriers. The enamel of newly erupted teeth has normal thickness, but is chalky white and becomes darker with age. The study protocol and patient consents were independently reviewed and approved by the Institution Review Board at the Seoul National University Dental Hospital. Informed consent was obtained according to the Declaration of Helsinki. This work was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A060010), a grant from the Korea Science and Engineering Foundation (KOSEF) through the Biotechnology R&D Program (No. M10646010003-08N4601-00310), a grant from the KOSEF through the Bone Metabolism Research Center (No. R11-2008-023-02003-0), and an NIDCR/NIH grant DE12769.

## 548/W/Poster Board #206

**Molecular mechanisms in the TRPV4 disorders.** *S. Tompson[1], N. Camacho[2], P. Krejci[1], B. Boyce[3], P. Katzman[3], A. Janssens[4], D. Rimoin[1], R. Lachman[1], B. Nilius[4], D. Krakow[2], D. Cohn[1].* 1) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Dept. of Orthopaedic Surgery, University of California at Los Angeles, Los Angeles, CA; 3) Dept. of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY; 4) Dept. of Molecular Cell Biology, Katholiek Universiteit, Leuven, Belgium.

Missense mutations in *TRPV4*, which encodes a $Ca^{2+}$-permeable ion channel, have been shown to cause a spectrum of dominantly inherited skeletal phenotypes ranging in increasing severity from autosomal-dominant brachyolmia, to spondylometaphyseal dysplasia Kozlowski type (SMDK) to nonlethal metatropic dysplasia (MD). Previous reports have suggested both autosomal recessive and dominant forms of MD. However, molecular analysis has demonstrated that the full spectrum of MD can result from heterozygosity for *TRPV4* mutations in 8/8 additional nonlethal MD and 3/3 lethal MD cases. Analysis of intracellular $Ca^{2+}$ levels in HEK cells expressing normal and mutant *TRPV4* demonstrated that the mutations activate the channel, increasing both the baseline and agonist-stimulated level of $Ca^{2+}$. This suggests the mutations exert their phenotypic effect by increasing intracellular $Ca^{2+}$. It has been shown in ATDC5 cells that overexpressing *Trpv4* or agonist activating endogenous protein causes upregulation of *Sox9* and its downstream targets. To determine whether mutant *TRPV4* produces a similar effect, dual luciferase reporter assays were performed with rat chondrosarcoma chondrocytes. *SOX9* reporter levels were increased when mutant *TRPV4*, but not wildtype, constructs were expressed. FACS analysis revealed that expression of mutant *TRPV4* reduced cell proliferation, without increasing apoptosis. Thus, activating mutations in *TRPV4* lead to increased *SOX9* transcription and decreased cell proliferation. Histology of long bone growth plates from 2 lethal MD cases revealed exuberant cartilage and extracellular matrix in the reserve zone with increased vascularity. Hypertrophic zones showed a reduced number of poorly aligned hypertrophic chondrocytes. Taken together, the data fit a model in which mutant TRPV4 in the reserve zone results in increased SOX9 and, consistent with published data, a consequent increase in extracellular matrix synthesis. Greater vascularity likely results from SOX9-dependent synthesis of VEGF, a key angiogenic regulator. As SOX9 is known to inhibit the differentiation of proliferating chondroctyes into hypertrophic cells, the decreased number and disorganization of hypertrophic chondrocytes seen in lethal MD would be predicted to result from increased *SOX9* expression in the growth plate.

## 549/W/Poster Board #207

**Loss of MMP13 or MMP9 function, or autocatalytic and transcatalytic inactivation result in impaired endochondral ossification and present as different forms of metaphyseal anadysplasia.** *B. Zabel[1,2], R. Keppler[1], K. Hilbert[1], V. Cormier-Daire[3], S. Nikkel[4], G. Nishimura[5], S. Unger[2], J. Spranger[1], A. Superti-Furga[1], E. Lausch[1].* 1) Centre for Pediatrics and Adolescent Medicine, Freiburg University Hospital, 79106 Freiburg, Germany;; 2) Institute of Human Genetics, Freiburg University Hospital, 79106 Freiburg, Germany;; 3) Department of Genetics and INSERM U781, Hôpital Necker Enfants Malades, Paris, France; 4) Department of Genetics, Children's Hospital of Eastern Ontario, Ottawa, Canada; 5) Department of Radiology, Tokyo Metropolitan Kiyose Children's Hospital, Kiyose, Tokyo, Japan.

Different families of proteases mediate extracellular matrix (ECM) turnover and cartilage remodelling in the growth plate, in particular the matrix metalloproteinases (MMPs). Many MMPs are presumed to play roles in bone formation and growth as they are able to cleave the most abundant proteoglycans and collagens of skeletal tissues. MMP9 and MMP13 catalyse the degradation of ECM components in the growth plate and at the same time cleave and release biologically active molecules stored in the ECM. Ablation of either Mmp9, Mmp13, or both, in mice causes severe distortion of the metaphyseal growth plate. We report that mutations in either MMP9 or MMP13 are responsible for the human disorder Metaphyseal Anadysplasia (MAD), for which a milder recessive variant and a more severe dominant variant are known. We found that recessive MAD is caused by homozygous loss of function of either MMP9 or MMP13, while dominant MAD is associated with missense mutations in the prodomain of MMP13 that determine autoactivation of MMP13 and intracellular degradation of both MMP13 and MMP9, resulting in a double enzymatic deficiency. A sequential action of MMP13 and MMP9 might explain the scarcely additive effect of the combined deficiency. Regarding possible downstream target(s) a large body of evidence supports that angiogenesis is a crucial step in bone formation. While ECM remodelling is essential for vascular invasion to occur, terminal chondrocyte differentiation and apoptosis depend on angiogenic factors. We suggest the existence of an activation cascade from MMP13 over MMP9 to VEGFA, and that this process is disturbed in MAD leading to impaired ossification.

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## 550/W/Poster Board #208

**Mitochondrial mutation screening in Greek non-syndromic sensorineural childhood onset hearing impaired patients.** *H. Kokotas[1], M. Grigoriadou[1], G.S. Korres[1], E. Ferekidou[1], J. Economides[2], D. Kandiloros[3], A. Giannoulia-Karantana[4], S. Korres[3], M.B. Petersen[1].* 1) Department of Genetics, Institute of Child Health, 'Aghia Sophia' Children's Hospital, Athens, Greece; 2) Department of Audiology-Neurootology, 'Aghia Sophia' Children's Hospital, Athens, Greece; 3) Department of Otorhinolaryngology - Head and Neck Surgery, Athens University Medical School, Hippokration Hospital, Athens, Greece; 4) Department of Pediatrics, Athens University Medical School, 'Aghia Sophia' Children's Hospital, Athens, Greece.

Hearing impairment is one of the most common sensory handicaps with a frequency of at least 1/1,000, whereas at the age of 80 years more than 50% of the elderly have developed hearing loss severe enough to impair communication. Mitochondrial mutations are present in less than 1% of the children with prelingual deafness, but are more frequent at a later age. In the Caucasian population at least 5% of postlingual, non-syndromic hearing impairment is due to known mtDNA mutations, representing the most frequent cause of hearing loss after the 35delG mutation in the GJB2 gene encoding connexin 26. The use of aminoglycoside antibiotics can cause hearing loss in genetically susceptible individuals. Several mtDNA mutations leading to non-syndromic hearing impairment have been reported. Hot spot mutation regions are the MTRNR1 gene, encoding the 12S rRNA, and the MTTS1 gene, encoding the tRNA for Ser(UCN) . Some of these mutations could turn out to be polymorphisms without clinical significance, and most of them (including the frequent A1555G mutation) each have a low penetrance when they occur without other mutations. However, on the background of additional 12S rRNA mutations or nuclear mutations in genes involved in tRNA modification (TRMU-MTO2 and GTPBP3 genes) or rRNA modification (TFB1M gene), the penetrance is higher. To investigate the prevalence of the MTRNR1 A1555G mutation and the MTTS1 A7445G, 7472insC and T7510C mutations in the Greek population, we tested 600 unrelated cases of non-syndromic neurosensory childhood onset deafness. DNA extracted from whole blood was analyzed for the mtDNA mutations using PCR-RFLP protocols. The homoplasmic A1555G mutation was detected in two familial cases, one of which was found negative and the other heterozygous for the common GJB2 35delG mutation. Four cases had the A7445G mtDNA mutation; two of which were heterozygous for the 35delG mutation, one harbored the 35delG mutation in compound hetorozygosity with the GJB2 W24X mutation, and one case was homozygous for the 35delG mutation. None of the cases presented with the 7472insC or T7510C mutations. We conclude that the A7445G mutation might be a rather frequent cause of non-syndromic neurosensory deafness in the Greek population and should be screened in cases with non-syndromic childhood deafness (together with the A1555G mutation). It is noteworthy, that in 5 of the 6 cases with a mitochondrial mutation, at least one mutation of the GJB2 gene was also present.

## 551/W/Poster Board #209

**Novel mutations and loci are associated with limb girdle muscular dystrophy type 2.** *S.E. Boyden[1,2], M.A. Salih[4], E.A. Estrella[1], A.J. White[1], M.Z. Seidahmed[5], R.R. Bennett[1], L.M. Kunkel[1,2], P.B. Kang[1,3].* 1) Program in Genomics, Children's Hospital Boston, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA; 3) Department of Neurology, Children's Hospital Boston and Harvard Medical School, Boston, MA; 4) Department of Pediatrics, College of Medicine & KKUH, King Saud University, Riyadh, Kingdom of Saudi Arabia; 5) Department of Pediatrics, Security Forces Hospital, Riyadh, Kingdom of Saudi Arabia.

Limb girdle muscular dystrophy (LGMD) is a progressive muscle degeneration disorder resulting in profound weakness that affects primarily the shoulder and hip muscles. There are 14 genes known in which a variety of mutations cause autosomal recessive LGMD type 2 (LGMD2), but 30% of LGMD patients lack a molecular diagnosis. We hypothesized there may be additional undiscovered LGMD2 mutations and/or genes, and we ascertained eight consanguineous pedigrees in which patients had recessive LGMD. DNA samples for all affected family members and at least one unaffected sibling were genotyped at 10,204 SNP markers and linkage analysis was performed in MERLIN. Three families showed linkage to overlapping intervals on chromosome 19q13 containing the LGMD2 gene fukutin-related protein (FKRP). Sequence analysis of FKRP identified a novel missense mutation, T314M, in two of these families that shared a haplotype across a portion of the linkage peak containing FKRP, indicating they likely inherited a single founder mutation from a common ancestor. In the third family we discovered a second novel FKRP missense mutation, V338L. Two additional families showed linkage to chromosome 17q21, a region containing the LGMD2 gene alpha sarcoglycan/adhalin (SGCA). One family carried the previously-reported R34H mutation in SGCA, while in the other we found a novel 5' splice site mutation IVS5 +5G>A. The G base is highly preferred (75-85%) at the +5 position in the consensus 5' splice site sequence, and skipping of exon 5 would result in deletion of 66 amino acids and a frameshift, while inclusion of intron 5 would result in a premature stop codon. Efforts to confirm the presence of a mis-spliced mRNA in this family are ongoing. A sixth family produced a suggestive maximum LOD score of 2.5 at chromosome 5q33-34, a peak that includes the delta sarcoglycan (SGCD) gene, and mutation screening is underway. The final two families yielded several suggestive linkage peaks at loci throughout the genome, none of them corresponding to the location of a known LGMD gene. These data suggest the existence of at least 1-2 unreported LGMD2 loci and we are pursuing mutation identification in these regions. We have shown linkage mapping in consanguineous pedigrees to be a productive method for discovering novel mutations and novel loci for a genetically and mutationally heterogeneous disorder even when an abundance of genes and mutations have been previously identified.

## 552/W/Poster Board #210

**Exon-level analysis of Mendelian disorder genes by array CGH finds novel deletions and duplications.** *S. Aradhya, R. Busin, A. Stafford, K.S. Hruska, B. Boggs, G. Richard, S. Bale, J.G. Compton.* GeneDx, Gaithersburg, MD.

Intragenic deletions and duplications typically account for a small proportion of disease-related mutations in Mendelian disorders. Although the frequency of these mutations may be higher, the lack of a robust technology has prevented their discovery on a larger scale. Array CGH with high-resolution coverage within individual genes provides an opportunity to detect small deletions and duplications that may have been missed due to technical limitations of previous technologies or because the relevant genes have not been screened for these types of mutations. To identify such mutations, we have constructed an oligonucleotide microarray with probes concentrated in the exonic regions of approximately 450 genes associated with disorders caused by haploinsufficiency or with recessive disorders (in which the identification of only a single mutation may suggest the presence of a deletion on the other allele). When we used this microarray to analyze 700 clinical cases, many of which were negative by sequence analysis, we identified 28 novel intragenic or whole-gene deletions and one novel intragenic duplication. Intragenic deletions were identified in FLCN, STK11, PTCH1, CREBBP, VHL, MMACHC, VPS13B, MOCS2, USH2A, GPC3, and KCNQ1. We identified whole-gene deletions in TWIST1, PTEN, NDP, STK11, CREBBP, FOXC1, and PTCH1. An intragenic duplication was found in FLNA. These results demonstrate the utility of using exon-level array CGH for detecting clinically significant changes that may account for a larger proportion of mutations in Mendelian disorders than previously thought.

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## 553/W/Poster Board #211

**High Throughput Genotyping Utilizing a Versatile Extraction Platform.** *T. Wayman[1], S. Mitchell[1,2], Y. Louie[1], C. Meadows[1], E. Lyon[1,3].* 1) ARUP Laboratories, Salt Lake City, UT; 2) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; 3) Department of Pathology, University of Utah, Salt Lake City, UT.

Increased demand for molecular diagnostics testing has created the need to invest in higher throughput automation platforms. Due to increasing sample volumes, moving to a 96 sample extraction for high volume assays has been a priority of ARUP Laboratories. Without further automation, additional technologists will be required to extract, run, and analyze the increasing sample load. A 96 sample DNA extraction would ideally be able to increase efficiency and lower costs with existing personnel by allowing a larger batch size of multiple assays. In addition to increasing throughput we would expect any new extraction instrument to improve all possible aspects of the assay and be able to be used on multiple downstream analysis platforms. In investigating the SX-96GC (Precision System Science - Chiba, Japan) we have found a new platform and extraction reagent (MagDEA-HTS) to improve the efficiency of multiple endpoint detections through: increased quality of DNA, lower repeat rate, lower cost per test, and increased quality of result. DNA extracted on the SX-96GC was observed at an average yield of 47 ng/uL with a CV of 31%. This is an improvement from previous extraction platforms utilizing the MagNA Pure LC DNA Isolation Kit I (Roche Applied Science - Indianapolis, IN) like the Radius (Protedyne - Windsor, CT) (ave. yield 30 ng/uL, CV 50%) and the MagnaPure (Roche Applied Science - Indianapolis, IN) (ave. Yield 28 ng/uL, CV 35%). A260/280 ratios improved from 1.8 on the Radius and 1.9 on the MagnaPure to 2.1 on the SX-96GC. Assays used for endpoint evaluation were Factor V Leiden and Factor II 20210G>A mutations on the LC480 (Roche Applied Science - Indianapolis, IN) using an ARUP developed assay and the CFV30LA 23 mutation cystic fibrosis panel (Celera - Almeda, CA) on the ABI 3130 (ABI - Foster City, CA). Repeat rates of assays have been observed at 11.2% on the Radius and 10.6% on the MagnaPure with an improvement to 5% on the SX-96GC. The extraction cost using the PSS-SX96GC lowered by 10% compared to the MagnaPure and 8% from the Radius. The lower repeat rate is a product of improved results, which in turn lowers cost. In addition the SX-96GC has the ability to extract samples in horizontal and vertical half plates that allows easier migration to multiple endpoint detection platforms. Additional time and cost savings are realized when using the dual processing instrument to set up PCR reaction plates from the primary extraction plates.

## 554/W/Poster Board #212

**Mutation spectrum of the ABCA4 Gene in Greek patients with Stargardt disease: Identification of a novel mutation and evidence of three prevalent mutated alleles.** *S. Kamakari[1,2], P. Stamatiou[1,2], T. Panagiotoglou[3], C. Tsika[3], N.P. Anagnou[2], M. Tsilimbaris[3].* 1) Biogenomica Center for Genetic Research and Analysis, Athens, Halandri, Greece; 2) School of Medicine, University of Athens, Athens, Greece; 3) Department of Ophthalmology, School of Medicine University of Crete, Iraklion, Greece.

Purpose: Mutations in the ABCA4, photoreceptor-specific ATP-binding cassette transporter 4, gene have been associated with autosomal recessive Stargardt (STGD) disease, characterized by central vision impairment. This is the first systematic study investigating the frequency and pattern of disease-associated mutations among Greek STGD patients, never previously genetically characterized for sequence variations in the ABCA4 gene. Methods: 25 unrelated and 2 related STGD patients were analyzed by the ABCR400 microarray. Polymerase chain reaction (PCR) amplification and direct sequencing of 4 selected or all 50 exons of the ABCA4 gene was performed on 5 patients. Results: Disease-associated mutations, including the novel, likely pathogenic splice site mutation c.4352+1G>A in intron 29, were detected in 20/29 unrelated patients analyzed (69%). Both mutant alleles were found in 12 (41.4%) and only one was detected in 8 (27.6%). The major pathogenic allele IVS40+5G>A accounted for 30.3% (10/33) of the mutant alleles. Other frequently mutant alleles were p.L541P alone or as a complex p.L541P/p.A1038V allele and p.G1961E, each accounting for 24.2% (8/33) of the mutated alleles. Notably, a young patient with unaffected parents and his paternal affected aunt with different disease severity, were found respectively homozygous for the p.L541P/p.A1038V and compound heterozygous for the p.L541P/p.A1038V and p.G1961E alleles thus resolving the inheritance pattern. Direct sequencing of all 50 coding exons of the ABCA4 gene in 2 patients with one detected mutation revealed the second mutation in both patients: the novel, heterozygous c.4352+1G>A mutation in intron 29 in one patient and a known mutation, p.W1449X, in the second. The latter mutation had remained unidentified by the ABCR400 microarray although included on the chip. Finally, towards a cost-effective screening strategy, selective screening of the frequently mutated exons 12/21/40/42 successfully identified mutations in 2/3 unrelated patients. Conclusions: This first report of the ABCA4 mutation spectrum underlying STGD disease in Greece further elucidates the distribution of ABCA4 mutations in European populations. The detection rate by the combined methodology was 69%. We identified a novel potentially pathogenic ABCA4 splice mutation and 3 prevalent disease-causing alleles. Further evaluation of the genotype-phenotype correlations will advance our knowledge of STGD's etiology in Greek patients.

## 555/W/Poster Board #213

**Mutation analysis of the NEMO gene in patients with Incontinentia Pigmenti.** *W. Lissens, M. Bonduelle, S. Seneca, S. Van Dooren, I. Liebaers.* Ctr Med Gen, UZ Brussel, Brussels, Belgium.

Incontinentia pigmenti (IP) is a rare X-linked dominant genodermatosis that is lethal in males. Females can survive because of X-inactivation mosaicism. The most frequent mutation is a deletion of exons 4-10 of the gene, NEMO that causes IP. Molecular analysis was performed in 99 female patients referred for NEMO gene analysis. The patients were first studied for the presence or absence of the exon 4-10 deletion by PCR: 46 patients carried this deletion. Upon request of the referring physician, 10 of the 53 patients without a deletion were further sequenced, and in 7 a mutation (six resulting in nonsense codons and one in a missense codon) was found. From 30 patients with a proven mutation DNA samples from the mothers were available for testing and in 7 of them a mutation was found (7/30 or 23%). In the end, no mutation was found in 46/99 patients. Our results are in agreement with other studies that show that the exon 4-10 NEMO deletion is a major mutation causing IP, and that many mutations occur de novo. However, the deletion frequency in our patients is lower than has been described in literature (46% versus 70-90%). This can probably be attributed to the fact that we performed deletion screening for each request for IP mutation analysis without any selection. Clinical data are being collected to verify this hypothesis.

## 556/W/Poster Board #214

**An Interactive Database of Single Gene Disorders Among Plain People.** *H. Cross[1, 2], A.H. Crosby[2].* 1) Dept Ophthalmology & Vision, Univ Arizona Sch Med, Tucson, AZ; 2) Medical Genetics, Clinical Developmental Sciences, St. George's University London, London SW17 0RE, UK.

For a half century, endogamous isolates of plain people throughout the United States and Canada, including Amish, Mennonite and Hutterites, have been subjects of genetic studies. More than 120 disorders due to single gene mutations have been described in these populations. Some are unique to the Amish, others to Mennonites and Hutterites, while others are found throughout these populations. Published reports are widely scattered and no centralized resource useful to members of these groups and their medical providers has been available. We have compiled this information and published it as a searchable online database (www.wohproject.org). Each disorder contains a section with a general clinical description using plain non-medical language for individuals and families. Another section summarizes the clinical features in more detail to allow rapid consultation by busy medical practitioners in their daily practice. Known metabolic and biochemical pathways are graphically illustrated. Genomic information and inheritance patterns are summarized in a section on genetics, with additional information on treatment, prognosis, diagnostics and relevant specialists. Links to general information, support groups, and disease networks are provided. Literature references with PubMed links are included for each disorder as well as links to OMIM. We anticipate that this database, searchable via any combination of clinical, genetic, reference and population terms, will prove to be a useful resource for families affected by these conditions as well as healthcare workers and researchers involved in their study.

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### 557/W/Poster Board #215

**Genetics of Congenital Stationary Night Blindness - Mutation Update and Clinical Variability.** *N.T. Bech-Hansen[1,2], R. Tobias[1], C. Kennedy[1], A. Jackson[1], N. Lodha[1], CSNB Consortium.* 1) Medical Genetics, University of Calgary, Calgary, AB, Canada; 2) Institute of Child and Maternal Health, Faculty of Medicine, University of Calgary.

Congenital stationary night blindness (CSNB) represents a group of low vision disorders (reduced visual acuity, impaired night vision, myopia, nystagmus, and strabismus) with abnormal retinal neurotransmission. Various analyses have shown mutations in *CACNA1F* in the patients with incomplete X-linked CSNB (CSNB2), in *NYX* in patients with complete X-linked CSNB (CSNB1) and in *GRM6* in patients with complete autosomal recessive CSNB (CSNB1B). In a total of 199 patients diagnosed with CSNB, 112 for X-linked iCSNB, 73 for X-linked cCSNB and 14 with ar-cCSNB, we have now identified a total of 101 unique mutations (57 unpublished) in 142 of these patients. Of the mutations, 65 were in *CACNA1F* (15 in splice sites), 29 in *NYX* (17 single-nucleotide substitutions or small in-frame deletions or insertions) and 7 in *GRM6*. No mutations were identified in *CAPB4*. *CACNA1F* mutations were distributed across the entire coding region, while *NYX* mutations predominated in conserved residues of the leucine-rich repeats. The *CACNA1F* mutations were identified among patients originally diagnosed with incomplete X-linked CSNB (iCSNB or CSNB2), Åland Island eye disease (AIED and AIED-like), and Åland eye disease (AED) suggesting that these conditions are essentially the same genetic condition. No mutations were identified in 53 of 199 CSNB patients (27 percent). We consistently observed the *CACNA1F* founder mutation (c.3166dupC, exon 27) among individuals with Dutch-German Mennonite ancestry. A 24-bp deletion mutation in *NYX*, which results in the loss of the RACPAACA amino acids in the N-terminal Cys-rich region of nyctalopin, was detected in 12 North American CSNB families. Of 164 CSNB mutations known worldwide, we note that 18 of 48 unique *NYX* mutations, 24 of 93 unique *CACNA1F* mutations and 7 of 17 unique *GRM6* mutations have been detected in more than one family. Preliminary analysis indicates that some of the clinical variability observed among CSNB patients can be accounted for by the type of CSNB mutation. The high success rate of detecting mutations in *NYX* and *CACNA1F* in our patient cohort supports the notion that X-linked CSNB is more common than the autosomal recessive form of CSNB, and that the proteins, Cav1.4 ($\alpha$1F) and nyctalopin, encoded by these two genes, together with the GluR6 receptor (encoded by *GRM6*) are evidently important for establishing and maintaining retinal neurotransmission function. Supported by FFB-Canada.

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## 558/W/Poster Board #216

**Muscle Protein Polymorphism of Percids.** *A. Islam[1], M. Khasanova[2], V. Chernov[2].* 1) Dept Ophthalmology, Schepens Eye Res Inst, HMS, Boston, MA; 2) Kazan Institute of Biophysics and Biochemistry, Russian Academy of Sciences, Kazan, Russia 420111.

We compared the muscle protein polymorphism of two pikeperches (*Sander lucioperca* and *S. volgense*) through intra- and intermyomeric composition of white muscles. The protein expressions were investigated by using denaturing 10% sodium dodecylsulfate-polyacrylamide gel electrophoresis. The muscle proteins were studied through within- and between-species morphological development, sexual maturity and age of the species. Myosin, actin and troponin have a distinct role in the contraction and length tension of muscle fibers of these species. There were no obvious intramyomeric differences found in the myosin heavy chain of the species. Myosin light chains (15-38 kDa) have different expression in different age groups of the two *Sander* species. The muscle protein of the fingerlings and adults *S. lucioperca* had high molecular weight (50 kDa) myosin in contrast to the other Percid species. The molecular weight of actins increased comparatively in low-age-group fish. Troponin regulates the increasing concentration of light-chain myosin in both species. The muscle proteins of both species in different sexual maturity have polymorphism in various age groups but there is no difference in protein polymorphism of the same aged group. The white smooth muscle protein composition and contractile properties affect the power production during fast, unsteady movements and swimming of these species.

## 559/W/Poster Board #217

**Predicting Functionality of Missense Mutations with Varying Levels of Evolutionary Depth.** *S. Hicks[1], S.E. Plon[2,3], D.A. Wheeler[2], M. Kimmel[1].* 1) Department of Statistics, Rice University, Houston, Texas; 2) Human Genome Sequencing Center, Baylor College of Medicine, Houston, Texas; 3) Texas Children's Cancer Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas.

Several computational algorithms have been developed to predict the impact of missense mutations on protein structure and function. Informative classification of missense mutations is highly dependent on the parameters used in each algorithm. Past research has shown a high predictive value for methods that use evolutionary sequence conservation, surprisingly with or without protein structural information. Though the methods differ in parameters, they all agree that, in general, protein evolution is correlated with protein function: mutations at conserved residues are more likely to be pathogenic and affect protein function compared to more diverse residues. The goal of the study is to investigate the prediction accuracy of the functionality of missense mutations by using both curated and uncurated alignments of varying evolutionary depth. The three methods, SIFT, Polyphen and Align-GVGD were evaluated using a set of missense substitutions from the *BRCA1* and *BRCA2* mutation database (BIC), with sufficient evidence of clinical neutrality. We compared a) curated alignments with varying levels of evolutionary depth (http://agvgd.iarc.fr/) and b) uncurated alignments automatically generated in Uniprot using the built in ClustalW feature with sequences included based on a criteria of 90% or 50% identity. The results show SIFT dramatically overcalls neutral variants as deleterious as others have previously noted. The online version of Polyphen does not allow a rapid query of alignments of varying levels of depths, but has a comparable sensitivity to Align-GVGD. When considering curated versus uncurated alignments, Align-GVGD only miscalls as deleterious 1 of 17 *BRCA1* and 2 of 30 *BRCA2* neutral variants from the BIC database using a curated alignment compared to zero miscalls using an uncurated alignment that could only be obtained at a criteria of 50% identity. Simply considering evolutionary depth, Align-GVGD has a high sensitivity that increases with depth in curated alignments; the sensitivity also increases in uncurated alignments depending on the identity level of the sequences included in the alignment. Analysis of neutral variants in *MSH2* or *MLH1* yields a similar sensitivity with both types of alignments, but for the *TP53* gene a similar result is only obtained under 50% identity in the uncurated alignments. Thus appropriate alignment depth is dependent on the particular gene of interest and should be further explored.

## 560/W/Poster Board #218

**A nonsynonymous SNP in *EDAR* is associated with tooth shoveling.** *R. Kimura[1], Y. Yamaguchi[2], K. Maki[2], M. Takeda[3], O. Kondo[3], T. Hanihara[4], M. Osawa[5], H. Ishida[6], S. Kawamura[7], H. Oota[7].* 1) TRO-SIS, University of the Ryukyus, Okinawa, Japan; 2) School of Dentistry, Showa University, Tokyo, Japan; 3) Graduate School of Science, The University of Tokyo, Tokyo, Japan; 4) Saga Medical School, Saga, Japan; 5) Tokai University School of Medicine, Kanagawa, Japan; 6) Faculty of Medicene, University of the Ryukyus, Okinawa, Japan; 7) Graduate School of Frontier Sciences, The University of Tokyo, Chiba, Japan.

Teeth display variations among individuals in the size and the shape of cusps, ridges, grooves, and roots. In addition, there are certain dental characteristics which are predominant in certain human groups, such as tooth shoveling of upper incisors that is major in Asian populations but rare or absent in African and European populations. The common characteristics of dental morphology are thought to be determined mainly by genetic factors. However, genetic polymorphisms associated with dental morphology have not been elucidated yet. In humans, the *ectodysplasin A receptor* gene (*EDAR*) as well as the *ectodysplasin A* gene (*EDA*) is know to be responsible for hypohidrotic ectodermal dysplasia, a genetic disorder causing abnormal morphogenesis of teeth, hair, and eccrine sweat glands. Human genome diversity data have revealed that the derived allele of a nonsynonymous single nucleotide polymorphism (SNP), rs3827760 that is also called *EDAR* T1540C, is predominant in East Asian populations but absent in populations of African and European origins. It has recently been reported that the 1540C allele is associated with Asian-specific hair thickness. The aim of this study is to clarify whether the nonsynonymous polymorphism in *EDAR* is also associated with dental morphology in humans or not. For this purpose, we measured crown diameters and tooth shoveling grades, genotyped *EDAR* T1540C, and analyzed the correlations between them in Japanese populations. To comprehend individual patterns of dental morphology, we applied a principal component analysis (PCA) to individual-level metric data, the result of which implies that multiple types of factors affect the tooth size. This study clearly demonstrated that the number of the Asian-specific *EDAR* 1540C allele is strongly correlated with the tooth shoveling grade. The SNP significantly affected PC1 and PC2 in PCA, which denotes overall tooth size and the ratio of mesiodistal diameter to buccolingual diameter, respectively. Our study revealed a main genetic determinant of tooth shoveling that has classically received great attention from dental anthropologists. Further studies using powerful DNA technology will lead to clearer understanding about genetic factors for phenotypic variations in tooth morphology such as Carabelli's tubercle, the numbers of cusps and roots, and the size balances shown in metric measurements.

## 561/W/Poster Board #219

**Recombination and gene conversion in 17q21.31 inversion polymorphism region.** *W. Chen[1,2], X. Hao[1,2], L. Deng[1,3], C. Zeng[1].* 1) Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, China; 2) Graduate university of Chinese Academy of Sciences, Beijing, China; 3) Faculty of Basic Medical College, Nanchang University, Nanchang, China.

17q21.31 is a common inversion polymorphism region with relatively large size (0.9Mb), which is ideal for studying the effect of inversion on LD pattern and finding signals of conversion events. Using SNP data of Human Genome Diversity Project from 1043 individuals of 51 populations in 7 geographical regions (Africa, Mideast, Europe, South Asia, East Asia, Oceania, and America), we computed the recombination rates in 17q21.31. After haplotype phasing, principle component analysis resolved two distinct clades. As these corresponding to previously identified haplotype groups resolved by tag SNPs, we also use H1 and H2 (inverted) to represent the two opposite orientations. Accordingly a worldwide distribution map of 17q21.31 inversion polymorphism was constructed. In contrast to most populations whose frequencies of H2 haplotypes were lower than 10%, ethnic groups in Mideast and Europe showed significantly increases in both the frequency (20-25%) and the diversity of haplotypes in H2 clade, suggesting that H2 was perhaps historically under positive selection in these two areas. Consequently more H2 haplotypes were resulted after the pressure no longer existed. Computed by composite likelihood method in 29 HGDP populations (>19 individuals each), a low recombination rate was seen in 17q21.31 in comparison with a 1 Mb non-genic region in 22q12, indicating the inversion polymorphism suppressed crossover during meiosis. To discover possible conversion events, next we compared haplotypes in HapMap CEU samples with the method described by Betran. Totally 41 candidate domains with signals of gene conversion were found in 17q21.31. These domains were shown in two patterns. First, 26 were in the type of conversion as expected. For another 15 candidates, H1 and H2 appeared surprisingly to have parts of their specific haplotypes exchanged in H1/H2 heterozygous individuals. For detailed analysis, 5 CEU samples of each type were then selected for clone re-sequencing to separate each haplotype from diploid individuals on candidate regions. Finally, we verified that 5 regions from the group of 26 candidates were all experienced conversion events. However, the rest 5 showing the pattern of haplotype exchange were actually caused by phasing error of the HapMap. Moreover, 2 of the 5 verified regions experienced gene conversion events also displayed higher recombination rate, showing the conversion effect on the genetic information change in the human genome.

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## 562/W/Poster Board #220

**HUMAN β-DEFENSIN 3: GENE COPY NUMBER VARIATIONS VARY GEOGRAPHICALLY.** *P. Fode[1], M. Theisen[2], D. Dodoo[3], M. Lenicek[4], L. Vitek[4], A. Viera[5], J. Freitas[5], I. Vind[6], P. Munkholm[6], N. Frimodt-Moller[1], P. Skytt Andersen[1].* 1) National Center for Antimicrobials and Infection Control, Statens Serum Institut, Copenhagen, Denmark; 2) Department of Clinical Biochemistry and Immunology, Statens Serum Institut, Copenhagen, Denmark; 3) Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana; 4) Laboratory of Hepatology, Department of Clinical Biochemistry and Laboratory Diagnostics, Charles University, Prague, Czech Republic; 5) Department of Gastroenterology, Hospital Garcia de Orta, Almada, Portugal; 6) Digestive Disease Centre, Medical Section, Herlev Hospital, University of Copenhagen, Copenhagen, Denmark.

In recent years much interest has focused on variations in the genome among humans e.g. copy number variations (CNVs). The focus for the current study is to determine copy number variations for the human β-defensin 3 gene (DEFB103) in five different demographic populations. The nature and extent of CNVs in various populations are largely unknown, but differences are expected. Using a single tube duplex real-time PCR assay the copy number for DEFB103 was determined for 736 samples using RNaseP as reference gene. DEFB103 copy number varied in the five populations with copy number ranging from 2 to 17 per diploid genome. The mean copy number was found to be significantly higher in the Portuguese (X=7.2) and Ghanian (X=6.7) samples compared to the Danish (X=4.6), Czech (X= 5.1) and English (X=4.4) samples. In addition, a higher inter-population CNV was observed in the Ghanian and Portuguese populations (2-17 copies per diploid genome) compared to the Danish, English and Czech populations (2-12 copies per diploid genome). The DEFB103 copy number was found to vary significantly. Several studies indicate that the CNV polymorphism in the DEFB cluster may have impact on susceptibility to inflammatory diseases such as Crohn's Disease and Psoriasis. Others have linked the copy number for some defensin genes with the mRNA expression levels and CNVs might have important consequences for susceptibility to infectious diseases. The genetic variation in the DEFB cluster may explain the genetic predisposition to many biologic events and the polymorphism might reflect genomic drift in the human population.

## 563/W/Poster Board #221

**Study of genetic effects of atomic-bomb radiation using BAC-aCGH: *De novo* copy number mutants detected by aCGH in a Japanese population.** *N. Takahashi[1], Y. Satoh[1], M. Kodaira[1], K. Sasaki[1], Y. Kodama[1], Y. Shimoichi[1], J. Kaneko[1], A. Miura[1], M. Imanaka[1], E. Hiyama[2], I. Fukuba[2], H. Katayama[3], J. Cologne[4].* 1) Dept Gen, Radiation Effects Res Fdn, Hiroshima, Japan; 2) N-BARD, Hiroshima Univ, Hiroshima, Japan; 3) Dept Info Tech, Radiation Effects Res Fdn, Hiroshima, Japan; 4) Dept Stat, Radiation Effects Res Fdn, Hiroshima, Japan.

[Purpose] To study the effects of atomic-bomb radiation on human germline cells at the genome-wide level, we have introduced bacterial artificial chromosome (BAC) DNA micro-array based comparative genomic hybridization (BAC-aCGH). We report the results obtained from 305 individuals by BAC-aCGH analyses. [Experiment] We used an array with about 2,500 BAC-clones distributed across human autosomes at an interval of about 1.2 Mb. We examined 265 offspring who had at least one parent exposed to high-radiation doses (≥1.0 Gray) and 40 offspring from unexposed parents. [Results and Discussion] We found a total of 1,534 copy number variants (CNVs) in the genome; of these, 97 CNVs were termed "rare" CNVs whose frequencies were less than 1%, while the frequencies of the remaining 1,437 CNVs observed at 32 regions (termed "polymorphic" CNVs) were 1% or more. With respect to rare CNVs, DNA from the offspring and their parents were examined by quantitative polymerase chain reaction to confirm the inheritance. Three rare CNVs independently identified in three offspring were not identified in either their parents and these are defined as "putative *de novo* mutants." Two mutants were amplification types and remaining one was a deletion type. For the putative *de novo* mutants, we confirmed whether they are "true" mutants or ones generated as a consequence of Epstein-Barr virus transformation and/or extended cell culture. As a result, three were true mutants, not artificial. We determined the parental origin of the *de novo* mutants using single nucleotide polymorphisms (SNPs) proximal to the mutated regions in the offspring. For this purpose, Affymetrix SNP Array 6.0 was used. The results demonstrated that all three *de novo* mutants occurring on the gametes originated from exposed fathers. This number of *de novo* mutants (three) is too small to reach a firm conclusion as to whether the mutation rate of the exposed group is significantly higher than that in those from unexposed group. Therefore, we will continue the study using the high-density array system to increase the number of loci examined in order to increase the number of *de novo* mutants to be identified.

## 564/W/Poster Board #222

**A detailed phylogeography of mtDNA haplogroup C1d: another piece in the Native American puzzle.** *U.A. Perego[1,2], N. Angerhofer[1], M. Pala[2], J.E. Gomez-Palmieri[1], A. Olivieri[2], K.H. Ritchie[1], B. Hooshiar Kashani[2], V. Carossa[2], H. Lancioni[3], N. Myres[1], A. Gómez-Carballa[4], B. Zimmermann[5], G. Huber[5], M. Bodner[5], W. Parson[5], A. Salas[4], H.-J. Bandelt[6], S.R. Woodward[1], A. Torroni[2], A. Achilli[2,3].* 1) Sorenson Molecular Genealogy Foundation, Salt Lake City, Utah, USA; 2) Dipartimento di Genetica e Microbiologia, Università di Pavia, Pavia, Italy; 3) Dipartimento di Biologia Cellulare e Ambientale, Università di Perugia, Perugia, Italy; 4) Facultad de Medicina, Universidad de Santiago de Compostela, Santiago de Compostela, Galicia, Spain; 5) Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria; 6) Department of Mathematics, University of Hamburg, Hamburg, Germany.

Recent studies based on complete mitochondrial DNA (mtDNA) sequences revealed that two almost concomitant paths of migration from Beringia led to the dispersal of the first Americans (Paleo-Indians) approximately 15-17 thousand years ago (kya). This first expansion was followed by later more restricted diffusion events from the same dynamically changing Beringian source. Thus, five pan-American (A2, B2, C1, D1, and D4h3a) and four geographically confined (D2, D3, X2a, and C4c) mtDNA haplogroups represent the current female legacy of the ancient migratory events that gave rise to the native populations of the double continent. Regarding haplogroup C1, all its members appear to belong to one of three branches: C1b (characterized by the control-region transition at np 493), C1c, and C1d (with the control-region transition at np 16051). These three sub-haplogroups are found throughout the Americas, thus supporting the scenario that they most likely differentiated at the early stages of the Paleo-Indian southward migration. If considered as three separate founders, C1b, C1c, and C1d would bring the currently known number of native pan-American lineages to seven. As a whole, the C1 haplogroup has an estimated age of 17.0-19.6 ky, while the three individual branches are dated 16.5-17.0 ky, 17.2-17.6 ky, and 7.6-9.7 ky, respectively. The extremely young age estimate of C1d has been attributed, at least for the moment, to a major under-representation of C1d mtDNAs (only nine complete sequences published to date) in the current Native American mtDNA phylogeny. We have addressed this issue in the current study by completely sequencing more than 60 novel mtDNAs belonging to haplogroup C1d, which were carefully selected on the basis of both control-region variation and geographic/ethnic origin. Phylogeographic analyses have provided not only an accurate evaluation of the expansion time of C1d in the Americas, but also a detailed picture of its current distribution in both general mixed and indigenous populations.

## 565/W/Poster Board #223

**Closely Spaced Multiple Mutations as Potential Signatures of Transient Hypermutability in Human Genes.** *J. Chen[1,2,3,4], C. Férec[1,2,3,4,5], D.N. Cooper[6].* 1) Institut National de la Santé et de la Recherche Médicale (INSERM), U613, Brest, France; 2) Etablissement Français du Sang (EFS) - Bretagne, Brest, France; 3) Faculté de Médecine et des Sciences de la Santé, Université de Bretagne Occidentale (UBO), Brest, France; 4) Institut Fédératif de Recherche (IFR) 148, Brest, France; 5) Laboratoire de Génétique Moléculaire et d'Histocompatibilité, Centre Hospitalier Universitaire (CHU) Brest, Hôpital Morvan, Brest, France; 6) Institute of Medical Genetics, School of Medicine, Cardiff University, Cardiff, United Kingdom.

Data from a diverse series of organisms suggested that transient hypermutability is a general mutational mechanism with the potential to generate multiple synchronous mutations, a phenomenon probably best exemplified by closely spaced multiple mutations (CSMMs). However, to date, its potential impact on the evolution of higher eukaryotes has not been widely appreciated. Here we attempted to extend the concept of transient hypermutability from somatic cells to the germline, using human disease-causing multiple mutations as a model system. Employing fairly stringent criteria for data inclusion, we have retrospectively identified numerous potential examples of CSMMs causing human inherited disease that exhibit marked similarities to the CSMMs reported in other systems. These examples include (i) eight multiple mutations, each comprising three or more components within a sequence tract of <100 bp, (ii) three possible instances of 'mutation showers' and (iii) numerous highly informative 'homocoordinate' mutations. Using the proportion of CpG substitution as a crude indicator of the relative likelihood of transient hypermutability, we also present evidence to suggest that CSMMs comprising at least one pair of mutations separated by ≤100 bp may constitute signatures of transient hypermutability in human genes. This analysis not only extends the generality of the concept of transient hypermutability but also provides new insights into what may be considered a novel mechanism of mutagenesis underlying human inherited disease. Finally, our findings raise serious concerns regarding current practices in mutation screening, which are likely to miss many potentially important secondary mutations linked in cis to the putative primary pathological lesion.

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## 566/W/Poster Board #224

**Genetic variation in unsampled ancestral populations.** *R. Jiang[1], C. Eng[2], E.G. Burchard[1,2], J. Wall[1].* 1) Institute for Human Genetics, UCSF, San Francisco, CA; 2) Department of Medicine, UCSF, San Francisco, CA.

Analyses of genetic variation are important to the study of human populations, both for inferring population history and for understanding the genetic basis of complex traits. Here, we show how genetic variation in ancestral populations can be analyzed from sampling recently admixed individuals. This two-step process involves estimating local ancestry across chromosomes from genome-wide SNP data, followed by targeted resequencing of regions inferred to be inherited from a particular ancestral population. We apply the method to Mexican-American samples from the NIGMS panel, and analyze genetic variation in the Native American population(s) ancestral to the panel. To our knowledge, this is the first large-scale study of autosomal resequencing variation in any Native American group.

## 567/W/Poster Board #225

**Decrypting the mtDNA gene pool of modern Panamanians.** *A. Achilli[1,2], U.A. Perego[2,3], M. Tribaldos[4], N. Angerhofer[3], K.H. Ritchie[3], J. Robinson[3], L. Milani[5], M. Lari[5], D. Caramelli[5], N.M. Myres[3], R. Cooke[6], J.M. Pascale[4], J. Motta[4], A. Torroni[2], S.R. Woodward[3].* 1) Dipartimento di Biologia Cellulare e Ambientale, Università di Perugia, Italy; 2) Dipartimento di Genetica e Microbiologia, Università di Pavia, Pavia Italy; 3) Sorenson Molecular Genealogy Foundation, Salt Lake City, Utah, USA; 4) Gorgas Memorial Institute for Health Studies, Panama City, Panama; 5) Dipartimento di Biologia Evoluzionistica, Università di Firenze, Firenze, Italy; 6) Smithsonian Tropical Research Institute, Panama City, Panama.

The Isthmus of Panama - the narrow neck of land connecting the northern and southern American landmasses - was a forced corridor for the Paleo-Indian expansion that originated from Beringia ~15-17,000 years ago. Archeological findings suggest that some descendants of the earliest migrants remained on the isthmus, while accounts from early European explorers witness the presence of two main indigenous groups (the Cueva and the Coclé) in pre-Columbian times - populations that have since disappeared due to disease, fights, and enslavement following the Spanish conquest. Today's indigenous groups total about 5.3% of the Panamanian population, mainly represented by the Ngöbé, Buglé, Kuna, Emberá, and Wounan tribes, which traditionally appear to have settled in Panama from surrounding regions after the autochthonous natives were decimated. However, there is no evidence that the ancestral indigenous gene pool was completely replaced. If this were the case, the populations of modern Panama should have retained at least a fraction of the native pre-Columbian gene pool, possibly at a variable extent, given the differential degree of geographical and genetic isolation of the different Panamanian communities during the past five centuries. To evaluate the nature and extent of the maternally inherited mitochondrial DNA (mtDNA) variation in modern Panamanians, DNA samples and historical records were collected from approximately 1500 volunteer participants living in the nine provinces and four indigenous territories of the Republic, and from a few pre-Columbian skeletal remains. Currently, mtDNAs for more than 500 subjects have been sequenced for the entire control region, thus including all three hypervariable segments (HVS-I, II, and III). Due to the recent gene flow from the Old World, we detected ~8% and ~13% of European and African mtDNAs, respectively, with a minor Asian component (~1%). However, the overwhelming majority (~78%) of Panamanian mtDNAs clustered into Native Pan-American haplogroups, mostly represented by A2 (49%), also identified in a pre-Columbian skeleton. In conclusion, despite the key location of the isthmus and the major construction efforts in the area (Panama Railroad and Panama Canal) that brought large numbers of slaves from Africa and immigrants from Asia, the analysis of mtDNA indicates an overall genetic continuity of female lineages between first and last inhabitants of Panama.

## 568/W/Poster Board #226

**hzAnalyzer: Analysis of contiguous homozygosity in eleven human sample populations using R and Java.** *T.A. Johnson[1,2], Y. Niimura[2], T. Tsunoda[1].* 1) Laboratory for Medical Informatics, Center for Genomic Medicine, RIKEN Yokohama Institute, Yokohama, Japan; 2) Department of Bioinformatics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan.

Reports have shown that human genetic variation is often non-randomly organized into regions of restricted diversity in which a limited range of haplotypes can be observed. To examine such patterns, we have developed methods to analyze the genome-wide and localized extent of contiguous homozygosity, which represents one simple means for reducing high-density genotyping datasets into a form by which populations can be compared for their relative haplotype diversity. To apply these methods, we developed hzAnalyzer, a suite of programs using R and Java and analyzed the HapMap phase 3 rel. 2 dataset that includes approximately 1.5 million SNPs for 1,184 individuals from 11 sample populations representing founders from Africa, East Asia, India, and Europe. We detected and analyzed segments of contiguous homozygous genotypes and present as a summary the mean±SD (X$10^8$bp) of total homozygous segment length (≥131,367bp) on autosomes. Based on similarity between sample means, we grouped them into Low, Intermediate, and High groups. The Low group consisted of all populations with substantially African origins (ASW=1.6±0.1, n=83; LWK=1.7±0.2, n=90; MKK=1.7±0.2, n=171; YRI=1.9±0.1, n=167), while the Intermediate group had those with mainly European or Indian origins (CEU=4.5±0.3, n=165; GIH=4.4±0.2, n=88; MEX=4.4±0.6, n=77; TSI=4.5±0.2, n=88). East Asians possessed the highest levels of extended homozygosity (CHB=5.6±0.2, n=84; CHD=5.6±0.3, n=85; JPT=5.7±0.3,n=86). Based on recent reports on the intersection between migratory patterns during human history and haplotype diversity, these categories likely represent relative migration distances from humanity's origins in Africa. However, this data also highlights differences that might have occurred due to subsequent population admixture, since the ASW and MEX populations likely represent varying degrees of admixture between European, African, or Asian founders. Interestingly, of all groups, ASW showed the lowest levels of total homozygosity, suggesting that admixture between different African populations as well as Caucasians led to higher levels of haplotypic variation. Conversely, the MEX population showed the highest standard deviation, substantial overlap with the East Asian distributions, and a long lower tail indicating that some individuals had greater haplotype diversity. These results suggest that the impact of migration and admixture on haplotypic variation can be complex and bears further study.

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## 569/W/Poster Board #227

**Haplogroup H of mitochondrial DNA, a far echo of the West in the heart of Central Asia.** *S.R. Woodward[1], U.A. Perego[1,2], J.E. Gomez-Palmieri[1], N. Angerhofer[1], D. Tumen[3], K.H. Ritchie[1], B. Hooshiar Kashani[2], M. Pala[2], A. Olivieri[2], V. Carossa[2], E. Myagmar[3], H. Lancioni[4], F. Panara[4], N. Babudri[4], D. Bayarlkhagva[3], M. Bayarlkhagva[3], A. Dyikanbaeva[5], A. Torroni[2], N.M. Myres[1], A. Achilli[2,4]*. 1) Sorenson Molecular Genealogy Foundation, Salt Lake City, Utah, USA; 2) Dipartimento di Genetica e Microbiologia, Università di Pavia, Pavia, Italy; 3) National University of Mongolia, Ulan Bator, Mongolia; 4) Dipartimento di Biologia Cellulare e Ambientale, Università di Perugia, Perugia, Italy; 5) American University of Central Asia, Bishkek, Kyrgyz Republic.

Through the millennia, Inner Asia played a pivotal role in shaping the history that greatly added to the cultural, ethnic, and genetic diversity observed throughout present Eurasia. Perhaps the two most significant phenomena witnessed in this part of the world were the ambitious expansion strategy employed by Mongolia's most prominent personality, Genghis Khan and the complex network known as the Silk Road that for nearly 3,000 years contributed to the exchange of goods and the transmission of philosophy, art, and science that laid the foundation for the great civilizations of China, India, Egypt, Persia, Arabia, and Rome, and in several respects to the modern world. Over the last few years, through an international collaborative effort, researchers at the Sorenson Molecular Genealogy Foundation were able to collect 2,727 DNA samples, informed consents, and genealogical data in Mongolia, Kyrgyzstan, and Kazakhstan. All the samples were sequenced for the three hypervariable segments of the mitochondrial DNA (mtDNA) control region to assess the genetic composition of the modern population of these countries. We identified ~600 different haplotypes that could be ascribed to more than 30 haplogroups and sub-haplogroups. As expected, most haplogroups are typical of modern East Asian populations, but intriguingly, many different Western Eurasian clades were also identified, with a particular high incidence of H (~8.0%), the most common haplogroup in Europe. This feature cannot be attributed to genetic drift since different H sub-lineages have also been identified, each of them represented by several different haplotypes. The mtDNA distribution profile in the heart of Central Asia suggests a direct link between this area and Western Eurasia that could be explained by ancient migrations or by more recent historical events, such as Genghis Khan's conquering efforts and trade or cultural exchanges along the Silk Route. To discriminate between these two possible scenarios, we are now analyzing a subset of these samples at the highest possible level of resolution - that of complete mtDNA sequences - focusing particularly on those H mtDNAs that seem to be the most informative considering their control-region haplotypes. Our preliminary data seems to be in favor of rather ancient genetic inputs from the West in shaping the peculiar mtDNA gene pool of Inner Asia's present-day populations.

## 570/W/Poster Board #228

**Genetic Associations with Mountain Sickness for Chinese Residents at the Tibetan Plateau.** *N. Buroker[1,2], X-H. Ning[2], Z-N. Zhou[4], K. Li[3], W-J. Cen[3], X-F. Wu[4], M. Ge[5], L-P. Fan[2], W-Z. Zhu[4], M. Portman[1,2], S-H. Chen[1]*. 1) Dept Pediatrics, RR335 HSB, University of Washington, Seattle, WA; 2) Divisions of Cardiology, Seattle Children's Hospital. Institute. Foundation, Seattle, WA USA; 3) Lhasa People Hospital, Tibet; 4) Laboratory of Hypoxia Physiology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, China; 5) Department of Molecular and Human Genetics, Baylor College of Medicine One Baylor Plaza, Houston, TX USA.

Acute (A) and chronic (C) mountain sickness (MS) are illnesses that occur among people visiting and inhabiting high altitude environments, respectively. Both diseases are influenced by genetics. Consequently, some individuals are genetically less fit than others when stressed by extreme high-altitude environment. We studied six polymorphisms in association with mountain illness among residents at the Tibetan plateau. The angiotensin-converting enzyme (ACE) insertion/deletion, angiotensinogen (AGT) M235T, angotensin II type 1 receptor (AT1R) A1166C and GNB3 promoter A(-350)G polymorphisms all associated with hypertension as well as two SNPs (apolipoprotein B, rs693 and tribbles homolog 1, rs17321515) associated with levels of LDL cholesterol and triglyceride, respectively, were studied with relation to AMS and CMS in normal Han and Tibetan populations. Among the hypertension polymorphisms, the ACE deletion (D) allele [OR= 1.71, CI = 1.04-2.8, p=0.036] and the AGT 235M allele [OR=2.71, CI=1.08, p=0.039] were associated with AMS and CMS, respectively, while the A allele of GNB3 was significantly (Fst, p=0.034) associated with AMS. Oxygen saturation (SO2a) was significantly lower for the AGTR-235MM genotype than for either the 235MT or 235TT genotypes. All polymorphisms were in Hardy-Weinberg equilibrium (HWE) for the Han and Tibetan controls. A departure from HWE was found for the apolipoprotein B SNP in the AMS study group. A significantly higher incidence of the TRIB1 SNP G allele was present in the CMS group compared to the normal Tibetan population. Pairwise linkage disequilibrium was found between the ACE and AGT M235T polymorphisms in the AMS study group.

## 571/W/Poster Board #229

**Statistical methods for estimating the rate of de novo mutation at FGFR3 in sperm samples from healthy men using high-throughput sequencing.** *S.P. Pfeifer[1], A. Goriely[2], G.A.T McVean[1], A.O.M. Wilkie[2]*. 1) Department of Statistics, University of Oxford, South Parks Road, Oxford OX1 3TG, UK; 2) Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DS, UK.

Some rare congenital disorders (such as Apert, Crouzon, Pfeiffer, Muenke, Costello and Noonan syndromes and achondroplasia) originate from spontaneous mutations in the germline of healthy fathers which are older than the average (paternal age effect). The prevalence of these specific mutations may reflect a protein-driven, positive selection of mutant cells according to the functional consequences of the encoded amino acid substitution. We aimed to use new sequencing technologies to quantify all possible point mutations at codon K650 (AAG) of the fibroblast growth factor receptor 3 (*FGFR3*) leading to achondroplasia. 78 sperm and 8 blood samples were sequenced using the Illumina sequencing technology. An attempt to use Illumina's inbuilt quality scores to estimate the rate of de novo mutations could not account for the underlying error structure. Therefore, a Bayesian approach was used to fit a model to the observed counts of each codon in the sequencing data to account for errors and bias derived from the rounds of PCR and digestion during the sample preparation, and the sequencing process. Titration data were analysed together with biological samples to validate our method down to the level of $10^{-5}$. Whilst mutation rates in blood were low, 73% of the total mutations quantified in the sperm samples were caused by a 1948A>G mutation. It reached high mutation levels (with a maximum of $2.1 \times 10^{-4}$) in sperm samples which were significantly correlated with donor age (Spearman rank r=0.34, P=0.002). Several other substitutions attained levels $>10^{-5}$ in a minority of sperm samples. These results show the utility of advanced statistical methods to estimate mutation rates in human sperm from high-throughput sequencing data down to the level of $10^{-5}$, whilst capturing subtle features of the machine and run dependent error structure.

## 572/W/Poster Board #230

**Analysis of mitochodrial DNA polymorphism in Chinese Han,Miao,and Tu populations.** *N. bin[1], z. hai yan[2], T. sai nan[3], y. zhao chu[4], Z. yong hua[5], C. yong[6]*. 1) medical genetics lab, Hunan family planning institute, Changsha, Hunan , China; 2) medical genetics lab,Hunan family institute,-Changsha,Hunan,China; 3) medical genetics lab,Hunan family planning institute,Changsha,Hunan,China; 4) medical genetics lab,Hunan family planning institute,Changsha,Hunan.China; 5) medical genetics lab, Hunan family planning institute,Changsha,Hunan,China; 6) medical genetics lab , Hunan family planning institute,Changsha,Hunan,China.

Analysis of mitochondrial DNA( mtDNA) is commonly performed in forensic investigations when the DNA in a sample is degreded or the amount is not sufficient for a STR analysis.However, mtDNA analysis has the draback of low discrimination power compared to what can be obtained by nuclear DNA analysis,owing to multiple individuals sharing identical mtDNA types in the HVI / HVII region.In this study,mtDNA sequences of the hypervariable regions HVI and HV IIand 15 SNP loci in mtDNA coding region were analyzed in 40 Chinese Han samples ,40 Miao minority ethnic group samples and 40 Tu minorityethni group samples from Hunan province,China as an initial effort to generate a database for forensic identification purposes.Comparing with Anderson sequence,98 polymorphic loci in HVI and 34 in HVII were found in total 120 individual samples, 90 and 65 haplotypes were then defined,respectively.Of 115 haplotypes,5 were shared by 2 individuals. When the HVI ,HVII and coding region were combined, only 1 haplotypes were shared by more than 1 individual.Thus,mitochondrial coding analysis can substantially increase the discriminatory power of mtDNA analysis.The random match probability and discrimination power in 120 individuls were 0.86% and 99.14%,respectively.

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## 573/W/Poster Board #231

**Identification of rare variants for ulcerative colitis and celiac disease by high-throughput sequencing.** *A. Szperl[1], C.C. van Diemen[1], A. Zhernakova[2], E.A.M Festen[1,3], P. van der Vlies[1], R.K. Weersma[3], C. Wijmenga[1].* 1) Gen Dept, UMC Groningen, Groningen, The Netherlands; 2) Complex Genetics Section, DBG-Department of Medical Genetics, University Medical Center Utrecht, The Netherlands; 3) Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

Celiac disease (CD) and ulcerative colitis (UC) are chronic inflammatory diseases of the small intestine with a complex genetic origin. It has been shown that these disorders co-occur in patients and families. Recent genome wide association studies (GWAS) suggest a common genetic background for UC and CD with five loci already associated to both diseases. The loci identified by GWAS, due to the association to common variants, confer small risks with odds ratio between 1.2 and 1.5. We hypothesize that each of these loci may also contain rarer variants with higher risks that are causative for development of disease. The aim of this study was to identify rare causative variants in immune-related genes associated with CD or/and UC. We amplified the coding regions and 50-100 bp flanking intronic sequence of 19 immune-related genes in pools of DNA from 50 UC patients, 100 CD patients and 100 controls and analysed the samples by single-read sequencing on the Genome Analyzer II (Illumina). We have sequenced ~52 kb per DNA pool and identified 69 known SNPs and 110 unknown variants (65 non-synonymous and 114 synonymous). We selected 14 variants for follow-up that each fulfilled the following criteria: a) had a coverage of more than 700 reads per basepair; b) were observed in different reads in both the forward and reverse direction; c) were previously unknown; d) had a frequency <5%; e) were observed in CD and/or UC but not in controls; f) were non-synonymous. We were already able to confirm one stop codon variant in UC. We subsequently genotyped this variant in 900 UC, 900 CD patients and 900 controls by TaqMan genotyping and identified one other heterozygous UC individual for this stop codon. The variant is not found in CD cases nor in controls. Functional assays for the newly confirmed variant is currently ongoing as well as full sequence analysis of the entire gene in the entire cohort. Follow-up of other variants is also ongoing. Our study has shown that rare variants in a gene that also contains common variants may underlay the association to UC. This is very reminiscent to the study of common and rare variants in IFIH1 associated with type 1 diabetes.

## 574/W/Poster Board #232

**On the size distribution of private microsatellite alleles.** *Z.A. Szpiech[1], S.B. Reddy[1], N.A. Rosenberg[1,2].* 1) Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI; 2) Department of Human Genetics, University of Michigan, Ann Arbor, MI.

Private microsatellite alleles tend to be found in the tails rather than in the interior of the allele size distribution. To characterize this phenomenon, we have investigated the size distribution of private alleles in a coalescent model of two populations, assuming the symmetric stepwise mutation model as the mode of microsatellite mutation. For the case in which four alleles are sampled, two from each population, we condition on the configuration in which three distinct allele sizes are present, one of which is common to both populations, one of which is private to one population, and the third of which is private to the other population. Conditional on this configuration, we calculate the probability that the two private alleles occupy the two tails of the size distribution. This probability is seen to be greater than the value that would be expected if there was no relationship between privacy and location in the allele size distribution. Results from our model can assist in interpreting the allele size properties observed for private microsatellite alleles in human populations.

## 575/W/Poster Board #233

**Direct estimation of the microsatellite mutation rate.** *J.X. Sun[1], A. Helgason[2], G. Masson[2], N. Patterson[4], D.E. Reich[3,4], K. Stefansson[2].* 1) Division of HST, Massachusetts Institute of Technology, Cambridge, MA, USA; 2) deCODE Genetics, 101 Reykjavik, Iceland; 3) Dept of Genetics, Harvard Medical School, Boston, MA, USA; 4) Broad Institute, Cambridge, MA, USA.

Characterizing the behavior of mutations is fundamental to our understanding of genetic variation. Attempts to directly observe DNA mutations arising from germline transmissions are confronted by two challenges: The large amount of DNA sequence that needs to be collected in order to observe a mutation (since the mutation rate in humans is estimated to be ~$2x10^{-8}$ per generation), and a poor signal-to-noise ratio, due to the fact that any modern genotyping technology has an error rate far exceeding the mutation rate. Using deCODE Genetics' database of over 95,000 Icelanders genotyped at over 3,000 microsatellite loci, we directly observed mutations in germline transmissions from pedigrees. Microsatellites are thought to have mutations rates as high as $10^{-3}$ per locus per generation. To overcome the genotyping error rate, which was estimated in this data set to be $\leq 10^{-2}$ per allele call after appropriate filtering, we carried out two independent analyses: (1) We restricted our analysis to mother-father-child trios, and required the mutated allele to be genotyped at least twice in both the child and in the transmitting parent to confirm mutant transmissions. This identified 2,124 mutant events from 5.62 million instances of parent-child transmissions, yielding a mutation rate estimate of $3.78x10^{-4}$ averaged across the markers that we analyzed. (2) We traced the haplotype affected by the mutation through local pedigrees, requiring that the mutant haplotype is observed in the affected proband's children, and simultaneously, that the wildtype haplotype is observed in the affected proband's siblings. This identified 788 mutant events from 1.59 million instances of parent-child transmissions, yielding a mutation rate of $4.96x10^{-4}$. Our collection of mutant events is significantly larger than previous studies. This allows for categorical analyses of microsatellite mutation rates partitioned based on the gender and age of the individual transmitting the allele, as well as the repeat type and cytogenetic position.

## 576/W/Poster Board #234

**Distribution of the CTG repeat polymorphism at the DMPK gene in a healthy Mexican population sample.** *J.J. Magaña[1], P. Cortés-Reynosa[1], R. Gómez[1], A. Miranda[1], N. Leyva[1], B. Cisneros[2].* 1) Genetics, National Rehabilitation Institute, Mexico City, D.F., Mexico; 2) Genetics and Molecular Biology Dept, CINVESTAV-IPN, Mexico City, D.F., Mexico.

Myotonic dystrophy type 1 (DM1) is the most common form of muscular dystrophy in adults, affecting 1/8000 individuals. DM1 is caused by expansion of the CTG trinucleotide repeats within the 3'-untranslated region (3'-UTR) of the DMPK gene. This repeat is polymorphic in normal individuals with alleles ranging from 5 to 37 repeats. Alleles exceeding a threshold of approximately 50 repeats and reaching up to a number of 4000 CTGs result in disease. The present study, analyze the variability at the DMPK gene polymorphism in 400 unrelated Mexican healthy subjects. CTG repeats were genotyped using capillary electrophoresis in an ABI PRISM 310 genetic analyzer. A total of twenty five alleles were found, ranging from 5 to 37 repeats. Distribution of alleles was bimodal, with peaks at 5 and 10-14 repeats. The most common allele had 13 repeats (38.8%) followed by the 11 and 5 repeats alleles (24.6% and 15% respectively). Alleles greater than 22 repeats had a frequency of 3.25%. As expected from such allelic distribution, the predominant genotypes were 11/13 and 13/13 repeats. Polymorphic distribution was compared with data from 8 different human populations (Caucasian European, Yugoslav, Japanese, Korean, Taiwan, African Negroids, African American and Chilean) and one Amerindian group (Cabecar, a tribal population of Costa Rica). We found that all populations were significantly different among them ($P < 0.01$). The 13 repeats allele was also the predominant allele in the Japanese population (27.35%), however its frequency is statistical different between the Japanese and our population. The frequency of the >22 repeats alleles in the Mexican population was similar than that of the others populations, with the exception of the African population, where these alleles exhibits low frequency. This fact may explain the low DM1 incidence in black populations. Generally, the 5 repeats allele is the most common allele in the analyzed populations; however, it showed lower frequency in the Mexican population. This particular distribution in the Mexican population could be attributable to our Native American Ancestry. We and others have evidenced the presence of a mixture of European, Native American and African ancestry in our population. Our findings confirmed the high variability of the CTG repeats at the 3'UTR of DMPK gene, which makes it a useful tool in forensic and ethnic admixture studies, and could explain also the different distribution of the disease in the world.

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## 577/W/Poster Board #235

***MECP2* gene coding sequence variations in newborn infants.** *J. Yu[1], Z. Qi[1], Y. Zhang[2], C. Iyama[2,3], H. Zeng[2], G. Hoffman[4], D. Kurtycz[4,5].* 1) Lab Med, Univ California, San Francisco, San Francisco, CA; 2) Waisman Center, University of Wisconsin-Madison, Madison, WI; 3) Department of Pediatrics, University of Wisconsin-Madison, Madison, WI; 4) Wisconsin State Laboratory of Hygiene, University of Wisconsin-Madison, Madison, WI; 5) Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI.

The *MECP2* gene codes for Methyl-CpG binding protein 2. Mutations in the gene are responsible for Rett syndrome (RTT) and other neurological disorders. Thus far more than 700 mutations/variations in the *MECP2* gene have been documented in affected individuals. However, the prevalence of the mutations/variations in general populations is unknown. We analyzed *MECP2* coding sequence changes in 1,000 randomly selected newborn infants and found that 35 infants (3.5%) carried 24 sequence variations, of which 14 were previously reported in patients and 10 were apparently new. This was the first observed distribution of *MECP2* mutations/variations in a general newborn population. The findings suggest that frequent coding sequence variations might be a genomic feature of the *MECP2* gene, which poses a potential challenge for clinical screening and testing of pathogenic mutations of the gene. The pathogenic significance of *MECP2* sequence variations without population studies needs to be carefully evaluated.

## 578/W/Poster Board #236

**Extensive population-based variation in drug metabolism is revealed by analyzing Hapmap DNA with the Affymetrix DMET Plus microarray.** *E. Mansfield[1], M. Sedova[2], C. Bruckner[1], R. Sapolsky[2], G. Mei[3], P. Dickinson[4], R. Wheeler[3], J. Burrill[1], C. Davies[3], S. Cawley[3], M. Shapero[2].* 1) Application Sciences Dept., Affymetrix, Santa Clara, CA; 2) Assay & Application Product Development Dept., Affymetrix, Santa Clara, CA; 3) Informatics Dept., Affymetrix, Santa Clara, CA; 4) Data Analysis Dept., Affymetrix, Santa Clara, CA.

**Background:** The DMET Plus assay is a comprehensive drug metabolism microarray capable of simultaneously genotyping ~2,000 common and complex functional variants in 225 drug-metabolizing enzymes, transcription regulators, and transporter genes. These genes have evolved as the major human hepatic detoxification system for processing environmental toxins and xenobiotics and show extensive genetic variation. Interpretation of the genetic profiles generated by the array is greatly aided by reports translating genotype data into well-recognized haplotype-based allele calls. **Methods:** Over 1,200 distinct DNA samples were analyzed, including 713 samples from the Hapmap extended-diversity panel. Genomic DNA extracted from blood samples or commercially available cell lines were used. Data was analyzed using Affymetrix' DMET Console software, followed by principal component analysis (PCA) and 2-class association analysis using HelixTree (Golden Helix, Bozeman, MT). **Results:** A 99.92% concordance between SNP6.0 and DMET Plus arrays was observed across the Hapmap samples at 219 overlapping SNPs. Analysis results and reports focused on the metabolic pathways of drugs of interest such as Tamoxifen, Warfarin, and Plavix will be used to illustrate the versatility of the array's analysis tools. The DMET Console software generates fully-annotated marker reports that include commonly recognized, haplotype-based allele names (e.g CYP2D6*7, NAT2*5F, GSTP1*C, etc.). Evidence-based reporting of variants with known phenotypic or structural alterations is documented in the translation summary. Star allele frequency distribution across the major populations in genes such as ABCG2, CYP2C19, CYP4F2, SLCO1B1, TPMT, and VKORC1 agree with published sources and are compiled in formats amenable to downstream genetic analysis. PCA reveals complete segregation of Asian, Caucasian and African samples by population of origin and stratification within these groups is evident when 7 Hapmap populations are compared. Among the ADME genes represented on the DMET Plus microarray, CYP3A4, PPARG, and VKORC1 show the greatest allele frequency differences worldwide. These 3 genes are the most abundantly expressed hepatic cytochrome P450, a transcription regulator, and the drug target for the blood thinner Warfarin respectively. **Conclusion:** The DMET Plus microarray and analysis tools facilitate highly-multiplexed genotyping of functional variants across full pharmacokinetic pathways.

## 579/W/Poster Board #237

**Medaka (*Oryzias latipes*) as a model to study human polymorphic functions.** *T. Katsumura, S. Oda, H. Mitani, S. Kawamura, H. Oota.* Dept Integrated Biosci, University of Tokyo, Kashiwa, Chiba, Japan.

Since the HapMap project, a lot of single nucleotide polymorphisms (SNPs) have been identified in human genome. Among the SNPs, we find highly differentiated non-synonymous SNPs among populations, which can be explained rarely by natural selection related to adaptation for each habitat. It is important to reveal a functional difference between distinct alleles. However, experiments using humans are almost impossible technically and ethically. We propose here the utility of the medaka fish (*Oryzias latipes*) as a model system for human polymorphisms. Medaka has a long time history of an experimental animal: inbred strains have been established, many lab-stocks from wild have been maintained, and molecular biological techniques are developed. Most importantly, genetic variations of medaka and its close relatives are known to be high.

To establish an efficient screening method, we first looked into genetic stratification of medaka. We examined the mitochondrial (mt) DNA diversity in the deme- and the grid-based sampling methods: the former was the sampling from local-wild schools in Kanto (the eastern part of Japan), while the later was the sampling from lab-stocks collected from various geographical regions. The population genetic statistics showed that the grid-based sampling provided larger polymorphisms than the deme-based one. Next, we focused on *cytochrome P450* (*CYP*) gene family because it is well known that each gene has highly polymorphic in humans and this gene family can be related in adaptation to circumstances. We found 20 *CYP* genes showing apparent orthology between medaka and human, and chose four *CYP* genes (*CYP1A*, *CYP1B1*, *CYP5A1*, and *CYP20A1*) harboring non-synonymous SNPs with different allele frequencies among human populations. We conducted SNP screenings for the medaka *CYP* genes and found non-synonymous SNPs at the same site or the vicinity in medaka genome where human *CYP* orthologs have SNPs. To assay functional difference among the *CYP* alleles, we made them express in a cell culture using the GAL4/UAS system and measured their enzymatic activities using a known substrate in human CYP.

This is first trial of comparative population genomics for CYP functions between medaka and humans, and opens up a new window to analyze the functional difference among human SNPs using medaka.

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## 580/W/Poster Board #238

**Joint Haplotype Structure of the Tumor Necrosis Factor and HLA Class I Blocks in European Americans: Implications for Disease Association Studies.** *B. Aissani[1], K. Zhang[2], R. Malhotra[1], K.M. Ogwaro[11], J. Tang[3], S. Shrestha[1], O. Martinez-Maza[4,5], L.P. Jacobson[6], R. Detels[7], J.P. Phair[8], J.J. Martinson[9], J. Margolick[10], R.A. Kaslow[1,3].* 1) Department of Epidemiology, University of Alabama at Birmingham. Birmingham, AL; 2) Department of Biostatistics, University of Alabama at Birmingham. Birmingham, AL; 3) Department of Medicine, University of Alabama at Birmingham. Birmingham, AL; 4) Department of Obstetrics and Gynecology. University of California at Los Angeles. Los Angeles, CA; 5) Department of Microbiology, Immunology and Molecular Genetics, University of California at Los Angeles. Los Angeles, CA; 6) Department of Epidemiology, Johns Hopkins University. Baltimore, MD; 7) Department of Epidemiology, University of California at Los Angeles. Los Angeles, CA; 8) Division of Infectious Diseases, Northwestern University. Chicago, IL; 9) Department of Infectious Diseases and Microbiology, University of Pittsburgh. Pittsburgh, PA; 10) Department of Molecular Microbiology and Immunology, Johns Hopkins University. Baltimore, MD; 11) Department of Psychiatry, University of Alabama at Birmingham. Birmingham, AL.

Within the human major histocompatibility complex (MHC), the tumor necrosis factor (TNF) block including the LTA +252 (A>G) and TNF -308 (G>A) polymorphisms, henceforth G-A haplotype, has been implicated in the susceptibility to numerous immune conditions. However, the high gene density and the complex pattern of linkage disequilibrium (LD), independent of the intermarker distance, have hampered the precise identification of disease genes in this region. To better understand the haplotypic structure of central MHC, we have examined the association between *HLA-B* and the TNF block haplotypes. **Design & Methods:** We analyzed data on 656 non-Hispanic European American men from the Multicenter AIDS Cohort Study (MACS) and 92 unrelated subjects from the CEU population of the international HapMap project. A set of 47 single nucleotide polymorphisms (SNP) located in a 70 Kb-long region spanning the TNF gene cluster (*LTA*, *TNF* and *LTB*) and flanking genes (*MMCD1*, *BAT1*, *ATP6VIG2*, *NFKBIL1* and *LST1*) were typed in the MACS sample. HLA-B alleles were identified to four-digit resolution by reference-strand conformation polymorphism and DNA sequencing-based typing methods. The HAPLORE algorithm was used for the statistical inference of SNP-HLA-B haplotypes. **Results:** Of a total of 337 haplotypes inferred in the MACS sample, 23 unique haplotypes occurred at a frequency > 1% and accounted for approximately 61% of all haplotypes. As expected due to the high level of polymorphism at *HLA-B*, most of these common TNF block haplotypes were split into HLA-B-specific sub-haplotypes. The LTA-TNF G-A haplotype is carried on two major ancestral HLA-B haplotypes: the immune disease-prone HLA-B*08 (AH 8.1) and the less extensively conserved B*4402 (AH 44.2). Our haplotype data are consistent with the reconstructed haplotypes in the CEU reference population, although only 15 common haplotypes were found in this small population. **Conclusion:** Our study in the MACS is among the largest to examine the TNF block and HLA-B haplotypes jointly. Of relevance to disease association with central MHC genes, we identified a sub-haplotype of the B*4402 (AH 44.2) lineage carrying the LTA-TNF G-A haplotype. Because AH 44.2 shares only a limited portion of sequence with the more extended haplotype AH 8.1, if both haplotypes can be unequivocally associated with the diseases previously associated only with AH 8.1, it would greatly reduce the extent of the candidate region(s)requiring more intensive inquiry.

## 581/W/Poster Board #239

**Estimating Population Genetic Parameters and Comparing Model Goodness-of-fit Using DNA Sequences with Error.** *X. Liu, Y.-X. Fu, T.J. Maxwell, E. Boerwinkle.* Human Genetics Center, School of Public Health, The University of Texas Health Science Center at Houston, Houston, TX.

Sequencing error can bias estimation of evolutionary or population genetic parameters from deep re-sequencing studies. Sequencing errors are even more of a problem when using so-called next generation sequencing methods or when sequencing large sample sizes. To overcome this problem, we propose a new method based on the composite likelihood of SNP configurations to simultaneously infer the population mutation rate $\theta=4N_e\mu$, population exponential growth rate $R$ and error rate $\varepsilon$. Using simulation, we demonstrate the interactive effects of various parameters, such as $\theta$, $n$, $\varepsilon$ and $R$ on the accuracy of the estimators. We compared our maximum composite likelihood estimator (MCLE) of $\theta$ with other $\theta$ estimators that take account for error. The results show that the MCLE performs well when the sample size is large or the error rate is high. Using parametric bootstrap, composite likelihood can also be used as a statistic for testing the model goodness-of-fit of the observed DNA sequences.

We applied our method to published exon data from ANGPTL4 in 1,832 African American and 1,045 European American individuals. Previous analysis showed a significant excess of rare alleles assuming a constant population size and no sequencing error. To test the robustness of this conclusion under an error model, we estimated that per base-pair MCLE($\theta$)=0.0017 and MCLE($\varepsilon$)=$3\times10^{-6}$ for African Americans, and MCLE($\theta$)=0.0011 and MCLE($\varepsilon$)=$4\times10^{-6}$ for European Americans. Using the estimated $\theta$ and $\varepsilon$, we simulated the null distribution of two neutrality test statistics (Achaz's Y and Y*). By comparing the results of the two tests using the original sequence data, we confirmed the conclusion of a significant excess of rare alleles in both African Americans and European Americans. Using the fact that the rare nonsynonymous changes were verified experimentally in these data, singletons should have a lower error rate than other SNPs. Because this information is not incorporated in our model, our MCLE($\varepsilon$) should be an upper limit of $\varepsilon$. Even with an error model with overestimated $\varepsilon$, Y and Y* rejected the null hypothesis of no excess rare alleles, which suggests that sequencing error cannot explain the excess of rare alleles. This leaves us alternative explanations that include purifying selection, population expansion, or mixed effects of multiple acting factors mentioned above.

## 582/W/Poster Board #240

**THE PREVALENCES OF INHERITED THROMBOPHILIC RISK FACTORS IN TURKISH PATIENTS EXPERIENCING RECURRENT MISCARRIAGE, INFERTILITY AND VENOUS THROMBOSIS : FACTOR V LEIDEN, PROTHROMBIN G20210A, AND METHYLENETETRAHYDRO FOLATE REDUCTASE C677T.** *B. Ozturk[1], H. Comert[2], B. Yazar[2], O. Ilbay[2], L. Erkan[2], N. Ercelen[2].* 1) Clinical Genetics/Pediatrics, ECU Brody Sch Med, Greenville, NC; 2) Genetics & Genomic Sciences Center, American Hospital, Istanbul, TURKEY.

Introduction: Inherited thrombophilia has has been associated with recurrent pregnancy loss, infertility and venous thrombosis. The aim of this retrospective study is to evaluate the prevelance of inherited thrombophilic risk factors in Turkish patients experiencing recurrent miscarriage, infertility and venous thrombosis. Materials and Methods: A total of 596 patients who referred to American Hospital, Genetics and Genomic Sciences center were included in this study. All of the patient group subjects had a history of recurrent miscarriage, infertility and/or venous thrombosis. Informed consent was obtained from all subjects. DNA samples were isolated from peripheric blood samples drawn from all the participants. 596 patients were analyzed for Factor V Leiden, 567 patients were analyzed for Prothrombin G20210A and 558 patients were analyzed for Methylenetetrahydro-folate Reductase C677T. Results: The prevalence of FVL, Prothrombin G2010A and MTHFR C677T mutations were detected for Turkish recurrent miscarriage, infertility and venous thrombosis patients. The prevalences of inherited thrombophilic risk factors were found for our patient group: FVL (26.84%), prothrombin G20210A (7.93%) and MTHFR C677T (55.55%). Conclusions: In the litearature prevelances of FVL1, Prothrombin G20210A2 and MTHFR C677T2 were reported as 7.9%, 2.74% and 40.56% respectively in healthy Turkish population. As a conclusion high prevalences of FVL, prothrombin G20210A and MTHFR C677T mutations in our patient group suggest that these inherited thrombophilic risk factors should be considered in patients with a history of recurrent miscarriage, infertility and/or venous thrombosis. References:
1) Akar N. Factor V 1691 G-A mutation distribution in a healthy Turkish population. Turk J Hematol 2009; 26:9-11. 2) Çeliker G., Can U., Verdi H., Yazici C., Ozbek N., Atac B. Prevalence of Thrombophilic Mutations and ACE I/D Polymorphism in Turkish Ischemic Stroke Patients. Clin Appl Thromb Hemost 2008; April.

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## 583/W/Poster Board #241

**ASSOCIATION Helicobacter pylori and mtDNA-A IN A SAMPLE OF MEXICAN NAHUA POPULATION.** *R. Penaloza[1], P. Negrete[1], U. Galicia[1], G. Gonzalez[2], J. Torres[2], L. Buentello[3], K. Sandoval[4], D. Comas[4], F. Salamanca[1].* 1) UIM Gen Humana, Inst Mexicano del Seguro Soc, Mexico City, Mexico; 2) UIM Enfermedades Infecciosas, Inst Mex Seg Social, Mexico City, Mexico; 3) Inst. Inv. Antropologicas, UNAM Mexico City. Mexico; 4) Depto. Biol. Molecular, Univ. Pompeu Fabra, Barcelona, Spain.

Background: Helicobacter pylori (Hp) infects more than 50% of the world's population, infection can persist in the human stomach and induce chronic gastritis for the lifetime, increasing the risk for developing peptic ulceration, noncardia gastric adenocarcinoma and gastric lymphoma; it is considered by WHO as a carcinogenic type I and there are some reports showing relatedness with susceptibility and some markers including mutations in mitochondrial DNA (mtDNA). Aim: To assess a possible association of mtDNA haplotypes and Hp infection in a sample of Mexican Nahua population. Material and Methods: .Twenty two Mexican Nahua individuals from Atocpan, Milpa Alta with previous informed consent were studied. The urea breathe test (UBT) and the string test were applied for diagnosis and isolation of H pylori. The string was inoculated immediately in blood agar plates with antimicrobials, packed in Jars with campy pack CO2 generators (Beckton Dikinson Co. USA). DNA was extracted of four ml of peripheral blood by standard method. HVI from D-loop was amplified by PCR and sequenced directly with product previously labeled by BigDye kit (Applied Biosystems, USA). Results were compared with Andrews et al. (1999) sequence corrected. Results and Conclusions: H pylori CagA was isolated from thirteen samples. Eight samples have mtDNA haplogroup A2, two haplogroup C1 and three D1, suggesting an association between H. pylori and mtDNA-A, haplogroup highly frequent in some Mexican Indigenous and Noth Amerindian populations.

## 584/W/Poster Board #242

**What should we call a microsatellite: A case study of human dinucleotides.** *K. Makova[1], Y. Kelkar[1], N. Candiello[2], S. Hile[2], F. Chiaromonte[1], K. Eckert[2].* 1) Center for Comparative Genomics and Bioinformatics, Penn State University, University Park, PA; 2) Department of Pathology, Gittlen Cancer Research Foundation, Penn State University College of Medicine, Hershey, PA.

Microsatellite sequences are abundant in the human genome and have mutation rates orders of magnitude higher than any other genomic sequences. Microsatellite mutations cause over 40 neurological diseases, implicated in several types of cancer, and allele-length polymorphisms at microsatellites are implicated as genetic risk factors in several other diseases. Microsatellites are also frequently used as markers in forensics and population genetics. However, there is a disagreement about what we should call a microsatellite. In this interdisciplinary project, to determine the threshold value at which a repetitive motif becomes a microsatellite, we utilized two approaches - a biochemical one and a computational (population genomic) one. The threshold was defined as the number of repeats for which the insertion-deletion (indel) frequency is higher than the average indel frequency in genomic DNA. In the biochemical approach, we analyzed the in vitro replication slippage error rates of DNA polymerase (Pol Beta) at repetitive sequences of different repeat numbers as well as at control sites. We discovered that GT repeats with 5-7 units acquire rates higher than these of control sites. In the computational approach, we studied microsatellite indel polymorphism in ten ENCODE regions, using the HapMap-II resequencing data for 48 humans. To find the minimal size of a microsatellite, we investigated the relationship between the proportion of polymorphic repetitive loci, and the size of the smallest repetitive allele. Similar procedure was followed for control sites. We found that repetitive loci with the smallest allele size of 4-5 repeats were rarely polymorphic. In contrast, ~20% of loci with 6-7 units were polymorphic, this proportion was higher than at control sites. Therefore, the results of the two approaches are in remarkable agreement and point towards the threshold of 5-7 units for GT microsatellites in humans. Thus, measuring the proportion of polymorphic loci depending on repeat number is an appropriate method to study the dynamic microsatellite mutational activity. In this context, we also characterized the mutational processes responsible for pushing a DNA sequence beyond the microsatellite size threshold. Additionally, we found that the microsatellite threshold size depends on a repeated motif.

## 585/W/Poster Board #243

**The heritability of endometriosis in a Utah population.** *P. Farrington, K. Ward, H. Albertsen, G. Frech, T. Maness, S. Dintelman, L. Wong.* Juneau Biosciences, LLC, Salt Lake City, UT.

Purpose: Endometriosis is a complex condition of uncertain etiology in which endometrial glands, stroma, and/or tissue normally confined to the inner lining of the uterus is found outside the uterus, most often in the pelvic area. Endometriosis is also characterized by inflammation, cysts, and scarring at affected sites, as well as by pelvic pain, dysmenorrhea, and infertility. Endometriosis affects up to 10% of reproductive-age women. The present study utilizes a population-based genealogical database and a list of women diagnosed with endometriosis to estimate its heritability. The present study seeks to extend the heritability study of the Icelandic population by Stefansson et al. (Hum Reprod 17, 555-9, 2002), with a different and more diverse population from the U.S. Intermountain West. Methods: Women who had endometriosis surgically confirmed by laparoscopy or laparotomy were ascertained for this study. All participants had their endometriosis-related medical records reviewed by a research Ob-Gyn physician to confirm the diagnosis. An extensive genealogy database (GenDB) was constructed using more than 15,000 genealogy sources in the public domain (birth, death, and marriage records from prior to 1930). This database was searched for the names, birth dates, and birthplaces of the four grandparents of the probands. Heritability-related parameters were determined for the probands' grandparents and compared to randomly chosen sets of control groups whose birth years and connectivity in GenDB closely matched the patient group. Summary of Results: Results indicate that in our population of mostly Utah individuals, in agreement with a previous study from the Icelandic population, patients with endometriosis are more closely related than matched controls, close family members have an increased relative risk for the disease, and the minimum number of founders required to account for the cases is smaller than for the controls. A preliminary analysis using 165 cases, excluding contributions from affected first-degree relatives and allowing for up to six generations of ancestors, revealed a significantly higher ($p<0.001$) average kinship coefficient (KC) of $2.07 \times 10^{-4}$ compared to matched control sets ($0.58 \pm 0.07 \times 10^{-4}$). When contributions from affected first- and second-degree relatives were omitted, the average KC was $1.04 \times 10^{-4}$ (155 cases) versus $0.49 \pm 0.07 \times 10^{-4}$ ($p<0.001$). Conclusion: A genetic component contributes to endometriosis.

## 586/W/Poster Board #244

**Fast and Accurate Inference of Long-Range Haplotypes for Large Cohorts of Unrelated Individuals.** *P.F. Palamara, A. Gusev, I. Pe'er.* Columbia University, New York, NY.

Current technologies allow the sampling of large cohorts of individuals using high-density SNP arrays. Unfortunately, genotype data obfuscates the phase of the sampled individuals, which is relevant in several applications such as disease-gene association studies and genealogy reconstruction. This technological deficiency is currently remedied by either trio data or computational phasing methods, that can be unreliable over long regions where recombination events are likely to occur. A recently introduced implementation relying on remote genetic relatives as surrogate parents has shown the great benefits of long-range phasing, although it has only been demonstrated for localized regions and would be computationally infeasible on a genomewide, population-wide scale. In this work we report methodology and software for haplotype inference in large cohorts of unrelated individuals genomewide. The method relies on GERMLINE, our recent efficient algorithm for the detection of segments identical-by-descent (IBD) between purported unrelateds. Compared to the currently used methods for the detection of IBD segments, GERMLINE allows a dramatic increase in speed and sensitivity, traded for limited false positives. We use shared segments identified in pairs of individuals to resolve ambiguous phase. We utilize information beyond IBD pairs, identifying subsets of haplotype carriers, thus propagating phase information within these sets. The proposed methodology is evaluated on real datasets for two large populations that present different levels of IBD sharing. Through results obtained on a variety of large sets of synthetic data we outline the dependency of our method's performance on two main parameters, namely the sample size and the expected chance of IBD sharing in the studied population. We finally show how long-range haplotype inference can be used to decrease the false-positive rate of the GERMLINE algorithm, exploiting the interdependency between phasing accuracy and specificity. Propagating the information derived from incorrect IBD segments, in fact, leads to detection of inconsistencies, which can be used to improve the detection of IBD.

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## 587/W/Poster Board #245

**The perfect phylogeny map of human genome and its applications.** *G. Zhang[1,2], R. Chakarborty[2], S. Xu[3], L. Jin[2,3].* 1) Department of Family Medicine, University of Cincinnati, OH; 2) Center for Genome Information, Department of Environmental Health, University of Cincinnati, OH; 3) 3. CAS-MPG Partner Institute of Computational Biology, SIBS, CAS, Shanghai.

Genealogies of human genetic variations will assist the understanding of how various forces (i.e. mutation, selection, recombination and demographic history) have shaped the extant patterns of our current genome. However, reconstructing the full history of a set of genetic variants is close to impossible due to the complexities introduced by recombination events. To overcome this difficulty, we introduced the concept of perfect phylogeny block of SNP markers. In contrast to commonly used block definitions based on linkage disequilibrium or haplotype block deversity, the proposed perfect phylogeny blocks can be physically overlapped and the mutation history of each variant within the block is well represented by a perfect phylogenic tree. Following this definition we have developed an efficient computational algorithm to find all perfect phylogeny blocks by using genotype data of high-density SNP markers. By applying our method to the recent released HapMap III data set, we constructed a high resolution perfect phylogeny map of human genome (http://tgx.uc.edu/ppb) and reconstructed the genealogy of each individual block. This map has two direct practical applications. First, the perfect phylogeny structure is more informative in identifying targets of natural selection than the traditional methods based on summary statistics on individual loci or haplotype diversity. Second, the perfect phylogeny block structure can facilitate robust inference of local recombination rates. Accordingly, we developed several methods that can interrogate the genealogy information of perfect phylogeny blocks for detecting signals of positive selection and estimation of local recombination rate. By comparing with other methods, we demonstrated that by integrating the genealogy information and by summarizing evidences from multiple overlapped blocks, these perfect phylogeny block based methods are able to detect positive selection signals with higher confidence and can accurately quantify fine-scale recombination rate.

## 588/W/Poster Board #246

**The architecture of long-range haplotypes shared across populations.** *A. Gusev[1], P.F. Palamara[1], A. Darvasi[2], P. Gregersen[3], I. Pe'er[1,4].* 1) Computer Sci, Columbia Univ, New York, NY; 2) Department of Genetics, Hebrew University, Jerusalem, Israel; 3) Long Island Jewish Medical Center, North Shore, NY; 4) Center for Computational Biology and Bioinformatics, New York, NY.

The availability of cost-effective, high throughput technologies to genotype common alleles has yielded an unprecedented wealth of genomewide data on human variation, deeply sampled within and across populations. Examining thousands of individuals now facilitates extensive evaluation of shared genetic segments across millions of sample-pairs. Analysis of such sharing has the potential of revealing recent genetic history of samples, both genomewide as well as for specific loci. In this work, we extend a method for efficiently identifying co-inherited shared segments in a large population of unphased genotype data. We apply this method to data from multiple populations and demonstrate the surprisingly pervasive amount of sharing within and between populations. The bulk of this sharing yields insights into population history and substructure. In particular, we use this sharing in a bottom-up clustering approach to differentiate between the varying populations of the International HapMap Project. Exploring the distribution of segment sharing in a more dense population of Ashkenazi Jews, we are able to make inferences into the effective population size and structure near the present. Looking at long inter-population sharing, likely indicative of positive selection, we expose hotspots of commonly shared haplotypes and report their unique functional and structural significance. Specifically, we found 16 commonly shared regions that span only 0.92% of the genome and account for 18% of sharing between continents. We establish significant overlap between these regions and long structural variants (p=0.00052 in long variants), suggesting functional importance. Our results have implications in both population genetics and genome structure; illuminating the history of heritable variation from a fresh angle focused on modern times; and discovering functional connections between the patterns of long range haplotype sharing and structural variants, an unexpected result pointing to haplotype-sensitive recombination rates.

## 589/W/Poster Board #247

**Variable gene copy number determination of *CCL3L1* related gene family cluster.** *M. Nyaku, S. Shrestha.* Department of Epidemiology, School of Public Health, University of Alabama at Birmingham, Birmingham, Alabama.

Copy number variations (CNVs) of the gene CC chemokine ligand 3-like1 (*CCL3L1*) have been implicated in several disease outcomes, specifically HIV-1 susceptibility. *CCL3L1* is a nonallelic isoform of CC chemokine ligand (*CCL3*) and a potent ligand for CC chemokine receptor 5 (CCR5), the major coreceptor for HIV. In comparison to other CCR5 chemokine ligands, *CCL3L1* exhibits the most superior suppressive characteristics against HIV-1. Studies have shown that individuals with *CCL3L1* copies greater than their ethnic population medians are less susceptible to HIV-1 infection and progression to AIDS. Several CNV assays have been developed for the *CCL3L1* gene. *CCL3L1* however, shares very significant homology with a cluster of genes localized to chromosome 17q12, namely chemokine (C-C motif) ligand 3 (*CCL3*), CC chemokine ligand 3-like 2 (*CCL3L2*), and CC chemokine ligand 3-like 3 (*CCL3L3*) Through pairwise and multiple alignments of these genes, we have shown that the homology between these genes ranges from 50% to 99% in complete gene sequences and above 70% in the exonic regions with *CCL3L1* and CCL3L3 being very identical. We have developed an algorithm that shows that previously utilized RT-PCR based CNV assays for *CCL3L1* are indeed not specific to *CCL3L1*. We aligned sense primers, anti-sense primers and probes used in seven previously described assays against pre-multiple alignments of all 4 chemokine genes. Each set of probe and primers aligned without a nucleotide mismatch with at least 2 out of the 4 genes. We have now developed gene specific CNV assays after carefully accounting for the high homology regions between these regions. Some of the inconsistencies in the association studies could be due to heterogeneity in these assays that are not specific for a single *CCL3L1* gene but rather non-specific combinations of the 4 genes in this genomic cluster. The applications of these new assays can be extended to ongoing *CCL3L1* CNV association studies in other human disease pathogenesis including psoriasis, Crohn's disease, rheumatoid arthritis, systemic lupus, type 1 diabetes and Kawasaki disease.

## 590/W/Poster Board #248

**Admixture between Ashkenazi Jews and Central Europeans.** *W. Klitz[1,2], L. Gragert[3], M. Maiers[3], M. Fernandez-Viña[4], Y. Ben-Naeh[5], G. Benedek[6], C. Brautbar[6,7], S. Israel[7].* 1) Pub Hlth, Univ California, Berkeley, CA; 2) Public Health Institute, Oakland, CA; 3) National Marrow Donor Program, Minneapolis, MN; 4) University of Texas, MD Anderson Cancer Center, Houston, TX; 5) Hebrew University, Jerusalem, Israel; 6) Hebrew University - Hadassah Medical School, Jerusalem, Israel; 7) Hadassah University Hospital, Jerusalem, Israel.

When distinct populations inhabit the same geographic space, culture often acts to restrict random mating in our species, while at the same preventing complete genetic privacy. The residency across Central Europe by the Ashkenazi Jews over the last thousand years is such a case. HLA typing from bone marrow donor registries in Israel, Poland and Germany were utilized to measure admixture between central European host populations and Ashkenazim. Inferred high resolution HLA A-B-DRB1 haplotype frequencies were generated from each population. A total of 1,676 Polish-origin-Ashkenazim and 13,556 Polish haplotypes were analyzed, along with a similar sample of ~5 million German haplotypes. The informativeness of HLA haplotypes is shown by the A-B-DRB1 haplotype 0101-0801-0301, the most common haplotype found in northern Europe. HLA B*0801 bearing haplotypes are present in the Near East, but those B*0801 haplotypes carry the HLA C allele Cw*0702 instead of the Cw*0701 found in 0101-0801-0301. The 100 most common haplotypes constituted 53% of the total Ashkenazi, and 45% of the Polish, and 43% of the German samples, reflecting the sizeable total fraction of very rare haplotypes familiar in population samples of the diverse HLA system. The most common Ashkenazi haplotype had a frequency of 6.14% (n = 102.9) and the 100th haplotype was present at 0.29% (n = 4.86). Comparable values for the Polish sample were 5.83% (n = 790.3) and 0.13% (n = 17.6), respectively. Haplotypes from one population compared to those haplotypes in a second could be classified into three categories: less frequent, statistically identical or more frequent. In the graph of the ordered 100 Polish haplotypes, the less frequent Ashkenazi haplotypes supply a possible signature of admixture from the Poles into the Polish Ashkenazim, while the haplotypes more frequent in Ashkenazim than Poles are candidates for movement of genes from the Ashkenazim to the Poles. The averaged frequency differences between these categories give an indication of population admixture. The analysis showed that 1.8% of Polish haplotypes may be of Ashkenazi origin and 0.6% of Ashkenazi of Polish origin. The sample from Germany, in which the initial generations of Polish-Ashkenazi history was spent, was useful in demonstrating consistency of haplotype frequencies by rank order. The results show clear evidence of admixture occurring in both directions between two largely HLA-distinct populations.

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## 591/W/Poster Board #249

**An Effective and Cost-Efficient Approach to High Throughput Analysis of Population Stratification.** *E.R. Londin[1], L.A. Mamounas[2], R. Zhang[2], L. Gutmann[2], R.A. Corriveau[1].* 1) Coriell Institute Medical Research, Camden, NJ; 2) National Institute for Neurological Disorders and Stroke, Bethesda, MD.

The NINDS Repository was established in 2002 to accelerate discovery of genetic risk factors for neurological disorders by providing a centralized and open resource of biomaterials, genotype and phenotype data, and collaborative support to researchers. Genetic ancestry is an important variable that impacts discovery of genetic risk for heritable disease, as well as susceptibility to disease itself. Self-reported ancestry has been tracked for individual subjects since inception of the Repository. However, considerable evidence indicates that self-reported ancestry and genetic ancestry are not equivalent for populations with admixture, as is the case in the United States and Canada, where most of the 26,000 NINDS Repository samples were collected. Moreover, self-reported indications of Hispanic and non-Hispanic generally reflect social constructs rather than genetic ancestry. Therefore, we have set out to develop a systematic approach for efficient and cost-effective determination of genetic ancestry applicable to high throughput collections such as the NINDS Repository. This will help ensure that genetic ancestry and population stratification does not confound the mission of genetic discovery. For preliminary studies we have used two sources of samples and data: (i) publicly available microsatellite (MSAT) data for HGDP-CEPH ancestry standards consisting of 1064 samples from 51 worldwide populations genotyped with 431 MSATs. (ii) Coriell-generated data representing a panel of 19 MSAT markers (ABI Identifiler panel plus four additional MSAT markers) genotyped on 8000 NINDS samples. Preliminary results obtained using both STUCTURE and principle component analysis (PCA) suggest that while a relatively small number of MSATs can provide adequate data for ancestry analyses, the panel of 19 MSATs currently used for NINDS Repository samples needs to be modified. We plan to test additional MSAT markers, chosen based on F-statistic (Fst) values, together with our original panel of 19 markers, on both NINDS samples and HGDP-CEPH standards to optimize the approach.

## 592/W/Poster Board #250

**The genetic structure of Native Americans: inferences from SNPs in genes involved in carcinogenesis, immunity and pharmacogenetics.** *E. Tarazona-Santos[1,2], G.B. Soares-Souza[1], J. Chevitarese[1], R.H. Gilman[4,5], M. Yeager[6], S.J. Chanock[2,3].* 1) Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brazil; 2) Section of Genomic Variation. Pediatric Oncology Branch. National Cancer Institute. National Institutes of Health. MD. US; 3) Laboratory of Translational Genomics. National Cancer Institute. National Institutes of Health. MD. US; 4) Asociación Benéfica PRISMA. Lima, Peru; 5) Bloomberg School of Public Health, Johns Hopkins University, MD, US; 6) Core Genotyping Facility. National Cancer Institute. National Institutes of Health. MD. US.

We have studied the genetic structure of South Amerindian populations in the context of worldwide diversity. We genotyped 1442 SNPs located on 400 genes involved in cancer, immunity and pharmacogenetics in the following 67 Western South Amerindians: (a) 22 Quechua highlanders from the Peruvian Andes; (b) 17 Quechua from San Martin, (c) 21 Matsiguengas from the Monte Carmelo community and (d) 7 Cayapas from Ecuador. Samples b-d are settled in the geographic region located between the Andean mountains (West) and the Amazon region (East). We also genotyped the Human Genome Diversity Project panel that include the following South Amerindians: 24 Karitiana, 21 Surui, 13 Piapoco-Curripaco, 25 Pima and 25 Maya. The following results emerged from SNPs and haplotype analyses: (1) Most of the loci fit the Hardy-Weinberg equilibrium in most populations. With the exception of the San Martin population (Fis = 0.06), none of the Western South Amerindian populations studied by us evidenced high levels of inbreeding or Wahlund effect. (2) Consistently with previous studies, South Amerindians and Oceanian populations show the lowest intra-population diversity when compared with autochthonous populations from other continents. (3) Within South America, Western South Amerindian populations show the highest intra-population diversity, consistently with an evolutionary model previously proposed by us, which suggest a higher long term effective population size and levels of gene flow among them. (4) Comparing the different continents, the between-population diversity is clearly the highest for South Amerindians (Fst = 0.19). This value is almost twice the level of differentiation observed for the worldwide population (Fst = 0.11). (5) We identified genes (such as COMT and CYPA1A, important in pharmacogenetics), with a high level of differentiation in Native Americans respect to other continents. Identifying SNPs with these characteristics is important because markers with large differences in allele frequencies among continental groups may produce false positive results in epidemiological studies involving complex traits with different incidences among continental groups. Our results provide important information about the genomic structure of South Amerindians, an underrepresented group in population genetic studies, in particular for genes involved in carcinogenesis, immunity and pharmacogenetics. Financial Support: NCI/NIH, Brazilian agencies CAPES and CNPq.

## 593/W/Poster Board #251

**Deep population structure in sub-Saharan African populations.** *J. Wall[1], M.P. Cox[2], F.L. Mendez[3], A.E. Woerner[2], M.F. Hammer[2,3].* 1) Institute for Human Genetics and Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA; 2) ARL Division of Biotechnology, University of Arizona, Tucson, AZ; 3) Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ.

We analyzed ~500 Kb of resequencing data from 91 different intergenic regions in samples from three sub-Saharan African populations: Mandenka from Senegal, Biaka pygmies from the Central African Republic and San from Namibia. We employed novel methodology to estimate the split times and migration rates between populations. We found strong evidence for split times that predate the exodus of modern humans out of Africa (e.g., > 100 Kya). In addition, we also found evidence of ancient admixture (with unknown 'archaic' human groups) in the recent history of both the Biaka and the San.

## 594/W/Poster Board #252

**Gorillas out of the mist: Unravelling the fog surrounding another genome.** *B. Yngvadottir, A. Scally, J. Simpson, Y. Gu, W. Whitener, A. Rogers, Z. Ning, J. Burton, M.A. Quail, H. Swerdlow, R. Durbin, C. Tyler-Smith, Gorilla Genome Consortium.* [The first two authors contributed equally to this work] Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

Gorillas are divided into two species based on their morphology and geographical location within Africa, eastern gorillas (*Gorilla beringei*) and western gorillas (*Gorilla gorilla*), each of which has two subspecies. Most field studies have been carried out on eastern gorillas, while genetic studies have been largely confined to western gorillas. The gorilla and human lineages diverged around eight million years ago and although humans are genetically more similar to chimpanzees over most of their genome, some regions are more similar to gorillas. A fuller picture of the gorilla genome is therefore expected to provide us with a better understanding of the differences between humans and chimpanzees by identifying which traits are uniquely human and which ones are unique to the chimpanzee as well as contributing to our understanding of gorilla biology. The genome of Kamilah, a female western lowland gorilla, has been sequenced with a coverage depth of 2x capillary and 35x Illumina, and an initial assembly has been created. While previous studies of the gorilla genome have relied on a limited number of loci, this assembly will enable us to perform a genomewide analysis at the inter-specific level and will be a useful resource for further biological and medical studies. In addition, we have sequenced to ~5x the genome of Mukisi, an eastern lowland gorilla, which will give us more information on genetic variation at the intra-specific level, within and between eastern and western gorillas. To this end, we are identifying different classes of variants including: 1) those shared by both gorilla species but no other closely related species 2) those that are not variable in Kamilah but are heterozygous in Mukisi and 3) vice versa. We intend to genotype these variants in a larger set of individuals from both species to get further insights into the genetic variation and evolutionary history of the species. This project will help us understand what makes gorillas unique as well providing us with a further understanding of our own species. For example, how different are humans and gorillas and what similarities do they share that exclude their chimpanzee cousin, how distinct are eastern and western gorillas, when did they differentiate, how much genetic variation is present within populations of each, how much gene flow has there been between these populations? We hope to answer these questions and begin unravelling the fog enveloping the gorilla genome.

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## 595/W/Poster Board #253

**Super Y-chromosomes in Eurasia and the impact of social selection and Neolithic transition.** *P.L. BALARESQUE[1,2], E. HEYER[2], M.A. JOBLING[1].* 1) Department of genetics, University of Leicester, Adrian Building, Leicester LE2 1WL, Leicestershire, United Kingdom; 2) Muséum National d'Histoire Naturelle - Centre National de la Recherche Scientifique-P7 Unité Mixte de Recherche 5145, Eco-Anthropologie, Musée de l'Homme, 75016 Paris, France.

Some Y-chromosomal haplotypes have been found at unusually high frequencies in Asian and European human populations. The massive spread of these lineages has been explained by the impact of social selection i.e. the high reproductive success of some males and their relative/descendants due to their high social status. The most well-known examples are the "Khan haplotype" and the "Manchou haplotype" in Asia, and the U'Neill haplotype in Ireland. But are these frequent haplotypes always associated with recent events of social selection, or could they be linked to much older processes? To address this question, we have surveyed ~ 3500 males in 97 populations from Turkey to Japan. We have focused on the 12 most frequently represented haplotypes in Eurasia and tested whether their expansions are linked to a specific factor such as language or subsistence methods. Our results show that both recent and ancient processes are responsible for the expansions of these lineages. The recent expansions (2000-3000 years) likely to be linked to social selection are prevalent in Altaic-speaking and pastoral populations. This might indicate a recent cultural change in the social organization of these populations. The ancient expansions (8000-10000 years) are over-represented in Indo-European speaking and sedentary farmer populations, and are likely to be the result of the Neolithic transition.

## 596/W/Poster Board #254

**Discovering Distant Relatives within a Diverse Set of Populations Using DNA Segments Identical by Descent.** *L. Hon, B.M. Henn, J.M. Macpherson, N. Eriksson, A. Wojcicki, L. Avey, S. Saxonov, J.L. Mountain.* 23andMe, Inc, Mountain View, CA.

Close familial relationships, such a parent-offspring relationships, are inferred readily from genotype and allele frequency data for a limited number of single nucleotide polymorphisms (SNPs) or short tandem repeat (STR) polymorphisms. However, researchers have not focused on accurately inferring the degree of relationship between individuals who share a common ancestor 3-10 generations ago; such inference requires high density genome-wide information not available until recently. In order to characterize relatively distant relationships for a sample, we analyzed the sharing of DNA identical by descent (IBD) in a large database of self-selected individuals of European ancestry, including a subset identified as Ashkenazim. Individuals from more endogamous ethnic populations, such as the Ashkenazim, share on average more DNA identically by descent than do individuals of Asian ancestry or of European ancestry broadly defined. Extensive population-level sharing of identical genomic segments complicates the prediction of relationship level for a pair of individuals since identical segments may reflect common ancestry older than the most recent ancestor for a pair of individuals in a pedigree. In order to understand the pattern of the observed population-level sharing, we simulated extended pedigrees using different populations to calculate the expected amounts of sharing for 1st through 10th cousins. Specifically we assessed the relationship between the length of the longest segment and the most recent common ancestor. From the simulations, we also determined bounds for predicted cousinships given a specific amount of segmental sharing. Using these bounds as a guide, we detected at least one distant relative, between 2nd-8th degree cousin, for 90% of individuals in our dataset of more than 5000 individuals with European ancestry. An even higher fraction of our Ashkenazi sample, 99%, had at least one distant relative between 2nd-7th degree cousinship in our dataset.

## 597/W/Poster Board #255

**Comparing spatial maps of population-genetic variation using Procrustes analysis.** *C. Wang[1], Z.A. Szpiech[1], N.A. Rosenberg[1,2].* 1) CCMB, University of Michigan , Ann Arbor, MI; 2) Department of Human Genetics, University of Michigan, Ann Arbor, MI.

Multivariate analysis techniques such as principal components analysis (PCA) and multidimensional scaling (MDS) are often used with population-genetic data used to produce "statistical maps" of sampled individuals or populations. With these techniques, each individual or population is represented as a point in a spatial coordinate system in such a manner that nearby placement of two points carries information about the similarity of the genotypes in the underlying individuals or populations. To quantitatively evaluate the relationship between pairs of maps generated in different ways from the same individuals, we have borrowed another multivariate analysis technique, namely Procrustes analysis. This approach separately transforms the maps, preserving relative distances among points within each map, to maximize a measure of the similarity of the placement of the points in one transformed map to the placement of the corresponding points in the other transformed map. We consider the Procrustes approach applied to two scenarios with human population-genetic data. In the first scenario, we compare positions on an MDS map constructed from individual SNP genotypes to a map of the geographic sampling locations of the individuals. In the second scenario, we compare positions on MDS maps constructed from different types of genetic markers studied in the same individuals. As statistical maps have become increasingly common as a tool for summarizing population relationships, our examples illustrate the potential of Procrustes-based quantitative comparisons for interpreting the results in these maps.

## 598/W/Poster Board #256

**European Population Genetic Substructure: Refining Ancestry Informative Markers for Distinguishing Among Diverse European Ethnic Groups.** *R. Kosoy[1], C. Tian[1], R. Nassir[1], A. Lee[2], P. Villoslada[3], L. Klareskog[4], L. Hammarstrom[5], H. Garchon[6], A.E. Pulver[7], M. Ransom[1], P.K. Gregersen[2], M.F. Seldin[1].* 1) Dept Biological Chemistry, Univ California, Davis, Davis, CA; 2) The Robert S. Boas Center for Genomics and Human Genetics, Feinstein Institute for Medical Research, North Shore LIJ Health System, Manhasset, NY; 3) Center for Applied Medical Research, University of Navarra, Pamplona, Spain; 4) Karolinska University Hospital, Stockholm, Sweden; 5) Karolinska Institutet at KUS Huddinge, Stockholm, Sweden; 6) Institut Cochin, Inserm U567, University Paris Descartes, Paris France; 7) Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD.

The definition of European population genetic substructure and its application to understanding complex phenotypes is becoming increasingly important. In the current study using over 4000 subjects genotyped for 300 thousand SNPs we provide further insight into relationships among European population groups and identify sets of SNP ancestry informative markers (AIMs) that can be used in a variety of genetic studies. In general, the graphical description of these principal components analyses (PCA) of these diverse European subjects showed a strong correspondence to the geographical relationships of specific countries or regions of origin. Clearer separation of different ethnic and regional populations was observed using PCA when northern and southern European groups were considered separately and PCA results were influenced by the inclusion or exclusion of different self-identified population groups including Ashkenazi Jewish, Sardinian and Orcadian ethnic groups. SNP AIM sets were identified that could distinguish the regional and ethnic population groups. Moreover, the studies demonstrated that most allele frequency differences between different European groups (defined by PCA) could be effectively controlled by analyses using these AIM sets. These European substructure AIMs should be widely applicable to ongoing studies to confirm and delineate specific disease susceptibility candidate regions without the necessity to perform additional genome-wide SNP studies in additional subject sets.

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### 599/W/Poster Board #257

**Genome-wide patterns of homozygosity in worldwide human populations.** *T.J. Pemberton[1], D. Absher[2], M. Flickinger[3], M. Feldman[4], R.M. Myers[2], N.A. Rosenberg[1], J. Li[1].* 1) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 2) HudsonAlpha Institute for Biotechnology, Huntsville, AL; 3) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 4) Department of Biological Sciences, Stanford University, Palo Alto, CA.

Genome-wide patterns of runs of homozygosity and their variation across individuals can provide a rich source of data for uncovering patterns of diversity, population structure, and history of human populations, and for facilitating the identification of genes underlying recessive traits via homozygosity mapping. We identified runs of homozygous genotypes in 53 populations of the Human Genome Diversity Project and 11 populations of the International HapMap Project by using a likelihood-based approach and data from >640K common SNPs. We observed considerable variation across populations in the number and length of runs of homozygosity. For a given individual, the per-individual count and total length of homozygous segments between 0.5 and 5 Mb are predicted extremely well by the geographic origin of the individual, with Africans having the lowest values and Native Americans having the highest values. However, the per-individual count and total length of homozygous segments larger than 5 Mb showed a different pattern, with more inter-individual variation and larger values in populations where isolation and consanguinity are more common. This difference reflects the distinct forces generating unusually long homozygosity runs compared to runs of intermediate length. Additionally, genomic regions containing homozygosity runs in many individuals and those with runs in unusually few individuals are both present in the human genome, and not all of these regions are explained by local recombination rate estimates. This pattern suggests that many forces, including inbreeding, recombination, selection, and random drift, have played a role in shaping genome-wide patterns of homozygosity in the human genome, and they provide important clues to the genomic background and population diversity that must be taken into account in homozygosity mapping of disease genes.

### 600/W/Poster Board #258

**The distribution of sex-specific human genetic variation in Ethiopia.** *C.A. Plaster[1], A. Tarekegn[2], E. Bekele[2], N. Bradman[1], M.G. Thomas[1].* 1) Genetics, Evolution, Environment, University College London, London, United Kingdom; 2) Department of Biology, University of Addis Ababa, Addis Ababa, Ethiopia.

Ethiopia has been proposed as a candidate location for the emergence of anatomically modern humans, and the source region for the expansion out of Africa. It is also a region of substantial cultural diversity as expressed in languages (Nilo-Saharan, Cushitic, Semitic, and Omotic language families), religions (Christians, Jews, Moslems and Animists), ethnic identities (over 80 groups) as well as many marginalised groups socially excluded on grounds of caste-like occupation, supposed origin, or both. The demographic history of Ethiopia over the past several thousand years has involved both sustained migration of Semitic speakers from the Arabian Peninsula as well as internal conquests of lands in the south. To investigate the demographic histories of ethnic groups we analysed a battery of SNPs and microsatellites on the non-recombining portion of the Y chromosome (NRY) and sequence variation in the Hypervariable Segment 1 (HVS1) of mtDNA (5756 samples from 45 ethnic groups). Commonly used summary statistics (gene diversity h, genetic distance Fst) were analysed within the context of non Ethiopian data e.g. West Africa (Igbo, Nigeria) and Europeans. We present preliminary results reporting a wide range of genetic diversity values within ethnic groups (h: NRY = 0.743 - 0.972, HVS1 = 0.962 - 0.996) and pairwise genetic distance values between groups (Fst: NRY = 0.000 - 0.294, HVS1 = 0.000 - 0.035). A clustering of Ethiopian groups was observed when using principal coordinate analyses with genetic distances, appearing midway between a West African Niger-Congo speaking group (Igbo of Nigeria) and an Indo-European speaking group (Greek Cypriots). Some south-western groups (e.g. Anuak) showed greater similarity to West-Africans while the culturally influential Amhara were more similar to Europeans. Gene flow between dominant Dawuro agriculturalists and excluded members of the Manja was sex-biased, with many more NRY haplotypes common to the two groups than mtDNA haplotypes, relative to the distribution of the two systems across all the ethnic groups. The marginalised group had a particularly low level of mtDNA HVS1 diversity (h = 0.705). Of particular interest is the extensive sharing of discriminating NRY and mtDNA haplotypes across many ethnic groups, suggesting either a) the creation or preservation of cultural diversity despite substantial inter-group gene flow or b) recent ethnogenesis of the currently extant groups.

### 601/W/Poster Board #259

**Genetic Diversity of the Ancient People in Mesoamerica.** *L. Wang[1], K. Kurosaki[2], N. Saitou[3], S. Sugiyama[4], S. Ueda[5].* 1) Medical & Animal Gen, Inst Gen, Chinese Academy Sci, Beijing, China; 2) Department of Legal Medicine, School of Medicine, Toho University, Tokyo, Japan; 3) Division of Population Genetics, National Institute of Genetics, Mishima, Japan; 4) Graduate School of International Cultural Studies, Cultural Symbiosis Research Institute, Aichi Prefectural University, Aichi, Japan; 5) Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo, Japan.

DNAs were extracted from the human remains buried in the Moon Pyramid at archaeological Teotihuacan site in Mexico. Nucleotide sequences of their mitochondrial D-loop and SNP sites were determined by the PCR-direct sequencing. To reveal the genealogy of mitochondrial DNA sequences of the individuals buried in the Moon Pyramid and assess their positions among Native Americans, we first constructed a network of the mitochondrial DNA from the contemporary Native Americans; the northern Native Americans (Haida, Bella Coola, and Nuu Chah Nulth), the central Native Americans (Huetar, Kuna, and Ngöbé), and the southern Native Americans (Yanomami, Zoro, Gavião, and Xavante), and compared them with those of the individuals from the Moon Pyramid. All of the mitochondrial DNA types from the Moon Pyramid individuals were unique, and clear genetic affinities were not observed between the Moon Pyramid individuals and any of the 10 Native American populations. To investigate genetic diversity among the contemporary central Native American populations, we constructed a phylogenetic tree of their mitochondrial DNA sequences using the neighbor-joining method. There was a major mitochondrial DNA sequence common to these three central Native American populations. However, there were a relatively small number of mitochondrial DNA types in each population, most of which were, moreover, unique to each Native American population. Next we compared the mitochondrial DNA sequences of the Moon Pyramid individuals with those of the ancient Mesoamerican people, ancient Maya people from the classic Copán site. We also used Huetar people as a reference for the contemporary central Native Americans. The distribution of the mitochondrial DNA types found in the ancient Native Americans is greatly different from that found in the contemporary Native Americans. These results show that genetic diversity in the ancient Native Americans was not as low as that in the contemporary Native Americans, suggesting an occurrence of bottleneck in the past.

### 602/W/Poster Board #260

**Genomic runs of homozygosity: population history and disease.** *J. Wilson, M. Kirin, C.S. Franklin, H. Campbell, R. McQuillan.* Pub Hlth Sci, Univ Edinburgh, Edinburgh, United Kingdom.

Runs of homozygosity (ROH), resulting from the inheritance from both parents of identical haplotypes, are abundant in the human genome. ROH length is determined partly by the number of generations since the common ancestor: offspring of cousin matings have long ROH, while the numerous shorter ROH reflect shared ancestry tens and hundreds of generations ago. We have previously shown in studies of European populations, that Froh, a multipoint estimate of individual autozygosity derived from genomic ROH, distinguishes clearly between subpopulations classified in terms of demographic history and correlates strongly with pedigree-derived inbreeding coefficients. Now in a global population dataset, our analysis of ROH allows categorisation of individuals into four major groups, inferred to have (a) parental relatedness in the 150 years (many south and west Asians), (b) shared parental ancestry arising hundreds to thousands of years ago through population isolation and restricted effective population size (Ne), but little recent inbreeding (Oceanians, African hunter-gatherers, some European and south Asian isolates), (c) both ancient and recent parental relatedness (Native Americans), and (d) only the background level of shared ancestry relating to continental Ne (east Asians, urban Europeans; African agriculturalists). Long runs of homozygosity are therefore a widespread and underappreciated characteristic of our genomes which record past consanguinity and population isolation and provide a unique record of individual demographic history. Individual ROH measures also allow quantification of the disease risk arising from polygenic recessive effects. We present preliminary data from a survey of the effects of ROH on quantitative disease-related traits and disease risk.

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## 603/W/Poster Board #261

**Patterns of gene flow in South Amerindians: inferences and reanalysis based on mitochondrial DNA data.** *L.W. Zuccherato[1], M.C.F. Silva[1,2], S. Fuselli[3], R.H. Gilman[4], E. Tarazona-Santos[1].* 1) Departamento de Biologia Geral, Universidade Federal de Minas Gerais, Brazil; 2) Fundação Hemominas, Minas Gerais, Brazil; 3) Università di Ferrara, Italy; 4) Asociación Benéfica PRISMA, Lima, Peru.

Between 2001 and 2003, we proposed the following scenario to explain the pattern of genetic diversity of South Amerindian populations: Western populations of South America, settled in the Andean area, showed higher long-term effective sizes and gene flow than Eastern populations (settled in Brazil and Paraguay). This scenario has caused a homogenization of the gene pool in the former but a divergence of populations in the latter. During the last five years, a considerable amount of data about the mitochondrial DNA diversity in South Amerindians have been generated, and we have studied three new Peruvian native populations presenting the following autochthonous Pan-American A-D haplogroup frequencies: Shimaa (n=37, A:3%, B:89%, C:8%, D:0%) and Monte Carmelo (n=28, A:31%, B:61%, C:8%, D:0%) from the Matsiguenga ethnic group; and the population of Rio Tambo (n=60, A:40%, B:55%, C:0%, D:5%) from the Ashaninka ethnic group. Interestingly, these populations are located in a geographic and environmental area of transition between the Andes and the Amazon that is underrepresented in human population genetics studies. Admixture analysis using 16 Ancestry Informative Markers confirms a very low (<10%) level of European admixture for these populations. An updated database of mtDNA data for South Amerindians has been implemented, containing 1758 individuals from 46 populations. By using the Isolation by Distance (IBD) model as null hypothesis, we confirmed that there is no correlation between geographic distance and genetic variability when populations from all South America were analyzed together, as well as when Eastern South Amerindian populations are considered, differently from our previous finding and suggesting that complex evolutionary scenarios have conceal the effect of geography on the genetic structure of these populations. On the other hand, Andean populations from Colombia to Chile do fit the IBD model, also when non-Andean populations settled in the area between the Andes and the Peruvian Amazon (Matsiguenga and Ashaninkas) are included in the analysis. In particular, the Ashaninka (Rio Tambo) and Monte Carmelo populations also present high level of intrapopulation diversity, which is suggestive of gene flow or a recent split from populations from the Andean region. Financial Support: Brazilian agencies CAPES-Ministry of Education, FAPEMIG and CNPq.

## 604/W/Poster Board #262

**Considerations on the use of shared controls in genome-wide association studies.** *G.K. Chen, D. Stram.* University of Southern California Los Angeles, CA.

Thanks to the large number of samples that have been collected for modern genome wide association (GWA) studies, researchers have been able to detect many genetic risk factors in common diseases with modest effect sizes (e.g., odds ratios~1.5). However, these variants alone usually account for only a small fraction of the population attributable risk for a disease and it is likely that much larger sample sizes are needed to elucidate the variants with even smaller effect sizes. To address this issue, we consider the strategy of sharing a set of common controls across different studies with the primary goal of cost efficiency. While this strategy can improve power to detect variants, it can increase the false positive rate when controls are not genetically matched to cases and the relative risk of disease is higher in one ethnicity than others. To quantify the effect of the inflation in type 1 errors, we compare false positive rates across various relative risks by simulating disease status in a set of 1,500 African-Americans spanning a wide range of admixture levels. Finally, we consider the merits and potential pitfalls of applying two well known methods that are designed to address this problem, genomic control and principal components analysis, as well as an extension of a method originally described by Bourgain et al (AJHG, 2003) which shares several properties of the former two methods.

## 605/W/Poster Board #263

**A comprehensive partitioning of associations of all orders among genome-wide SNPs.** *M.K. Uyenoyama[1], D. Fusco[2], Y.-J. Li[3].* 1) Biology, Duke Univ, Durham, NC; 2) Computational Biology and Bioinformatics, Duke Univ, Durham, NC; 3) Center for Human Genetics, Duke Univ, Durham, NC.

We introduce a full partitioning of genomic SNP variation, including analogues of linkage disequilibrium (LD) and all higher-order disequilibria. Even in the absence of population structure, 2n(2n+1)/2-1 independent forms of genetic associations can exist among n biallelic SNP loci. In contrast, most genome-wide studies describe only two kinds of genetic association, homozygosity and LD. Classical population genetics theory suggests that the analysis of higher-order associations may provide a basis for inferences about population structure. For example, partial inbreeding generates identity disequilibrium (correlation in heterozygosity) across the entire genome, even between unlinked loci. Our partitioning of genotypic associations simplifies schemes previously proposed for inbred populations. All of our measures of association constitute functions of Bernoulli random variables, a structure particularly well-suited for analytical explorations using coalescence arguments. Because classical measures of multilocus association are generally estimated as covariances, they represent properties of samples, not of individuals or haplotypes. In contrast, each zygote or haplotype can be scored under our definition, with sample-wide associations obtained by summing the individual scores. This property opens the analysis of genome-wide associations to a large corpus of dimension reduction procedures, including principal components analysis (PCA). We describe an exploration of pairwise associations in simulated data. Our analogue of identity disequilibrium is a major contributor to the first principal component (PC1) over both long and short recombinational distances (correlation = -0.67). Our analogue of LD tends to be negatively correlated with identity disequilibrium over short scales and is a major contributor to PC2 over longer scales. Regular systems of inbreeding and population subdivision generate entire ensembles of associations. We present analytical distributions for measures of association in the absence of crossing-over. For example, the distribution of our analogue of LD becomes more concentrated near zero as the rate of substitution increases, but has a pronounced negative skew and a negative expectation. Lastly, we describe pairwise and higher-order associations in human genomes using HapMap data.

## 606/W/Poster Board #264

**Homozygosity scan: a valuable addition to the clinical geneticist's armamentarium.** *F. Alkuraya[1,2,3].* 1) Dept Gen, KFSH & RC, Riyadh, Riyadh, Saudi Arabia; 2) Department of Pediatrics, King Khalid University Hospital and College of Medicine, King Saud University, Riyadh, Saudi Arabia; 3) Department of Anatomy and Cell Biology, College of Medicine, Alfaisal University, Riyadh, Saudi Arabia.

Consanguinity (union between related individuals) is an ancient practice that is still common in multiple cultures. Coefficient of inbreeding (percentage of genome that is identical by descent) of the progeny of consanguineous union ranges from <1% (third cousin parents) to 12.5% (double first cousins). The consequence of increased coefficient of inbreeding on the frequency of autosomal recessive conditions is well established. On the other hand, this same phenomenon increases the probability of disease causing mutations to reside in blocks of autozygosity. This has made it possible for researchers to identify disease causing mutations by pursuing hypothesis-free genomewide search for blocks of autozygosity. This has been particularly helpful in the setting of very rare disorders that are much more likely to present in a homozygous (really autozygous) state than combined heterozygous state. Our group has similarly utilized this phenomenon to identify novel disease causing genes. Increasingly, we have also realized that consanguineous populations lend themselves to “shortcuts” that make the practice of clinical genetics easier in a number of ways, again in the setting of autosomal recessive disorders. Genetically heterogeneous conditions, for example, represent a major challenge in outbred populations where prioritization scheme for mutation analysis usually follows published frequencies of the involved genes.In the setting of consanguineous parents, however, a much more efficient prioritization scheme is homozygosity scan which is becoming increasingly easier and cheaper to perform thanks to the advent of SNP chip-based genotyping platforms. This scan can easily eliminate genes and often identifies one or very few genes for downstream mutation analysis.In our experience, this has brought about significant reduction in cost, impressively boosted the yield of mutation analysis and dramatically enhanced patient care. This approach has allowed us to revisit genes that have been excluded by major referral centers on the basis of lack of identifiable mutation, only to leave us uncover deep intronic mutations that we could only pursue because those were the only genes highlighted by the homozygosity scan. In summary, we have shown that consanguinity can be taken advantage of not only in the research arena but, as importantly, in the practice of clinical genetics. We show that homozygosity scan is a valuable addition to the clinical geneticist's armamentarium.

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## 607/W/Poster Board #265

**An African village in Colombia.** *N. Ansari Pour[1], Y. Monino[2], C. Duque[3], N. Gallego[3], G. Bedoya[3], A. Ruiz-Linares[1,3], M.G. Thomas[1], N. Bradman[1].* 1) Dept of Genetics, Environment and Evolution, University College London, United Kingdom; 2) Dept of Linguistics, CNRS, France; 3) Institute of Molecular Genetics, University of Antioquia, Colombia.

The Palenque, who live in Palenque de san Basilio some 70km from Cartagena in Colombia and speak a Spanish Creole (Palenquero), have an oral history of the community's formation that includes, early in the seventeenth century, an initial founding group of escaped slaves establishing a village and subsequently maintaining their independence in the face of Spanish military presence in the region. They developed a thriving community that has continued to the present day. Interestingly linguists have identified some 200 words in regular usage that originate in a Kikongo language that is still spoken in the Republic of Congo. The most likely source of this vocabulary has been identified as the Yombe who live mainly in Pointe-Noire on the west coast of the Republic of Congo. In this study we investigated whether similarity in the distribution of the paternally inherited non-recombining portion of the Y chromosome (NRY) in the Palenque and in 42 sub-Saharan ethnic groups supported linguistic data that Yombe individuals were the most likely principal male members of the founding group. All the sub-Saharan groups in the dataset, which included representatives of the Yombe and seven other Congolese groups, could have contributed individuals to the Atlantic slave trade (n = 2870) and no group contributed less than 26 samples (median = 54), all of which were unrelated at the paternal grandfather level. On analysing 15 SNP and six microsatellite markers on the NRY using pairwise Fst and applying a sign test to the distribution of NRY haplotypes among groups with low pairwise Fst values we established that Yombe were the most similar to the Palenque (Fst = 0.015, Sign Test p-value = 0.001) thereby supporting the prior hypothesis based on linguistic analysis. Analyses of mtDNA to elucidate the likely maternal origins of the Palenque are in progress.

## 608/W/Poster Board #266

**Improved resolution of the human Y-chromosomal phylogeny using targeted next-generation sequencing.** *Q. Ayub, Y. Xue, C. Tyler-Smith.* Human Evolution, The Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, CB10 1SA, United Kingdom.

The non-recombining part of the Y chromosome provides unique insights into male-specific aspects of human genetics and history. We are using next-generation Illumina sequencing to fully re-sequence targeted regions of the Y and resolve the Y-chromosomal phylogeny by characterization of additional single nucleotide polymorphisms (SNPs) on lineages of interest. Initially ~6 Mb of Y sequence (NCBI36:Y-chromosome: 12,308,579-18,230,132) is being generated for an African haplogroup A male. The strategy involves sequence enrichment by long template PCR of genomic DNA (10-20 ng/reaction) using overlapping fragments of 5.5 - 6.5 kbp. Currently ~70% of primer pairs work using a standard touchdown PCR protocol. Fragments obtained from a single individual are pooled and used for library preparation and Illumina sequencing. Re-sequencing generates accurate high coverage data; SNP calling and their subsequent validation will be presented. Most SNPs are expected to be rare but some are likely to resolve deep divisions within African populations. Subsequently, we aim to (1) determine the time depth of the human Y phylogeny, (2) resolve multifurcations in the major lineages by discovering additional SNPs on the relevant and (3) discover SNPs that mark any lineage of particular interest. In addition, we will be able to provide a subset of all primers that work well with this protocol to investigators who are interested in Y-chromosomal phylogenies so that comparable standard datasets can be generated for use by the community.

## 609/W/Poster Board #267

**Identifying ancestry of genomic regions in admixed individuals.** *A. Brisbin[1], K. Bryc[2], J. Degenhardt[2], A. Reynolds[2], A. Auton[3], J.G. Mezey[2], H. Ostrer[4], C.D. Bustamante[2].* 1) Center for Applied Mathematics, Cornell University, Ithaca, NY; 2) Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY; 3) Wellcome Trust Centre for Human Genetics, Oxford, OX3 7BN, United Kingdom; 4) Departments of Medicine, Pediatrics (Genetics) and Pathology, New York University School of Medicine, New York, NY.

Identifying ancestry along each chromosome in admixed individuals is of great interest for admixture mapping, removing confounding in association mapping, and identifying recent targets of selection. We present a Principal Components-based forward-backward algorithm for determining ancestry along each chromosome from a high-density, genomewide set of SNP genotypes of admixed individuals. We compare our method as applied to genotype and phased haplotype data, and find that the phased haplotype data allows confident assignment of ancestry to a larger proportion of the genome, in diverged populations where admixture is difficult to assess. We apply our method to a dataset of Hispanic/Latino populations and identify regions of excessive ancestry that may be recent targets of selection and could serve as candidate regions for admixture-based association mapping. Additionally, application of the method to the CEPH Human Genome Diversity Panel finds surprising signatures of admixture within and among isolated and anthroplogically defined populations.

## 610/W/Poster Board #268

**Genetic Differences of the Northern and Southern Han Chinese.** *C.H. Chen, M.H. Su, T.H. Lu, J.Y. Wu, Y.T. Chen.* Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

**BACKGROUND** Genetics evidences suggested that the Han people could be genetically differentiated into northern and southern Hans, separated by the Yangtze River. Two recently available data sets of Hans, the Chinese Han in Beijing (CHB) and the Chinese Han in Denver (CHD) of the HapMap Project Phase III, were widely-referred but not yet fully examined accordingly. This study was aimed (1) to conduct a norm that could more accurately summarize the genome-wide genetic profiles of northern and southern Hans; (2) to genetically localize the two Han groups of the HapMap Project.

**METHODS** Self-reported language/location group data and genotype data (Illumina Hap550duov3 chip) of 2,400 Hans were obtained from the National Clinical Core and the National Genotyping Center at Academia Sinica, Taipei, Taiwan. Data of three Asian samples, CHB, CHD and Japanese in Tokyo (JPT), were obtained from the HapMap project. Multidimensional scaling analysis on genome-wide identity-by-state (IBS) pairwise distances was performed to extract genetic information. Discriminant functions were then derived based on subsets of subjects having four grandparents with a same origin. Predict rate was estimated for the language/location groups.

**RESULTS** Minnan and Hakka, two major Han groups in Taiwan, were correctly predicted as southern Hans (99.3% and 98.3%). Hans with origins in more northern or southern locations were also correctly predicted (> 95%). However, subjects from the places along the Yangtze River were difficult to predict. The overall predict rate was greater than 90%. For the HapMap Asian groups, the JPT group was completely separated from the Hans but showed a close relationship to the northern Hans; 64.6% of the 82 CHB subjects were categorized as northern Hans while only 24.3% for the 70 CHD subjects.

**SUMMARY** Our algorithm was able to differentiate between the northern and southern Hans while the genetics differences between the two HapMap Han samples demand further analysis.

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## 611/W/Poster Board #269

**The origin of the population on Ontong Java, a Polynesian Outlier in the Solomon Islands, based on the genetic diversity of mtDNA.** *M. Christiansen[1], R. Kuschel[2], A.E. Christensen[3], P.L. Hedley[1,4], C.M. Hagen[1], D. Schmidt[1], F.H. Aidt[1], D.V. Møller[1].* 1) Dept Clinical Biochemistry and Immunology, Statens Serum Inst, Copenhagen, Denmark; 2) Institute of Psychology, University of Copenhagen, Copenhagen, Denmark; 3) Department of Geography and Geology, University of Copenhagen, Denmark; 4) Department of Biomedical Sciences, University of Stellenbosch, Cape Town, South Africa.

Ontong Java (also known as Lord Howe Atoll) is a large atoll in the South West Pacific consisting of 120 islands located 220 km north of Santa Isabel in the Solomon Islands. The total population comprises 3,000 persons, of which 1,850 reside on the island. The remaining population live elsewhere in the Solomon Islands or abroad. Ontong Java is one of numerous Polynesian Outliers in Melanesia; there is also an apparent Micronesian cultural influence and little is known about the origin of the population. In order to establish the origin of the maternal gene pool we collected cheek swabs from 36 persons, representing all unrelated "ramages" (where the members are unrelated at least beyond first cousins) on Ontong Java. Mitochondrial DNA was extracted from 32 samples and the control region was sequenced. The presence of the COII/tRNA$^{Lys}$ 9 bp deletion was also ascertained and 24 persons (75%) were carriers. In total 10 different haplotypes were identified. Eight haplotypes, comprising 24 persons, belonged to the B4a haplogroup, occuring with a near 100% prevalence in central Polynesia, and two haplotypes, identified in eight persons, belonged to the M7c1 haplogroup, previously found in Micronesia and South East Asia. Twenty-one individuals (66%) had the full Polynesian motif. The B4a cluster comprised a star-like subcluster in an MJ network and three more distant haplotypes. The greater diversity in the B4a haplotypes compared to the M7c1 haplotypes suggests the the Polynesian-derived population may be older than the likely Micronesian-derived population element. An alternative and supplementary explanation is the extensive inter-island contact among the Central Polynesian Outlier atolls. The present population of Ontong Java most likely has a mixed Polynesian and Micronesian origin on the maternal side. The genetic diversity in the B4a haplogroup is compatible with a single expanding Polynesian-derived population in combination with additions from other Polynesian Outliers.

## 612/W/Poster Board #270

**Incompatibility of current Finnish mitochondrial diversity with simulations of assumed settlement history.** *M. Heger[1], J. Palo[2], T. Sundell[1,3], A. Sajantila[2], P. Onkamo[1].* 1) Department of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland; 2) Department of Forensic Medicine, University of Helsinki, Helsinki, Finland; 3) Institute for Cultural Research, Department of Archaeology, University of Helsinki, Helsinki, Finland.

Traditionally, geneticists studying Finnish population history have assumed a model where Northern and Eastern Finland were mostly uninhabited until the 16$^{th}$ Century A.D. and were then settled by small family groups from South-Western Finland. The reduced genetic diversity and the distinct Finnish disease heritage are seen as consequences of these founder effects. Y-chromosomal diversity is indeed reduced in the present population, especially in the eastern parts of the country. However, mitochondrial diversity is not heavily reduced compared to South-Western Finnish or other European populations. This discrepancy has been explained with the higher mitochondrial mutation rate having restored mitochondrial diversity in these populations since the founder effects.

In our view it seems unlikely that even with high mitochondrial mutation rates mtDNA diversity could be restored over a mere 17 generations after the alleged tight bottlenecks. Archaeological evidence also suggests a different settlement history, e.g. settlement beginning in South-Eastern instead of South-Western Finland.

In this study we use simuPOP, a state-of-the-art forward simulation tool, to simulate datasets corresponding to Finnish mitochondrial diversity under the traditional model and compare them with actual present-day Finnish data. We show that current mitochondrial variation is unlikely under this model, increasing the credibility of alternative hypotheses.

## 613/W/Poster Board #271

**Phylogeographic characterisation of mtDNA lineages from Northern Thailand.** *J. Horst[1], B. Zimmermann[2], M. Bodner[2], S. Amory[2, 3, 4], L. Fendt[2], A. Röck[5], D. Horst[6], B. Horst[7], T. Sanguansermsri[8], W. Parson[2], A. Brandstätter[9].* 1) Institut für Humangenetik, Universität Münster, Münster, Germany; 2) Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria; 3) Laboratory of Molecular Anthropobiology, Institute of Legal Medicine, Strasbourg, France; 4) International Commission on Missing Persons, Sarajevo, Bosnia and Herzegovina; 5) Institute of Mathematics, University of Innsbruck, Innsbruck, Austria; 6) Pathologisches Institut der Ludwig-Maximilians-Universität München, Germany; 7) Department of Surgical Pathology, Columbia University, New York, USA; 8) Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand; 9) Division of Genetic Epidemiology, Innsbruck Medical University, Innsbruck, Austria.

Footprints in the genetic composition of Thai mitochondrial DNA (mtDNA) lineages have been left by the immigration of ethnical minorities from surrounding countries into Thailand. The entire mtDNA control region (1122 bp) was typed in 190 unrelated male volunteers from the Northern Thailand province of Chiang Mai following highest quality standards. For a more precise haplogroup classification, selected SNPs from the mtDNA coding region were genotyped. Quasi-median networks were constructed for visualization of character conflicts. The data were put into population-genetic relationships with other Southeast Asian populations. We found several new, so far undescribed mtDNA lineages. Although the frequencies of the Thai haplogroups were characteristic for Southeast Asia in terms of haplotype composition and genetic structure the Thai population was significantly different from other Southeast Asian populations.

## 614/W/Poster Board #272

**Mobile elements reveal small population sizes in the ancient ancestors of Homo sapiens.** *C.D. Huff[1], J. Xing[1], A.R. Rogers[2], L.B. Jorde[1].* 1) Department of Human Genetics, University of Utah, Salt Lake City, UT; 2) Department of Anthropology, University of Utah, Salt Lake City, UT.

Because the insertion of a new mobile element in any small genomic region is a rare event, the genealogy of a region that contains such an insertion is older than that of the rest of the genome. Remarkably, the analysis of polymorphic mobile insertions in the complete genomes of just two humans provides substantial information about ancient human population size. In a simple model with a sample size of two and a constant population size, the expected time to most recent common ancestor (TMRCA) is doubled if a rare insertion is present in one of the samples. We test this model by examining single nucleotide polymorphisms (SNPs) around polymorphic Alu insertions from two human genomes: the NCBI reference genome (hg18) and the genome of Craig Venter (HuRef). We find that genealogies of genomic regions that contain polymorphic mobile elements are insensitive to the effects of recent bottlenecks and expansions but instead are shaped largely by the forces of ancient population history. The estimated TMRCA for regions containing a polymorphic insertion between HuRef and hg18 is 1.99x the genomic average ($p < 10\text{-}300$), as predicted. We employ coalescence simulations conditioned on the presence of a rare insertion under different demographic models. By comparing the likelihood of these models, we estimate that the effective population size of human ancestors living prior to one million years ago is between 10,000 and 20,000, and we can reject all models where the ancient effective population size is larger than 25,000. This result implies an unusually small population size for a species spread across the entire Old World, particularly in light of the estimated effective population sizes of chimpanzees (21,000) and gorillas (25,000), which together inhabit only one small part of a single continent.

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## 615/W/Poster Board #273

**On the borderline between the east and the west: the maternal genetic background of Karelians.** *S. Koivumäki[1], T. Lappalainen[2], P. Lahermo[2].* 1) Medical Genetics, University of Turku, Turku, Finland; 2) 2 Finnish Genome Center, University of Helsinki, Helsinki, Finland.

Introduction: The frontier between Finland and Russia represents one of the most conspicuous socioeconomic gaps in the world. Based on the mean gross national product, there is a ten-fold difference between Russian Karelian Republic and Finnish Karelia. Otherwise these populations share the same geophysical environment. For these reasons, Karelia has been a very interesting field of research for multifactorial disease studies. However, this area has undergone many demographic incidents, such as wars and famine, which may cause local differences in the gene pool. In this study, we wanted to elucidate the maternal genetic background of Karelians. Materials: Blood samples were collected from healthy unrelated individuals without known foreign background from four Karelian districts; Aunus(n=218), Viena(n=87), Tver(n=61) and Finnish Karelia (n=70), The sample collection was performed according to the Basic Principles of the Declaration of Helsinki. Methods: The entire mitochondrial DNA was sequenced in 32 reactions per sample with the BigDye® Terminator v3.1 Cycle Sequencing Kit in the Applied Biosystem's 3730 Genetic Analyzer sequencing machine. Sequence alignments were made by the SeqScape® Software, Version 2.5 (Applied Biosystem). Results: Haplogroup H was very common in all populations. However, H1a is almost absent in Finnish Karelia. Also U and its subhaplogroups were common. Specially U5b1b1 reached over 16% in Viena Karelians. U4 was most common among Tver Karelians. Conclusions: The maternal genetic background seem to be complex in this area. There is clear regional differences. Also there is solid evidence of gene flow from various sources. Representation of the clearly Asian haplogroups is strikingly low.

## 616/W/Poster Board #274

**Female to male breeding ratio in the history of modern humans.** *D. Labuda[1,2], J-F. Lefebvre[2], P. Nadeau[2], M-H. Roy-Gagnon[2, 3].* 1) Département de pédiatrie, Université de Montréal, Montreal, PQ, Canada; 2) Centre de Recherche CHU Sainte-Justine, Université de Montréal, Montreal, PQ, Canada; 3) Département de médecine sociale et préventive, Université de Montréal, Montreal, PQ, Canada.

Was the genetic contribution of men and women to successive generations the same? As a population, did we have fewer fathers than mothers? Was polygyny present among hominid lineages to influence relative divergence rates of autosomes and sex chromosomes? Students of genetic variation of the uniparentally inherited mitochondrial and Y-chromosome DNA confronted these questions, fewer addressed it by looking at the DNA diversity of autosomes and sex chromosomes (Hammer et al. 2009, Keinan et al. 2009) with equivocal results. Our approach is different: we analyzed the ratio of the population recombination rate, $\rho$, between autosomes and the X chromosome. The chromosome X recombines only in the female meiosis whereas autosomes undergo cross-overs in both male and female germ lines such that their relative $\rho$ reflects changes in the breeding ratio, $\beta$. The estimate of $\beta$ is calculated from the observed chromosomal $\rho$'s, obtained by InfRec (Lefebvre and Labuda 2008), after their calibration with the average chromosomal recombination rates known from pedigree data. We have tested our approach using coalescent simulations under different input parameters' values and various demographic scenarios. For the HapMap populations we obtained $\beta$ of 1.4 in Yoruba from West Africa, 1.2 in European and 1.0 in East Asian samples. This suggests that in the history of modern humans the reproductive variance between men and women did not drastically differ, thus consistent with the prevalence of monogamy or mild polygyny in the human lineage. Known incidences of polygyny may be of recent origin, related to raise of agriculture and shift from hunter-gathering to food producing economies, and therefore not sufficiently common to leave a strong genetic signature in the recombinational record. (*Supported by* GenomeQuebec/Genome Canada and Canadian Institutes of Health Research).

## 617/W/Poster Board #275

**Testing models of sex-biased demography using patterns of linkage disequilibrium.** *K.E. Lohmueller[1,2], C.D. Bustamante[1], A.G. Clark[2].* 1) Dept Biol Stat & Comp Biol, Cornell Univ, Ithaca, NY; 2) Dept Molec Biol & Gen, Cornell Univ, Ithaca, NY.

The X-chromosome is an important tool for studying differences in demographic history between males and females. Since males have only one X-chromosome, the ratio of the effective population size ($N_e$) of the X chromosome is expected to be ¾ that of the autosomes (i.e. $N_X/N_{Auto} = 0.75$) when assuming an equal number of males and females and a constant population size. Departures from this ratio are indicative of sex-biased demographic processes. Recently, a study by Hammer et al. (Plos Genetics 2008; 4: e1000202) found that $N_X/N_{Auto} > 0.75$. Conversely, a different study by Keinan et al. (Nat Genet 2008; 41:66-70) found no departure from the ¾ ratio in an African population, and $N_X/N_{Auto} < 0.75$ in non-African populations. By focusing on patterns of sequence diversity and SNP frequencies, both of these studies are dependent on having an accurate estimate of the mutation rate from divergence data as well as accurate calling and genotyping of low frequency SNPs. As an alternative measure that is not dependent on the mutation rate and low frequency SNPs, we estimated $N_X/N_{Auto}$ from patterns of linkage disequilibrium (LD) summarized by an estimate of the population scaled recombination rate, $\rho$. In particular, using data from the CEU (individuals having Northwestern European ancestry) and YRI (individuals from Ibadan, Nigeria) populations in HapMap project, we estimated $\rho$ on chromosome 7 and chromosome X using Hudson's composite likelihood estimator. Under the standard neutral model for autosomal data, $\rho = 4N_e r$, where $r$ is the per region recombination rate per generation. We obtained an estimate of $r$ from the deCODE genetic map and converted our estimates of $\rho$ into estimates of $N_e$. We find $N_X/N_{Auto} \approx 0.75$ in YRI and $\approx$ 0.64-0.69 in CEU. We are using coalescent simulations to investigate the consistency of these estimated ratios with various models of sex-biased demography. In particular, we are testing whether simple models with equal numbers of males and females can explain these patterns, or whether we need to invoke sex-biased demographic events. Since patterns of LD are calibrated by a genetic map, rather the mutation rate as inferred from an outgroup, our analysis provides an independent method to verify the conclusions of Hammer et al. or Keinan et al.

## 618/W/Poster Board #276

**Isolation and gene flow among 10 Polynesian Outliers and their geographic neighbors within Melanesia and Micronesia: A mtDNA perspective.** *J.K. Lum[1,2,3], R.M. Garruto[1,2], A. Kaneko[4], M.G. Vilar[1,3].* 1) Department of Anthropology, Binghamton University, Binghamton, NY; 2) Department of Biological Sciences, Binghamton University, Binghamton, NY; 3) Laboratory of Evolutionary Anthropology and Health, Binghamton University, Binghamton, NY; 4) Unit of Infectious Disease, Karolinska Institute, Stockholm, Sweden.

Polynesians are descendants of the first settlers of Remote Oceania, characterized archaeologically by the Lapita Cultural Complex that coalesced in the Bismark Archipelago 3,400 years ago. Within a few centuries of its formation, evidence of the Lapita Cultural Complex is found extending through the Solomon Islands, Vanuatu, New Caledonia, and Fiji into the Western Polynesian archipelagoes of Tonga and Samoa in a range expansion so rapid it has been referred to as archaeologically instantaneous. After a substantial pause the remaining archipelagoes of Eastern Polynesia and the peripheries of the Polynesian triangle (Hawai'i, Easter Island, and New Zealand) were settled within the past 2,000 years. At about the same time that Western Polynesians first began to colonize islands farther East, they also began to voyage back West into Melanesia and Micronesia to settle islands that were often remote and ecologically marginal, but occasionally included villages on high island otherwise settled by Melanesians (e.g. Fila on Efate). These culturally and linguistically Polynesian populations located geographically within Melanesia and Micronesia, are collectively known as the Polynesian Outliers. All previously examined Polynesian populations are nearly fixed for mtDNA haplogroup B sequences. Although thought to be derived from this relatively restricted Western Polynesian mtDNA gene pool, over the past 1,500 years, the different Polynesian Outliers have interacted to varying degrees with their neighboring Melanesian and Micronesian populations reflecting a range of physical and cultural isolation resulting in the potentially diverse populations observed today. In order to characterize the extent of gene flow between Polynesian Outlier populations and their geographic neighbors we compared mtDNA HVS1 sequences from 253 individuals representing ten Polynesian Outlier populations and 481 individuals from 17 geographically adjacent populations from Melanesia, Micronesia, and Polynesia. These comparisons revealed substantial variation (0% to 90%) in inferred maternal lineage replacement within Polynesian Outlier populations. Populations with minimal inferred maternal gene flow tended to be small, remote islands in Micronesia, the Solomon Islands, and northern Vanuatu, while populations in central and southern Vanuatu and on high islands surrounded by Melanesian populations were substantially admixed.

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## 619/W/Poster Board #277

**Accurate inference of individual ancestry geographic coordinates within Europe using small panels of genetic markers.** *P. Paschou[1], J. Lewis[2], P. Drineas[2].* 1) Dept of Molecular Biology & Genetics, Democritus University of Thrace, Alexandroupoli, Greece; 2) Rensselaer Polytechnic Institute, Troy, NY, USA.

The study of genomewide datasets of thousands of individuals of European ancestry supports the close correspondence between genetic distances and geographic coordinates within Europe, especially when information from hundreds of thousands of genetic markers is used. In fact, Principal Components Analysis (PCA), summarizing genetic variation over the top two principal components (PCs), results in plots that are surprisingly reminiscent of geographic maps of Europe. We set out to discover those markers that are most closely correlated with geographic origin, seeking to predict individual ancestry at a fine level, and even for closely spaced populations. To this end we analyzed a previously described subset of the Population Reference Sample (POPRES). We focused on 12 populations and 1224 individuals for which geographic coordinates (longitude and latitude) of individual origin are given for at least 20 individuals per population. First, we performed a complete leave-one-out crossvalidation experiment using 447,212 SNPs, and a simple nearest neighbors approach to infer geographic coordinates. This resulted in extremely high accuracy, placing individuals within an average longitudinal error of 2.2 degrees, and an average latitudinal error of 0.88 degrees. Next, we applied an algorithm that we have previously described to select the top 5,000 SNPs that correlate well with population structure as captured by PCA. We then filtered highly correlated SNPs using standard linear algebraic algorithms for the column subset selection problem. We thus selected 500 maximally uncorrelated markers, which have a Pearson correlation coefficient of 0.92 with PC 1, and 0.83 with PC 2. We extensively validated the effectiveness of such SNP panels for genetic ancestry testing by once more performing a complete leave-one-out crossvalidation experiment on the 1224 studied individuals (approx. two weeks of CPU time in commodity hardware). Using 500 carefully selected SNPs we can place individuals within a few hundred kilometers of their reported origin (average longitudinal and latitudinal error of 4.7 and 1.9 degrees respectively). Finally, we crossvalidated our best panel of 500 SNPs on the HapMap CEPH European individuals, placing them accurately on the Northwestern corner of Europe. Not surprisingly, our SNP panel includes markers that are either within genes reported to be under selective pressure in Europeans, or in high LD with such genes.

## 620/W/Poster Board #278

**Limited distribution of a common cardiomyopathy-associated variant in India.** *T.S. Simonson[1], Y. Zhang[1], C.D. Huff[1], J. Xing[1], W.S. Watkins[1], D.J. Witherspoon[1], S.R. Woodward[2], L.B. Jorde[1].* 1) Department of Human Genetics, University Utah, Salt Lake City, Utah; 2) Sorenson Molecular Genealogy Foundation, Salt Lake City, Utah.

Cardiomyopathy is a leading cause of heart failure in South Asia. Myosin binding protein C (MYBPC3) is expressed in the heart muscle, where it regulates the cardiac response to adrenergic stimulation and is important for the structural integrity of the sarcomere. Mutations in the *MYBPC3* gene are associated with hypertrophic or dilated cardiomyopathies. A 25-base-pair deletion in intron 32 causes skipping of the downstream exon and is associated with familial cardiomyopathy. The deletion is found only in South Asian individuals. In order to better characterize the distribution of this variant among South Asians, we determined its frequency in 660 individuals from 24 populations, including neighboring populations from Pakistan and Nepal. Consistent with previous reports (Dhandapany *et al.* 2009, *Nature Genetics*), the heterozygote frequency is as high as 19% in some of our Indian samples, although it is not present in any of the populations we sampled outside of India. The limited geographic distribution of this mutation is surprising considering its estimated age of 30,000 years before present. The differences in the deletion frequencies among populations in India are consistent with patterns of variation we observe among Indian populations based on high-density SNP chip data (Xing *et al.* 2009, *Genome Research*). For example, genome-wide SNP data indicate that caste populations are relatively homogeneous, whereas tribal Indian populations are more distinct from caste populations and each other, reflecting the effects of genetic drift. The South Indian Khonda Dora tribal population, who exhibit genetic similarity to Southeast Asians, has the highest frequency of the deletion (19% of individuals are heterozygotes), whereas the mutation is completely absent in our Irula sample, another South Indian tribal group. More than 15% of the Mala and Yadava (lower and middles castes, respectively) are heterozygotes; all other Indian populations sampled, including upper-caste Brahmins, exhibit heterozygote frequencies less than 10%. Our results indicate the *MYBPC3* deletion is primarily found among Indian populations and that its distribution is consistent with genome-wide patterns of variation in this region.

## 621/W/Poster Board #279

**Evidence for Population Bottleneck in Stone Age Finland from archaeological and genetic perspective.** *T. Sundell[1,2], M. Heger[1], J. Palo[3], A. Sajantila[3], P. Onkamo[1].* 1) Department of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland; 2) Institute for Cultural Research, Department of Archaeology, University of Helsinki, Helsinki, Finland; 3) Department of Forensic Medicine, University of Helsinki, Helsinki, Finland.

Investigation of population continuation has been a confounding issue in Finnish archaeology. Around 6,000 years ago Finland seems to have undergone a powerful and rapid development shown by the appearance of new cultural manifestations. This can be clearly seen in archaeological material. Furthermore the amount of dwelling sites increases threefold from the previous era only to plummet after 3,750 calBC. Thus, archaeologically there is emerging evidence for a population bottleneck. Genetically, it has long been assumed that the reduced genetic diversity of the Finns and the specific 'Finnish Disease Heritage' could be explained by founder effects and bottlenecks.

Since archaeological specimen, in the form of crop remains and bones i.e. aDNA, is scarcely found and almost non-existing from the Stone Age due to the poor preservation of bone material caused by the acidic soil in Finland, direct measurement of past population densities has been somewhat arbitrary. However, through the application of Bayesian archaeology and population genetic simulations it has become possible to estimate prehistoric population demography, framed by present day population diversity. The first estimations of ancient population densities show that based on radiocarbon datings (N=937), consisting of all independent findings in Finland, the demographic fluctuations have been more significant in the eastern part of Finland with the population size reaching its peak approx. 3750 calBC in the typical Comb Ceramic Culture, only to rapidly decline in few centuries.

In this multidisciplinary study we will shed new light on the prehistory of the Finns by integrating evidence from genetic and archaeological data. Bringing together apparently completely different types of information: rich archaeological data existing from the Finnish prehistory spanning from 10,000 years ago together with coalescence histories of mtDNA and Y chromosomal haplotypes in our country, equals to more profound conclusions. The questions to be answered are: when exactly did the archaeologically justified and genetically often assumed population bottlenecks take place in our past and how deep were they? How can the possible bottlenecks be seen in our gene pool and thus present genetic variation?

## 622/W/Poster Board #280

**Genetic relationships among the ancient Chinese populations viewed from discrete cranial traits.** *J. Tan[1,4], W.Q. Fu[1,4], Y.G. He[2], L.M. Li[1], Y. Wang[1], Z. Xu[1], B.S. Li[1], X.D. Chen[1], K.X. Han[3], H. Li[1], L. Jin[1,2].* 1) State Key Laboratory of Genetic Engineering and MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, China; 2) Chinese Academy of Sciences and Max Planck Society Partner Institute for Computational Biology, SIBS, CAS, Shanghai 200031, China; 3) Institute of Archaeology, Chinese Academy of Social Sciences, Beijing 100710, China; 4) Equally contributed. This study was supported by National Natural Science Foundation of China (3057101), Chinese National Science Fund For Distinguished Young Scholars (30625016), and Key Project of CMOST Natural Science Foundation (30890034).

The discrete cranial traits are informative in revealing the genetic relationship of human populations. Given little available knowledge on these traits, especially their underlying genetic determinants, the primary aim of this study is to select a small number of traits that are sufficiently informative to represent genetic differentiation among East Asian populations. We studied overall 51 traits for 1,578 skulls from 19 necropolises, and found that 5 traits could capture the largest variation in East Asian populations studied. They are accessory mandibular foramen, palatine torus, mandibular torus, mastoid foramen extra-sutural, and infraorbital suture. The analysis on these 5 traits resulted in similar population relationships to that using all 51 traits. The study on discrete cranial traits could not only facilitate exploration of the genetic relationship of populations, and could also allow identification of the genes underlying these anthropological traits.

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## 623/W/Poster Board #281

**Ancestry reflects history: genetic implications for epidemiologic studies in admixed populations.** *M. Via[1,2], C.R. Gignoux[2,3], L. Fejerman[2,4], G. Toro Labrador[5], J. Viera Vera[6], K. Beckman[7], E.G. Burchard[1,2,8], J.C. Martínez Cruzado[5].* 1) Dept Medicine, University of California, San Francisco, CA; 2) Institute for Human Genetics, University of California, San Francisco, CA; 3) Program in Pharmaceutical Sciences and Pharmacogenomics, University of California, San Francisco, CA; 4) Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA; 5) Dept. Biology, University of Puerto Rico, Mayagüez, PR; 6) Dept. Biology, University of Puerto Rico, Río Piedras, PR; 7) Dept. Genetics, Cell Biology and Developmental Biology, University of Minnesota, Minneapolis, MN; 8) Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA.

Admixture among human populations is not a random process. However, the effect that historical and geographical factors have exerted on the distribution of admixture and its potential impact on epidemiological studies within a population or geographic region has not been analyzed. We modeled the geographical variation of admixture in the island of Puerto Rico using spatial analysis tools. A population-based sample of 642 Puerto Rican individuals collected throughout the island was genotyped for 106 ancestry informative markers (AIMs). Individual ancestry estimates (IAE) were calculated through a Bayesian approach and location at the census block level was determined for each sample. The place of birth for the grandparents was used to compare the distribution of admixture across different generations. Average ancestry values for the Puerto Rican population were 25.05%, 22.77%, and 52.18% for the African, Native American, and European contributions, respectively. There were significant differences in the ancestry proportions between municipalities and between main geographic regions in the island (Kruskal-Wallis $p < 10^{-4}$). African ancestry clustered in the eastern part of the island (Moran's I $p < 10^{-9}$), reaching a maximum of 51.3% in the municipality of Loíza. Native American ancestry was significantly clustered in the northern part of the island (Moran's I $p < 10^{-9}$) even though regional variation in this ancestral component was smaller. Similar results were obtained when samples were relocated according to the grandparents' place of birth (Moran's I $p < 10^{-9}$, both for African and Native American ancestry). The locations of historical sites from the colonial period (i.e. sea ports associated with the slave trade and the sugar mills) were associated with the geographical distribution of African ancestry, but the influence of these factors showed regional variation according to the results of geographically weighted regressions. These results suggest that the design of genetic studies in Puerto Rico must take into account regional differences in ancestry. Sampling in different regions of the island results in significant differences in statistical power for admixture mapping studies. These results have important implications for genetic association studies of complex traits and drug response.

## 624/W/Poster Board #282

**Analysis of Genomic Admixture in Costa Rica Population.** *Z. Wang[1,2], K. Yu[1], G. Thomas[1], L. Burdette[1,2], A. Hutchinson[1,2], R. Herrero[3], S. Wang[1], A. Hildesheim[1], S. Chanock[1].* 1) Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland, United States of America; 2) Core Genotyping Facility, Advanced Technology Program, SAIC Frederick, Inc. National Cancer Institute - Frederick, Frederick, Maryland, United States of America; 3) Proyecto Epidemiologico Guanacaste, San Jose, Costa Rica.

Costa Rica (CR) population is a unique population representing a typical admixture of major continental ancestral populations. 1,301 samples collected from participants in a population-based study conducted in the Guanacaste region of CR were genotyped on a custom Illumina iSelect chip harboring 27,635 SNPs. The SNPs on the chip were selected based on multi-ethnic tagging strategy for three HapMap populations: CEU, YRI and JPT+CHB and cover 1,000 candidate genes/regions for a range of cancers. This data set was sufficiently large for the investigation of population substructure in our CR study and the examination of linkage disequilibrium (LD) patterns. Three HapMap major continental populations and a Native American population from the Illumina iControl DB were used as the reference populations for these analyses. Our preliminary results indicate that the Guanacaste CR population was formed mainly by a three-way admixture with 42.5%, 38.3% and 15.2% Native Indian, European, and African respectively. In addition, 4.0% residual genetic component derived from Asians was observed in our CR samples. Both model based STRUCTURE program and Principal Component Analysis (PCA) revealed consistent substructure pattern for the CR population. The magnitude of LD in the CR population seems to be smaller than all the reference populations except YRI. A more detailed knowledge of the underlying genetic structure of the CR population would be informative to assess its population genetic history and to assist in the interpretation of investigations of complex diseases in the CR or a comparably admixed population. Funded by NCI Contract No. HHSN261200800001E.

## 625/W/Poster Board #283

**Admixed ancestry and stratification of regional gene pools of Quebec.** *C. Bherer[1,2,3], D. Labuda[1,4], L. Houde[2,5], M. Tremblay[2,6], H. Vézina[2,6].* 1) CHU Sainte-Justine Research Center, Montréal, Québec, Canada; 2) Département de Biologie Moléculaire, Université de Montréal, Montréal, Québec, Canada; 3) Interdisciplinary Research Group in Demography and Genetic Epidemiology, Université du Québec à Chicoutimi, Chicoutimi, Québec, Canada; 4) Département de Pédiatrie, Université de Montréal, Montréal, Québec, Canada; 5) Département de Mathématiques et d'Informatique, Université du Québec à Trois-Rivières, Trois-Rivières, Québec, Canada; 6) Département des Sciences Humaines, Université du Québec à Chicoutimi, Chicoutimi, Québec, Canada.

In Quebec, studies of different molecular polymorphisms have shown that the French Canadian gene pool is as diverse as its source European populations and, contrary to what was previously anticipated, does not display more homogeneity. To better understand the genetic structure of the contemporary population, we analyzed the origins and contribution of 7,798 immigrant founders identified in the genealogical ascendance of a sample of 2,221 subjects representative of the French Canadian population of Quebec. As expected, French founders are the most important in number (n=5,326) in all Quebec regions. They contribute for about 90% of the regional gene pools, except for regions located in the easternmost part of the province (76%), which are characterized by more diverse origins. Although this study supports the French founders' importance, it also puts in the balance arguments in favor of the heterogeneity of the founding pool. The majority of immigrants landed as single member of their family, originating from all the regions of France. In addition, nearly all subjects have mixed origins, including French and non-French. Taken together, these results put into perspective the idea of the homogeneity of the origins of the French Canadians and of a pan-Quebec founder effect. The differential descent and genetic contribution of immigrant founders across regions points to the stratification of the French Canadian population of Quebec, showing a east-west gradient of diversity. These results will contribute to optimize study design in gene mapping studies relying on the founder effect in the French Canadian population of Quebec.

## †626/W/Poster Board #284

**Genome-wide patterns of population structure and admixture among Hispanic/Latino populations.** *K. Bryc[1], C. Velez[2], M. Hammer[3], R. Hernandez[4], A. Reynolds[1], A. Auton[5], T. Karafet[3], H. Ostrer[6], C.D. Bustamante[1].* 1) Dept Biol Statistics/Comp Biol, Cornell Univ, Ithaca, NY; 2) Human Genetics Program, Department of Pediatrics, New York University School of Medicine, New York, NY; 3) ARL Division of Biotechnology and Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ; 4) Department of Human Genetics, University of Chicago, Chicago, IL; 5) Wellcome Trust Centre for Human Genetics, Oxford, OX3 7BN, United KingdomDepartments of Medicine, Pediatrics (Genetics) and Pathology, New York University School of Medicine, New York, NY; 6) Departments of Medicine, Pediatrics (Genetics) and Pathology, New York University School of Medicine, New York, NY.

In order to document genome-wide patterns of variation in Hispanics/Latinos (HL's) we genotyped individuals from five distinct populations recruited in the US: Mexico, Colombia, Ecuador, Dominican Republic and Puerto Rico. We present population structure results from an extensive genome-wide SNP dataset compiled by merging Affymetrix 500K and Illumina 650K data from these populations together with the Human Genome Diversity Panel, HapMap, Mao et al (2005), and POPRES studies. We apply Principal Component Analysis (PCA) and a clustering method, frappe, to infer admixture and genetic relationships of 262 HL individuals with 467 Africans, 715 Europeans, and 210 Native Americans comprising a total of 88 populations. We observe substructure within Native Americans, and, as expected, find that the admixed HL populations show Native American ancestry derived from local Native American populations. We find striking differences in estimated population-wide mean African, European and Native American ancestry proportions which are consistent with historical admixture and proximity to slave trade routes. The Dominican Republic and Puerto Rico, located on islands along slave trade routes, show high levels of African Ancestry (means 41.7% and 23.6% respectively) with less Native American Ancestry (11.5% and 18.9%). Colombians show a wide range of both African and Native American ancestry, though they have an overall mean of slightly higher Native American ancestry (36.3%) and lower African ancestry (11.7%) than the highly-African Dominicans and Puerto Ricans. Ecuadorians show the highest Native American mean ancestry (54.0%) with low estimated mean African Ancestry (7.3%). Mexico shows the largest range of Native American ancestry (11.0% - 79.0%) with an overall mean of 50.1% Native American ancestry and the lowest African ancestry (5.6%). Our study shows a broad range in admixture proportions across different HL individuals as well as different admixture patterns across populations. We also compare this genotype data with mtDNA and Y chromosome genotypes and use simulations to estimate ancient male and female sex ratios in each HL population. Lastly, we discuss implications of population structure for genome-wide association studies in admixed populations such as HL's, especially when recruited in the United States.

† Trainee Award Finalist

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## 627/W/Poster Board #285

**Population Structure in Brazilian and other Worldwide Human Populations Revealed by SNP arrays.** *S.R. Giolo[1,2], J.M.P. Soler[3], M. de Andrade[4], J.E. Krieger[2], A.C. Pereira[2].* 1) Department of Statistics, Federal University of Parana, Curitiba, PR, Brazil; 2) Laboratoty of Genetics and Molecular Cardiology, Heart Institute, University of Sao Paulo, SP, Brazil; 3) Department of Statistics, University of Sao Paulo, SP, Brazil; 4) Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN, USA.

Brazilians are one of the most admixture populations in the world, formed by extensive interethnic crosses between Amerindians, Europeans, Africans and Asians. Although there are several studies that have investigated the genetic structure of several populations, the worldwide coverage remains incomplete with, for instance, populations from South America being under-represented in databases of human genetic variation. Only in such continent, almost 200 millions of people live in Brazil, which represents around 52% of the South America population and 3% of the world's inhabitants. An investigation of the genetic structure of this population is therefore of interest since it can provide complementary insights to other large studies of genetic variation. To this end, we have analyzed patterns of genetic variation across 365,116 single nucleotide polymorphisms (SNPs) genotyped using Affymetrix SNP array 6.0 and/or Illumina Human 1M in 1,129 unrelated individuals from twelve worldwide populations: one Brazilian and eleven of the International HapMap Project, Phase III, which include Amerindian, European, African, and Asian ancestry. In our analysis we have considered only the SNPs that all these populations had in common, excluding those with more than 5% missing entries, not in Hardy-Weinberg equilibrium and with minor allele frequency smaller than 1%. Principal components analysis applied to this data revealed discernible genetic differentiation among these populations and also that the large degrees of racial miscegenation experienced by the Brazilian population over centuries play an important role in its pattern of genetic variation. In this work, we have also analyzed whether small panels of markers could effectively capture the genetic variation revealed by all 365,116 markers. In this regard, we found that around 500 SNPs could efficiently reproduce the revealed population structure.

## 628/W/Poster Board #286

**Spatial autocorrelation in Asia using Principal Components of HLA Haplotype Frequencies.** *L. Gragert[1], M. Maiers[1], W. Klitz[2].* 1) Bioinformatics, National Marrow Donor Program, Minneapolis, MN; 2) Public Health Institute, Oakland, CA.

Geographic patterns of genetic diversity have corroborated the isolation-by-distance model of human migration. Visualizing genetic differences between humans using principal component analysis (PCA) has been accomplished using SNP data from multiple markers across the genome to compare individuals. Previous work using thousands of SNPs has shown that principal component values can be used to map the locations of individuals in Europe with a high degree of accuracy. We were able to accomplish a similar feat using a set of 3 linked HLA loci, the most polymorphic gene system in humans. The high dimensionality of full haplotype frequency distributions can be reduced to a few dimensions that capture the majority of the population variation. Visualizing haplotype frequency differences between populations is valuable for examining population history and barriers to gene flow. PCA maps were generated from 2-digit HLA A-B-DR haplotype frequencies of 7 different Asian populations (18,620 Chinese, 11,076 Filipino, 1,242 Hawaiian, 33,970 Indian, 8,753 Japanese, 20,605 Korean, and 13,507 Southeast Asian). We performed Mantel correlation tests between matrices of great circle distance between the population centers and Euclidean distance between PCA coordinates for the first 5 PCA dimensions. We detected correlation between PCs 3 and 4 and geographic distance with a correlation coefficient of 0.725 and a P-value of 0.028. The PCA chart for PCs 3 and 4 showed large separation of Indian and Hawaiian populations from the others in a way that closely mirrors the map of Asia. This work demonstrates the utility of the HLA system for population studies and provides new genetic evidence for examining human population relationships and the colonization of the Asian continent.

## 629/W/Poster Board #287

**A new statistical method to infer population admixture events using genetic variation data.** *G. Hellenthal[1], D. Falush[2], S. Myers[1].* 1) Dept Statistics, Univ Oxford, Oxford, United Kingdom; 2) Department of Microbiology, Environmental Research Institute, University College Cork, Cork, Ireland.

We present a novel statistical method that uses densely-spaced Single-Nucleotide-Polymorphism (SNP) data to identify the major admixture events occurring throughout a population's history. The model has several advantages over leading available analytical approaches in this area, such as principal-components-analysis and STRUCTURE. In particular it can simultaneously (i) take advantage of the information inherent in patterns of linkage disequilibrium, i.e. non-random associations amongst neighbouring SNPs along a chromosome, (ii) efficiently analyse hundreds of individuals at hundreds of thousands of SNPs genome-wide, and (iii) allow for relatively straight-forward interpretation and direct inference of key historical parameters, such as the proportions and times of major admixture events. Using simulated data matched to currently available human datasets, we show that our model can identify and accurately date admixture events that have occurred between 7 and 150 generations ago. As our technique exploits the rich information in genetic data to infer details of a population's admixture history, it marks a powerful complement to anthropological research and can help to resolve a number of existing controversies. We present results from applications of our model to two datasets: (1) SNP data from 22 distinct genetic regions for individuals from three chimpanzee populations in Africa; (2) genome-wide 650K SNP data for individuals from 53 world-wide populations of the Human Genome Diversity Panel (Science 319, 1100-1104). We highlight a number of intriguing new insights from these analyses. For example, the chimpanzee analysis showcases the model's ability to infer the relative divergence among populations. The human analysis identifies several important admixture events, some of which are historically well-established (e.g. identification of recent European genetic influx into the Maya Native American population), others that can be placed into a clear historical context (e.g. an East Asian genetic influx into several Central and South Asian populations dated precisely to the era of the Mongol empire), and some that are to our knowledge novel (e.g. admixture in the Cambodian population between a Central/South Asian source and an East Asian source dated to around the period of the Cambodian Empire).

## 630/W/Poster Board #288

**Analysis of Assortative Mating Patterns in Humans.** *S. Kim[1], R. Jiang[1], E. Jorgenson[2], J. Wall[1], J. Witte[1].* 1) Inst Human Gen, Univ California, San Francisco, San Francisco, CA; 2) Ernest Gallo Clinic and Research Center, Emeryville, CA.

Assortative mating patterns in plants are well document, but among mammalsin particular humanssuch mating patterns remain unclear. Understanding assortative mating in humans can provide insight to genomic regions involved with positive or negative selection. To help clarify mating patterns in humans we have developed two methods to detect genetic signatures of assortative mating. The first method is a likelihood-based approach which measures overall genetic similarity at a given locus compared to a panmictic population. The second method is a variance components approach that includes an over-all genetic similarity matrix to adjust for potential confounding due to population stratification. We apply both of our methods to the HapMap data to identify genomic regions that are potentially involved with assortative mating (e.g., MHC). In addition, we provide a simulation-based approach to identify the degree of positive assortative mating required to generate the genetic variation observed in the current human population.

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### 631/W/Poster Board #289

**World-wide patterns of genomic inbreeding estimates in unrelated individuals from the HGDP-CEPH diversity panel.** *A.L. Leutenegger[1,2], M. Sahbatou[3], H. Cann[3], E. Génin[1,2].* 1) INSERM, U946, Paris, France; 2) Univ Paris-Diderot, UMR-S 946, Paris, France; 3) Fondation Jean Dausset, CEPH, Paris, France.

We present inbreeding coefficients estimated from genotypes at 642,914 autosomal SNPs (Illumina650Y) for 940 unrelated HGDP-CEPH individuals from 51 populations (Li et al., Science, 319:1100-4, 2008).

FEstim (Leutenegger et al., AJHG, 73:516-23, 2003) was used to estimate the inbreeding coefficients from these individuals' genomic information. It is a maximum likelihood method that uses a hidden Markov chain to model the dependencies along the genome between the (observed) marker genotypes and the (unobserved) homozygosity-by-descent status. This estimation requires a marker map without linkage disequilibrium (LD); this is not the case for very dense SNP maps. We developed a procedure to generate multiple LD free sub-maps and to combine their information. 75% of the original map could be captured using 1,000 sub-maps.

Inbreeding levels were highest in the Americas, the Middle East and Central South Asia. In some populations of these regions, they reach values above 0.1 (similar to what is expected from double-first cousin or uncle-niece marriages). The lowest levels of inbreeding were found in Europe, East Asia and sub-Saharan Africa. For the Mbuti Pygmy and San samples, results were not always consistent between the different random maps. This may be related to the fact that these populations are known to have allele frequencies different from the other sub-Saharan populations. Indeed, inbreeding coefficient estimates depend on allele frequencies, and because of limited population sample sizes, these frequencies were estimated at the regional level. An allele frequency sensitivity analysis of these populations is underway.

### 632/W/Poster Board #290

**Genetic Landscape of Eurasia Viewed from Large Allele Frequency Differences.** *H. Li, K. Cho, J.R. Kidd, K.K. Kidd.* Department of Genetics, School of Medicine, Yale University, New Haven, CT 06520.

The diversification leading to modern human populations in Eurasia is one of the most important topics in the study of human expansions after leaving Africa. Most studies of Eurasia populations have used either limited markers or involved insufficient population coverage. We chose 68 markers based on large allele frequency differences among a few Eurasian populations and then typed them on 1766 individuals from 34 populations representing all subdivisions of Eurasia. Analyses using the STRUCTURE program showed a clinal east-west division when K=2, with a median border dividing Central Asia along the Ob River, the Kazakh highland, the western side of Pamir Mountains, and the southwestern side of the Himalayas. We fit curves to the STRUCTURE loadings using distances of the population coordinates from the median border. The genetic structure changed dramatically only within 2000km on each side of the border. At higher values of K the western populations of East Asia are the first to be distinguished (at K=3): Mongols, Tibetans, Qiang, and Baima, are most distinct from the more eastern populations. At K=4 Southwest and South Asians are distinguished from the Europeans; At K=5 Southeast Asians and at K=6 Central Asians are successively distinguished from eastern East Asians. Several more isolated populations such as Samaritans, Atayals, or Micronesians were distinguished in different independent runs when K=7 providing no clear anthropological information. South Asians were always clustered with Southwest Asians with pronounced similarity to Central Asians. The failure to distinguish South Asians maybe due to the selection of the markers with large allele frequency differences specifically between Europeans and East Asians. We also tested for statistical differences in the allele frequencies for all pairs of clusters when K=6. The results showed significant borders (P<0.0001) including those between western East Asians and eastern East Asians or Central Asians; however, insignificant borders were observed between Southwest Asians and Southeast Asians or western East Asians, neither was between Central Asians and eastern East Asians. This indicates substantial gene flow in North Asia between eastern East Asians and Central Asians, and in South Asia between South Asians and Southeast Asians. Using increased population and marker coverage, this study helps to understand the details of genetic diversity and landscape of Eurasians.

### 633/W/Poster Board #291

**Characterizing the history of sub-Saharan African gene flow into southern Europe.** *P. Moorjani[1], N. Patterson[2], J. Hirschhorn[1,3], D. Reich[1,2].* 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Broad Institute, Cambridge, MA; 3) Divisions of Endocrinology and Genetics and Program in Genomics, Children's Hospital, Boston, MA.

Recent analyses of whole-genome SNP data sets have suggested a history of sub-Saharan African ancestral contribution into southern Europe but not in northern Europe, consistent with previous analyses based on the Y-chromosome and mitochondrial DNA. However, there has been no characterization of the proportion of African admixture in southern Europe, or of its date. Here we analyze data from ~450,000 autosomal SNPs in the Population Reference Sample, ~650,000 SNPs from the Human Genome Diversity Panel, and ~1.5 million SNPs from the HapMap Phase 3 Project, and studied patterns of correlation in allele frequencies across populations to confirm the evidence of African ancestry in many southern European populations but not in northern Europeans. Using methods that can infer admixture proportions in the absence of accurate ancestral populations, we estimated that the proportion of sub-Saharan African ancestry in Spain is 2.4 +/- 0.3%, in Tuscany 1.5 +/- 0.3%, and in Greece 1.9 +/- 0.7% (1 standard error). We also studied the decay of admixture linkage disequilibrium with genetic distance, which provided a preliminary estimate of the date of African gene flow into Spain of roughly 60 generations ago, or about 1,700 years ago assuming 28 years per generation. This date is consistent with the historically known movement of individuals of North African ancestry into Spain, although it is possible that this estimate also reflects a wider range of mixture times.

### 634/W/Poster Board #292

**Recovering mitochondrial DNA lineages of extinct Amerindian nations in extant homopatric Brazilian populations.** *V.F.G. Oliveira[1], F.C. Parra[1], H. Gonçalves-Dornelas[1], C. Rodrigues-Carvalho[2], H.P. Silva[2], S.D.J. Pena[1].* 1) Biochemistry, UFMG, Belo Horizonte, Minas Gerais, Brazil; 2) Museu Nacional do Rio de Janeiro, Rio de Janeiro, Brazil.

Brazilian Amerindians suffered a drastic population decrease in the past 500 years. Most autochthonous groups which existed in the early 16th century, especially those in the eastern seashore have vanished. Their mitochondrial haplotypes, however, still persist among Brazilians. Our studies show that in Brazil circa 50 million people carry mitochondrial lineages of Amerindian origin. Such reservoir is a rich potential information source about extinct Amerindian nations. Our objective was to test if through the study of the present-day rural populations that inhabit the region anciently occupied by Botocudo Indians, we could identify and rescue the mitochondrial lineages from this largely extinct Amerindian group. We have called this methodology "Homopatric Targeting". We studied 174 individuals living in a small village in the northeast part of the state of Minas Gerais, a territory previously occupied by Botocudos. Pedigree analysis revealed 74 persons without matrilineal relationship, from which DNA samples were obtained. One hundred individuals from surrounding cities were used as control group. The screening of A, B, C and D mtDNA lineages was done by RFLP. We identified 20 Amerindian lineages, which had their HVRI and HVRII sequenced. We finally obtained 13 Amerindian haplotypes, of which 7 appeared to be novel, since they were not present in available databanks. Among the 13 haplotypes we observed a significant excess of haplogroup C (70%) and absence of haplogroup A, the most common in the control group. The novelty of the haplotypes and the excess of the C haplogroup suggested that we indeed might have identified Botocudo lineages, although genetic drift might also explain some of these differences. In order to validate our strategy, we studied teeth extracted from 12 ancient skulls of Botocudo Indians (approximately 200 years ago) from the anthropological collection of the National Museum of Rio de Janeiro. We recovered mtDNA sequences from all the teeth, identifying only 4 different haplotypes (a very low haplotypic diversity of 0.78), two of which were present among the lineages observed in the extant individuals. This validates "Homopatric Targeting" as a useful new strategy to study the peopling and colonization of the New World, especially when direct analysis of the genetic material is not possible.

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## 635/W/Poster Board #293

**Two major post-glacial genetic sources for the peopling of Sardinia.** *M. Pala[1], A. Achilli[1,2], A. Olivieri[1], U.A. Perego[1,3], B. Hooshiar Kashani[1], V. Carossa[1], S.R. Woodward[3], A. Torroni[1].* 1) Dipartimento di Genetica e Microbiologia, Università di Pavia, Pavia, Italy; 2) Dipartimento di Biologia Cellulare e Ambientale, Università di Perugia, Perugia, Italy; 3) Sorenson Molecular Genealogy Foundation, Salt Lake City, Utah, USA.

Sardinia remained unconnected with the mainland even when the sea level was lowest during the Last Glacial Maximum and was probably the last of the large Mediterranean islands to be colonized by modern humans. Due to its geography and history, the island has been characterized by a rather strong isolation that allowed its population to preserve specific genetic and cultural traits. Indeed molecular analyses from both classical and uniparental markers often placed present day Sardinians as “outliers” within the European genetic landscape. In order to clarify timing and dynamics of the peopling of the island, we analyzed the mitochondrial DNA (mtDNA) variation of 922 Sardinian subjects. By sequencing the control region and surveying diagnostic coding region markers we were able to describe in detail the pattern of haplogroup distribution on the island. Three haplogroups - U5b3, H1, and H3 - were found to encompass approximately 35% of the Sardinian population and their frequency distribution was specifically evaluated. These three haplogroups were also analyzed at the highest level of molecular resolution - that of complete mtDNA sequences. The analysis of U5b3, which involved the complete sequencing of 52 mitochondrial genomes, revealed that the ancestral homeland for U5b3 was the Italian Peninsula. According to our reconstruction, in contrast to mtDNA haplogroups that expanded from other refugia, the Holocene expansion of haplogroup U5b3 towards the North was restricted by the Alps and occurred only along the Mediterranean coasts, mainly towards nearby Provence (southern France). From there (about 7-9,000 years ago), a sub-clade of this haplogroup moved to Sardinia, possibly as a result of the obsidian trade that linked the two regions, leaving a distinctive signature in about 4% of today’s islanders. The sequence data obtained from 22 Sardinian mitochondrial genomes belonging to haplogroups H1 and H3 instead highlighted the role played by another European glacial refuge area - Iberia - as major source of founder lineages for the Sardinian gene pool. In conclusion our study reveals that concomitant post-glacial population re-expansions from two distinct geographic sources, the Franco-Cantabrian and the Italian refuge areas, have largely determined the present-day Sardinian genetic landscape. These initial major founding genetic inputs were then extensively shaped through the millennia by both founder effect and genetic drift.

## 636/W/Poster Board #294

**Genetic diversity of European population isolates in the context of their geographic neighbors.** *J. Novembre[1], K. Lohmueller[2], K. Veeramah[1], A.R. Boyko[2], A. Tonjes[3], P. Kovacs[3], I. Klimes[4], M. Stumvoll[3], C.D. Bustamante[2].* 1) Dept Eco & Evo Biol, Interdepartmental Program in Bioinformatics, Univ California, Los Angeles, Los Angeles, CA; 2) Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY; 3) Department of Medicine, University of Leipzig, Leipzig, Germany; 4) Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Mapping traits in population isolates provides an opportunity to simplify the challenges of complex trait mapping because such populations likely have enhanced levels of linkage disequilibrium and reduced genetic heterogeneity for the underlying traits. Here we analyze high-throughput SNP genotyping data to compare genomic-scale patterns of variation in several European population isolates (Adygei, Basque, Orcadian, Roma from Slovakia, Sardinians, and Sorbs) and contrast their patterns of variation to geographical proximal populations. Our results reveal insights for the demographic history of each of these unique populations, suggest substantial variation among these population isolates in patterns of diversity, and highlight the importance of population selection in genome-wide association mapping.

## 637/W/Poster Board #295

**The population frequency of alleles that cause cystinosis, cystic fibrosis, congenital glaucoma and galactosemia in an isolated Old Order Amish community.** *T. Rupar, V. Siu.* Departments of Pediatrics, Biochemistry, and the Children’s Health Research Institute University of Western Ontario, London, Ontario, Canada.

An Old Order Amish community in Ontario, Canada is a very conservative Anabaptist group that originated from the Alsace-Lorraine district bordering Switzerland and France in Europe. There has been little influx of new genes into the community since the founding members arrived in Ontario in 1825. The population of the community is about 900 and includes 220 married couples within 10 church districts. All marriages are consanguineous and about 25% of marriages are isonymous. Several genetic disorders that are rare in the general population are present in increased frequency in this population. There are eight surnames in the population with 37% of the community having the most common surname and the two most frequent surnames accounting for 55% of the community. With support from the community, a program was established in 2002 to screen all newborn and some older children and adults for nephropathic cystinosis, congenital glaucoma, cystic fibrosis and galactosemia. These disorders were selected for newborn screening because clinical experience indicated a relatively high incidence in the population and early diagnosis and intervention improves outcomes for affected children. Mutations known to be present in the community are cystinosis (CTNS G339R), cystic fibrosis (CFTR Δ F 508 and c3905insT), congenital glaucoma (CYP1B1 c1410 del 13 and E387K), and galactosemia (GALT Q199R). Since 2002, DNA has been isolated from umbilical cord blood for newborn and buccal swabs from older children and some adults. Genotypes for the six tested alleles were obtained for about 390 individuals when data from the newborn and older individuals are combined. **Results**: The carrier frequencies in the combined newborn and older population are cystinosis 16%, cystic fibrosis 16% with c3905insT the more common allele, galactosemia 11%, and congenital glaucoma 11% with the E387K the more common allele. A total of 6 alleles were tested with a maximum possible of 12 mutations in one individual. The frequency of mutations in individuals with no mutations was 59%, one mutation 32%, two mutations 8%, and three or more mutations 1%. The maximum number of mutations identified in one individual was 5. Haplotype analysis of normal and mutant alleles was performed for both of the mutations that cause cystic fibrosis and congenital glaucoma and the results indicate that for both diseases the two mutations are present on different haplotypes.

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## 638/W/Poster Board #296

**Genetics of celiac disease in the Saharawi population.** *J. Romanos[1], C.C van Diemen[1], G. Trynka[1], L. Franke[1], A. Zhernakova[2], S. Teresi[3], G. Iacono[4], R. Francavilla[5], S. Castellaneta[5], A. Ravelli[6], M.D Cantarero[7], C. Catassi[8], C. Wijmenga[1].* 1) Dept Genetics, UMCG, Groningen, Groningen, Netherlands; 2) Dept of Medical Genetics, University Medical Center Utrecht, the Netherlands; 3) Dept of Clinical Chemistry, Children's Hospital "G. Di Cristina", Palermo, Italy; 4) Dept of Pediatric Gastrienterology, Children's Hospital, Palermo, Italy; 5) Dept of Pediatrics, University of Bari, Italy; 6) Dept of Pediatrics, University of Brescia, Italy; 7) Pediatrician of Health Center of Illescas, Toledo, Spain; 8) Dept of Pediatrics, Università Politecnica delle Marche, Ancona, Italy.

Celiac disease is an autoimmune disorder of the small intestine caused by a reaction to gluten, a protein present in wheat, barley and rye. Celiac disease occurs in genetically predisposed individuals of all ages and it is estimated to affect approximately 1% of the European population. Symptoms include diarrhea, failure to thrive and abdominal distention. The only treatment is a life-long gluten free diet. Celiac disease is a complex disorder with a strong genetic component: 95% of the patients carry at least one of the risk alleles HLA-DQ2 and HLA-DQ8, which contribute to 40% of celiac disease heretability. This means that having HLA risk variants is necessary but not sufficient to develop the disease. The first genome-wide association study (GWAS) in celiac disease and its follow-up have identified 10 new genetic loci that contribute significantly towards celiac disease risk. Seven of these contain genes controlling adaptive immune responses, including IL2/IL21 (4q27), RGS1 (1q31), IL18RAP (2q11-2q12), CCR3 (3p21), IL12A (3q25-3q26), TAGAP (6q25), TNFAIP3 (6q23.3) and REL (2p16.1) and SH2B3 (12q24). The highest prevalence of celiac disease in the world (5.6%) has been described in the Saharawi, an African population living in Western Sahara and of Arab-Berber origin. It is yet unknown whether this results from a higher frequency of celiac disease risk alleles or that other genes are contributing to increased risk in this population compared to other populations. Our aim is to study the genetics of celiac disease and the involvement of susceptibility celiac genes in the genetically isolated population of Saharawi. We conducted a GWAS in 166 individuals, including 38 perfect trios, using the Illumina Infinium 610K HD beadchips. Analysis of the data revealed the linkage disequilibrium (LD) structure in this population to be different to that of Caucasians. Moreover, we were able to replicate the association of the HLA region to celiac disease. We investigated in detail the previously described IL12A/SCHIP region and showed association to two SNPs from this region to celiac disease in the Saharawi population. In order to increase the power of the study, we are currently genotyping 192 additional individuals. Moreover, the LD structure will help in narrowing down the associated regions initially identified in Caucasian populations. This information should assist us in identifying the causal celiac genes.

## 639/W/Poster Board #297

**DISCERNING KIR HAPLOTYPES.** *M. Maiers[1], S. Spellman[1], C. Vierra-Green[1], H. Noreen[2], M. Stewart[2], N. Yu[3], T. Lebeveda[3], E. Reed[4], R. Rajalingam[4], C. Hurley[5].* 1) Bioinformatics, National Marrow Donor Program, Minneapolis, MN; 2) University of Minnesota, Minneapolis, MN, USA; 3) American Red Cross, Dedham, MA; 4) UCLA, Los Angeles, CA; 5) Georgetown University.

KIR (Killer Immunoglobulin-like Receptors) whaplotype frequency analysis has been hampered by a lack of solid reference data with as many as 42 haplotypes described in the literature from a variety of sources determined using indirect and sometimes incompatible methods. We describe an analysis utilizing only reference haplotypes confirmed by genomic sequencing. A total of 509 European American samples were typed for 14 KIR loci (2DL1-5, 3DL1-3, 2DS1-5 and 3DS1). All samples have complete high resolution HLA data (HLA-A, B, C, DRB1/3/4/5, DQA1, DQB1, DPA1 and DPB1) and ~85% have cell lines available. Each sample was typed in duplicate by two of three different contracted laboratories. The typing was completed using a two-tiered approach: KIR gene presence/absence was determined by low-resolution methods followed by high resolution allelic typing using a combination of SSO, SSP and SBT. We developed software to interpret our data in light of the 7 gene-content haplotypes. We found that over 97% of the observed presence/absence genotypes could be described as combinations of 6 of these reference haplotypes. Furthermore, KIR genes that are located in different positions on the reference haplotypes (e.g. KIR2DL5, KIR2DS3/5) and framework KIR genes (e.g. KIR3DL2, KIR3DL3) exhibited allele-specific variation that correlated with gene-content haplotype. This analysis suggests a simpler picture of KIR haplotype and allele variation than described in earlier studies.

## 640/W/Poster Board #298

**Pedigree and kinship analysis of a large founder population in rural mid-Michigan.** *B.C. Schutte[1,2,3,4], R.A. Fisher[2], J.D. Bonner[3], C. Wei[5], Q. Lu[5], K.H. Friderici[1,2,4], J.L. Elfenbein[6], E. Wilch[4], D.L. Schutte[7].* 1) Microbiol and Molec Gen, Michigan State Univ, East Lansing, MI; 2) Pediatrics and Human Development, Michigan State Univ, East Lansing, MI; 3) Comparative Medicine and Integrative Biology, Michigan State Univ, East Lansing, MI; 4) Genetics PhD Program, Michigan State Univ, East Lansing, MI; 5) Epidemiology, Michigan State Univ, East Lansing, MI; 6) Communicative Sciences and Disorders, Michigan State Univ, East Lansing, MI; 7) College of Nursing, Michigan State Univ, East Lansing, MI.

Previously, we identified a cluster of individuals with an autosomal recessive form of deafness from a small region of mid-Michigan. Eleven cases are homozygous for the 35delG mutation of GJB2, and four cases are heterozygous for 35delG and a novel deletion located upstream of GJB2. We hypothesized that affected individuals descend from a founder population and the population has a high degree of kinship. Using public records and personal interviews, we constructed a genealogical database that included the extended families and ancestors of individuals who are deaf from this rural Mid-Michigan region. We used a novel algorithm similar to the connected components algorithm to cluster individuals into extended pedigrees and used the methods of Crow and Mange to calculate the degree of kinship based on isonymy. The genealogical database contains 27,726 records and includes 1681 surnames and 6328 marriages from ancestors and descendents of the founders. Although our analysis grouped the individuals into 336 pedigrees, 25,899 individuals (93%) were assigned to a single, exceptionally large, pedigree. The coefficient of isonymy indicates that, on average, matings from the founder population share a common ancestor 8 generations past. A 2001 analysis of isonymy in 247 US cities (including 10 Michigan cities) showed that matings within these cities share a common ancestor 9 to 12 generations past. We conclude that most individuals within the genealogical database, including the 15 individuals who are deaf from the previous study, belong to a common founder population, and the individuals in this population are highly related. Future studies will directly measure the kinship coefficient using pedigree-based methods of Wright. Given the high degree of relatedness/isolation of this immigrant founder population, their participatory history and their proximity to our institution, we developed a partnership with this community to 1) identify genetic and environmental factors involved in common health-related conditions and 2) to translate those discoveries.

## 641/W/Poster Board #299

**Genome-wide SNP analysis of Ashkenazi Jews reveals unique population substructure.** *S.M. Bray[1], J.G. Mulle[1], A.F. Dodd[1], A.E. Pulver[2], S.T. Warren[1].* 1) Human Genetics, Emory University, Atlanta, GA; 2) Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, Maryland.

The Ashkenazi Jews (AJ) are a genetic isolate that has been widely utilized in genetic studies of both mendelian and complex disorders. However, the genetic variation and population structure of the AJ have been previously investigated with relatively few individuals and few genetic markers. We have now genotyped a large AJ cohort with the Affymetrix 6.0 genome-wide SNP array. After strict quality control filters, genotype data at 775K SNPs in 466 unrelated AJ individuals were available for analysis. To investigate the genetic structure of the AJ relative to other populations we used principle components analysis (PCA) as well as the *frappe* clustering algorithm. When merged with the worldwide Human Genome Diversity Project dataset, PCA shows the AJ are distinct from all other groups, including both European and Middle-Eastern populations. Further PCA using AJ genotypes combined with a large European dataset again validates the separation of AJ from European populations. Interestingly, principle component one seems to largely separate European and Middle-Eastern populations geographically according to latitude with the AJ fitting South of Europe and North of the Middle-East. Additional analysis using the *frappe* population clustering algorithm is consistent with a unique population signature for the AJ. Limiting the *frappe* clustering to only two population groups, specifying k=2, reveals that AJ cluster more closely to Europeans than Middle-Eastern populations but when allowing three populations, k=3, AJ form a group distinct from both the Middle-East and Europe. Compared to European populations, AJ also show an increase in genome-wide linkage disequilibrium, consistent with possible founder effects. These findings will aid in the design and use of AJ in case-control and association studies and clearly demonstrate the genetic separation of AJ from other populations.

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## 642/W/Poster Board #300

**Fine-scale Population Structure in Worldwide Ethnic Populations as Revealed by Identical by Descent Segments.** *B.M. Henn, L. Hon, J.M. Macpherson, N. Eriksson, A. Wojcicki, L. Avey, S. Saxonov, J.L. Mountain.* 23andMe, Inc, Mountain View, CA.

It is well established that human population genetic diversity reflects continental-level geographic divisions, and that within-continental geographic and linguistic differences contribute to population structure between regions. The continental and regional differences reflect ancient demographic events, such as early migrations Out of Africa into Eurasia. However, it is not clear how recent demographic processes occurring on the order of hundreds, rather than thousands, of years affect patterns of genomic diversity. We analyzed the sharing of DNA identical by descent (IBD) inferred from 580,000 SNPs using a large database of individuals with European ancestry, including a subset identifying as Ashkenazim. We also explored the pairwise distributions of identical by descent segments in populations from the Human Genome Diversity Panel (HGDP-CEPH), a diverse set of ethnic groups from across the world. We observed that the average number and sizes of shared genomic segments differ substantially across the ~55 populations. The different patterns are likely attributable to differences in population histories such as recent bottlenecks and sub-structure. In particular, populations that are highly structured will contain individuals that share elevated amounts of IBD, indicative of recent common ancestry through multiple ancestors. In order to understand the pattern of the observed population-level sharing, we simulated extended pedigrees using empirical data from several populations and calculated the expected amounts of sharing for 1st through 10th cousins. We assumed random mating within each population for the simulations. Interestingly, the average sharing in the simulated distant cousins was consistently less than the observed average sharing in each population sample. This finding indicates the presence of fine-scale population structure for many ethnic groups, such as the Ashkenazim, within the last 10 generations (200-300 years).

## 643/W/Poster Board #301

**Scale Effects and Recent Brain Evolution: Theory and Preliminary Evidence.** *G. Jones.* Dept Economics, George Mason Univ, Fairfax, VA.

What forces have driven human evolution since the grand human diaspora? In this paper, I argue that the scale effects so central to endogenous growth theory in the field of economics (e.g., Kremer's widely-cited "Population Growth and Technological Change: 1,000,000 B.C. to 1990," Quarterly Journal of Economics, 1993) have been important drivers of human brain development since the diaspora. Scale effects have made prominent appearances in recent explanations of continent-level outcomes. For instance, in Kremer's model, big continents create larger, denser, faster-growing populations. In Diamond's Guns, Germs, and Steel model, wide continents raise the chance that an innovation will arise at a given latitude, an innovation which can then disperse across that latitude, enriching those who live on wider continents. In both models, the Malthusian nature of pre-Industrial Revolution existence imposes strong conditions on the general equilibrium outcome. My model takes those channels as given, and works out the theoretical implications for the divergent evolution of human brains on these continents. Brains are biologically costly, so evolution will only select for larger brains if there is a substantial payoff. And since larger brains tend to have higher levels of intelligence [corr(Brain Size, IQ)= 0.4 in recent in brain-scan studies], larger brains tend to have more processing and memory power. Under certain parameter values, Kremer's and Diamond's models both imply that the payoff to a big braina brain that can better adopt someone else's ideaswill be higher on wider, larger continents. Thus, we would expect human populations living on larger, wider continents to develop larger, more powerful brains. I model this relationship formally. This result should only hold on average: intra-group diversity is central to evolutionary theory, and massive intra-group diversity is an important fact of quantitative human genetics. The main purpose of the paper is to set forth the model, but I include some tests of its implications. I discuss whether, as the model predicts, human brain size and average IQ correlate positively with continent size and continent width. Indeed, evidence generally supports this hypothesis. Further empirical testing of the model's predictions will occur as future researchers employ genetic diversity databases. I plan to present the results in a manner intelligible to non-economists.

## 644/W/Poster Board #302

**Natural selection -a and the pattern of Identity by Descent in the human genome.** *R. Nielsen, A. Albrechtsen, I. Moltke.* Depts. of Integrative Biol. and Statistics, Univ California, Berkeley, Berkeley, CA.

Most forms of natural selection will tend to increase the degree of Identity By Descent (IBD) in the population. Individuals in a population will in average be closer related to each other in a region that has been targeted by selection than in regions that have evolved neutrally. We show mathematically that even when selection is acting on standing variation the amount of IBD in a region can be increased dramatically. We have also developed a fast method based on Hidden Markov models for identifying pairwise IBD, and a somewhat slower but more precise method based on Markov Chain Monte Carlo (MCMC) for identifying all sets of IBD sharing among multiple individuals. We apply these methods to large scale human SNP data sets and identify a number of extreme IBD patterns, the most extreme of which is located in the Major Histocompatibility Complex (MHC) region - a known target of selection. We do extensive work to verify that the patterns observed are not caused by genotyping errors, variation in recombination rates, or by random genetic drift. The new IBD based method for detecting selection has the advantage over other haplotype based methods that it maintains high power when selection is acting on standing variation.

## 645/W/Poster Board #303

**Copy number variation among rhesus macaques: evolutionary and phenotypic implications.** *O. Gokcumen[1,2], R.S. Smith[1,2], E.J. Vallender[3], M.E. Blake-Kinnin[2], R.E. Mills[1,2], W.E. Johnson[2,3], C. Lee[1,2].* 1) Department of Pathology, Brigham and Women's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) New England Primate Research Center, Southborough, MA.

A substantial amount of genomic variation is now known to exist in humans and other primate species. Recent studies have shown copy number variants (CNVs), which are segments of DNA that can range in size from hundreds to millions of base pairs in length and have different number of copies between individuals, constitute a significant portion of the genetic diversity within primate genomes. CNVs in humans have already been associated with susceptibility to certain complex diseases, dietary adaptation, and several neurological conditions. However, despite these exciting developments, the important role of CNVs in primate evolution and genetic diversity is still largely unknown. To explore the evolution and distribution of CNVs in non-human primates, we have sampled from rhesus macaques (Macaca mulatta), which have been the preferred model animals for biomedical and evolutionary research. For this study, we have designed a custom, array-based comparative genomic hybridization (aCGH) platform with 1,000,000 species-specific oligonucleotide probes. This platform allowed us to identify more than 500 CNVs (more than 25% of these CNVs observed in multiple individuals) with a median size of approximately 30,000 base pairs, among 10 unrelated macaque individuals. The average number of CNVs observed in a single individual was approximately 75, which is more than a threefold increase from the previous studies. This improvement is due to the substantial increase in the resolution of the platform used in this study, which enabled us to detect CNVs as small as 4,000 base pairs. Using the same platform, we also conducted cross-species experiments, hybridizing macaque samples to a human reference. We confirmed the previous observations that segmental duplications were significantly enriched at macaque CNV loci. Furthermore, we have correlated the macaque and human CNVs to identify hot-spots of CNV genesis. We also revealed CNVs in several loci that are conserved, genic or previously associated with expression of phenotypic variation. Interestingly, we found that most of the annotated genes that are copy number variable in macaques are immune system related, suggesting evolutionary pressures (or lack thereof) that induce or tolerate variation in these loci.

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## 646/W/Poster Board #304

**Extreme Evolutionary Disparities Seen in Positive Selection Across Seven Complex Diseases.** *E. Corona[1,2,3], J. Dudley[1,2,3], A. Butte[1,2,3].* 1) Biomedical Informatics, Stanford Univ, Stanford, CA; 2) Department of Pediatrics, Stanford University School of Medicine, Stanford, CA; 3) Lucile Packard Children's Hospital, Palo Alto, CA.

Genome-wide association studies (GWASs) have successfully illuminated disease-associated variation. But whether human evolution is heading towards or away from disease susceptibility remains an open question. We analyzed the seven diseases studied by the Wellcome Trust Control Case Consortium (WTCCC), to calculate the relative selective pressure at every significant loci. Results reveal striking differences between the seven studied diseases. We find evidence of recent positive selection in favor of alleles increasing the risk of Type 1 Diabetes (T1D), Crohn's Disease (CD), Hypertension (HT), Rheumatoid Arthritis (RA), and Bipolar Disorder (BD). Risk-associated alleles (defined as the allele most strongly associated with disease among associated SNPs) for Type 2 Diabetes (T2D) fall largely within the random neutral region, and Coronary Artery Disease (CAD) shows less positive selection than expected by random. When only protective alleles are considered (defined as the allele least strongly associated with disease among associated SNPs), we find that SNPs only associated with T1D, CD, and RA appear to exhibit significant signatures of positive selection. There is significant asymmetry in the 96 SNPs strongly associated with T1D (p-value ≤ 0.005) showing strong signs of positive selection, with 79 SNPs selecting for the risky allele, and only 17 SNPs selecting for the protective allele. Furthermore, selection patterns of Coronary Artery Disease (CAD) fall far below the expected levels of random, implying stable allele frequencies. Results reveal the evolutionary trajectories of T1D and CD favor risk alleles, possibly due to their simultaneous role in protection from infectious diseases. These results inform on current understanding of disease etiology, thus aiding efforts to discover novel approaches to disease treatment and prevention.

## 647/W/Poster Board #305

**Timing of selective pressures on geographically-restricted selected alleles.** *J.L. Kelley[1], J.K. Pickrell[1], R.R. Hudson[2], M. Przeworski[1,2].* 1) Human Genetics, University of Chicago, Chicago, IL; 2) Ecology and Evolution, University of Chicago, Chicago, IL.

Genome scans for selection have identified a number of loci at which an allele appears to have reached intermediate frequency due to positive directional selection. Interestingly, the geographic distribution of these putatively selected alleles vary markedly: in particular, some putatively selected alleles are at high frequency only in Europe or Asia (such as SLC24A5, TYRP1, and EDAR), while others are at high frequency throughout Eurasia (e.g., KITLG and ENAM). One interpretation of these geographic patterns is that the selected alleles currently at high frequency across non-African populations arose when humans first colonized these environments and were subsequently selected for in all populations, whereas locally selected alleles arose more recently and have not had the time to spread to other regions. An alternative is that geographically-restricted alleles are not on average younger but instead that their variable frequencies across populations reflect local differences in selective pressures. To distinguish between these possibilities, we estimated the time to the most common ancestor (tMRCA) for two sets of putatively selected alleles: those at high frequency only in Europe or only in Asia, and those at high frequency throughout Eurasia. If locally restricted alleles are simply too young to have spread, we would expect them to have a more recent tMRCA, on average. Alternatively, if their distribution reflects geographic differences in selective pressures, the ages should not differ systematically. Preliminary analyses reveal no significant difference in the onset of selection on the two sets of alleles, with most selected pressures estimated to predate the Neolithic. We evaluate this hypothesis using forward simulations of demography and selection. Our findings suggest that the distribution of selected allele frequencies reflects geographically varying selective pressures rather than differences in the timing of selection.

## 648/W/Poster Board #306

**Homing in on signals of positive selection in the human genome by large-scale re-sequencing.** *M. Hu[1], Q. Ayub[1], A. Assuncao[2], N. Huang[1], Q. Long[1], M. Quail[1], H. Swerdlow[1], J. Burton[1], C. Tyler-Smith[1], Y. Xue[1].* 1) Sanger Inst, Cambridge, United Kingdom; 2) The European Bioinformatics Inst, Cambridge, United Kingdom.

The detection of positive natural selection in the human lineage is of great interest for studying human adaptations to specific environmental conditions and understanding human history. Sabeti et al. reported 22 regions in the human genome that showed the strongest signals of recent positive selection based on their analyses of HapMap phase II data [Nature 449, 913-918, 2007]. However, most of these regions were hundreds of kb in length, and the biologically functional elements that had undergone selection could not be identified. We have amplified two of them, both about 300 kb long, in 27 HapMap individuals from the selected population using overlapping long PCR, and re-sequenced to high depth using Solexa/Illumina technology. The reads were mapped to the reference human genome sequence and SNPs were called using SSAHA [Ning et al. Genome Res. 11, 1725-1729, 2001]. We filtered out calls that are likely to be false positives, including SNPs with low read depth, within the PCR primers, near indels, within PCR failure gaps as well as heterozygous calls with significant differences between the read depths of the two alleles, and obtained high-quality sequence data. Haplotypes were then inferred using PHASE [Stephens et al. Am J Hum Genet 68, 978-989, 2001; Am J Hum Genet 73, 1162-1169, 2003]. Diversity was calculated and neutrality tests (Tajima's D, Fay and Wu's H) were performed using custom perl scripts. We successfully narrowed down the positively selected targets to ~10 kb for each region. One target contains a predicted miRNA gene [Assunção et al., unpublished] with sequence variants specific to the putatively selected haplotype. The other contains a small mRNA expressed in hippocampus, and is a possible protein coding gene. This target also lies ~10 kb downstream from a sperm antigen protein gene, SPAG6, which plays important roles in sperm motility. Our study indicates that using re-sequencing data in multiple individuals, we can identify positive selection targets in the human genome with a high resolution, so the biological elements that have undergone positive selection can be followed up.

## 649/W/Poster Board #307

**Lactase Persistence; Multiple causal mutations in sub-Saharan pastoralists.** *B.L. Jones, C.J.E Ingram, M.G Thomas, N. Bradman, D.M Swallow.* University College London, London, United Kingdom.

*Background* Milk is the primary source of nutrition for newborn mammals, including humans. The majority of human adults, estimated at approximately 65%, are unable to digest lactose (the main carbohydrate in milk) effectively since lactase expression is down-regulated after weaning, as it is in other mammals. In some humans however, lactase expression persists into adulthood (lactase persistence, LP) allowing adult consumption of milk from other species, and the frequencies of this trait vary throughout the world. A C-T SNP -13910 bases upstream from the lactase gene (*LCT*) is associated with LP in Europe. The *-13910*T* is rare in milk drinking groups in Africa although two other variants (*-13915*G, -14010*C*) have been shown previously to be significantly associated with LP and in an accompanying abstract (Ingram *et al*) we confirm a third locus (*-13907*G*) and present a fourth candidate SNP. However some LP individuals have also been identified who carry none of these alleles. *Aims* To examine the distribution across Africa of these and other allelic variants; to examine other regulatory regions in population groups in which enhancer alleles are lacking. *Results* The geographic and ethnic distribution of *-13907*G, -13910*T, -13915*G, -14009*G*, and *-14010*C* in 10 different countries and 15 distinct ethnic groups across Africa (n=1221 individuals) is presented here. Several other variants in this enhancer region are also described here for the first time. These tightly clustered enhancer variants are more frequent in pastoralist milk drinking groups than agriculturalist populations and are associated with several different *LCT* core haplotypes. Two further candidate regulatory regions have been sequenced in the same populations including a 1000bp region immediately upstream from *LCT* where novel variants have been found. *Conclusions* The data support the notion that many different mutations do have a functional role in LP, and that the trait has arisen independently several times, being subject to the positive selection conferred by the increased ability to digest milk lactose by people in pastoralist societies.

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## 650/W/Poster Board #308

**Evolutionary insights into a common genetic variant for dyslipidemia, Gln241His variant of *MLXIPL*.** *K. Nakayama[1], L. Munkhtulga[2], Y. Yanagisawa[1], T. Gotoh[1], K. Yamanaka[1], T. Ishida[3], Y. Koda[4], Y. Kagawa[5], S. Iwamoto[1].* 1) Division of Human Genetics, Jichi Medical University, Shimotsuke, Japan; 2) Health Science University of Mongolia, Ulaanbaatar, Mongolia; 3) Department of Biological Sciences, Graduate School of Science, the University of Tokyo, Tokyo, Japan; 4) Division of Forensic Medicine and Human Genetics, Kurume University, Kurume, Japan; 5) Kagawa Nutrition University, Sakado, Japan.

*MLXIPL* is recently identified as a novel gene that influences plasma concentrations of triglycerides in humans. The gene product of *MLXIPL* is a transcriptional factor that is mainly expressed in the liver and upregulates the expression of lipogenic genes in response to glucose influx into hepatocytes. In addition to its importance in the genetic predisposition to dyslipidemia, *MLXIPL* has been of interest for a presumptive role in adaptation to famine in modern human evolution. We investigated phenotypic effects, distribution and evolution of *MLXIPL* gene variants in a total of 3,667 individuals of 20 worldwide populations. A nonsynonymous SNP of *MLXIPL*, Gln241His variant (rs3812316), was strongly associated with plasma concentrations of triglycerides in the tested populations ($P$ = 5.8E-6, 11mg/dl decrease per minor His allele). The tested populations generally showed low frequencies of His allele (0.02 0.12) with the notable exception of three Central Asian populations including Mongolians (0.23), Tibetans (0.26) and Uygur (0.21). The glutamate residue was evolutionarily highly conserved and an in silico analysis demonstrated a possible damaging effect of the replacement with histidine residue. Characterizations of SNPs surrounding Gln241His exhibited strong linkage disequilibrium in the genomic region spanning 200kb. Haplotypes carrying His allele formed a distinctive cluster that was separated from haplotypes carrying Gln allele by many mutational steps. In the Mongolians, moreover, a significant deviation of Tajima's $D$ statistics ($D$ = 3.38: $P$<0.001) and "twin-peak" observed mismatch distribution were detected in the 200kb region. Such an allele frequency skew was not detected in HapMap JTP and CHB panels. The global high prevalence of Gln allele, which predisposes to higher triglyceride levels, is consistent with a hypothesis that *MLXIPL* was a "thrifty gene" that permits more efficient conversions of excess carbohydrates to fat at the times of food abundance. On the other hand, the high frequency of His allele and the non-neutral pattern of the surrounding SNPs in the Mongolians suggest that the His allele, which reduces glucose utilization and lipogenesis in the liver, likely had an metabolic advantage in nomadic ancestors of the Central Asians, whom relied on fatty acids for their dietary carbon source.

## 651/W/Poster Board #309

**Exploring the Genetic Basis of Human Uniqueness.** *J.P. Noonan[1], S. Sholtis[1], H. Breslawski[1], L. DeMare[1], C. Pease[2], J.M. McGrath[2], P. Zumbo[3], S. Mane[3], A. Ayoub[4], P. Rakic[4].* 1) Department of Genetics, Yale University School of Medicine, New Haven, CT; 2) Department of Comparative Medicine,Yale University School of Medicine, New Haven, CT; 3) W.M. Keck Foundation Biotechnology Resource Laboratory, Yale University School of Medicine, New Haven, CT; 4) Department of Neurobiology, Yale University School of Medicine, New Haven, CT.

The evolution of biological differences that distinguish humans from other species - such as increased brain size, bipedalism, and the dexterity of the human hand - likely required genome sequence changes that altered development. Although it has long been thought that gene regulatory changes have been a major driver of human evolution, evidence for human-specific developmental regulatory functions has remained elusive. We are investigating this fundamental question from two perspectives: through the identification of developmental regulatory sequences with human-specific functions, and by comparative analysis of gene expression of human, rhesus macaque and mouse at the earliest embryonic stages when biological differences emerge. Using mouse transgenic reporter assays, we have identified a set of developmental enhancers that are highly conserved across non-human vertebrate species but show extreme human-specific sequence divergence (HACNSs). These elements are located near genes that encode developmental transcription factors such as EN1, GLI2, and RUNX1T1 and act as enhancers in the developing brain, limb, and other structures. As a paradigmatic case we are focusing on the detailed functional characterization of the most rapidly evolving enhancer, HACNS1, in vivo. HACNS1 is located downstream of GBX2, an essential developmental transcription factor, and drives human-specific gene expression in the developing mouse limb, most notably in the mouse equivalent of the primordial thumb at embryonic day 13.5. To test the hypothesis that HACNS1 has influenced the evolution of human-specific limb development by altering the expression of nearby genes, we are characterizing transgenic mice that express a Gbx2 cDNA under the control of the HACNS1 enhancer. We are combining these cis-regulatory studies with global transcriptome sequencing of developing cortex and limb in human, rhesus and mouse. We are using laser capture microdissection to recover cortical stem cells and cortical plate neurons and have obtained robust, biologically meaningful transcriptome profiles from these samples using RNA-sequencing on the Illumina Genome Analyzer. These studies provide the foundation for an integrated understanding of how changes in developmental regulatory programs contributed to human evolution.

## 652/W/Poster Board #310

**Adaptive events in the evolution of innate immunity.** *D.J. Wilson[1], H. Quach[2], E. Patin[2], J. Manry[2], L. Barreiro[1,2], L. Quintana-Murci[2], M.F. Przeworski[1].* 1) Dept Human Gen, Univ Chicago, Chicago, IL; 2) Department of Genomes and Genetics, Institut Pasteur, Paris, France.

A major goal of human population genetics has been to detect adaptive change in the ancestry of modern humans and their hominid relatives. To date, practical considerations have focused this inquiry towards whole gene approaches, in which selection coefficients are estimated assuming that an entire coding region experiences selection uniformly, with no heterogeneity in selective pressure across sites. Consequently, the genes in which positive selection is detected are enriched for multiple amino acid substitutions, and adaptive changes that occur against a background of selective constraint tend to be missed.

As a first step towards developing a method to detect changes in selection pressure acting on specific sites over time, we present a new approach that jointly analyzes patterns of polymorphism and divergence from multiple species. Our intention is to employ both the flexibility of phylogenetic models of molecular evolution and the signal provided by polymorphism within populations. We motivate our work by a study of polymorphism and divergence between humans and other hominids (chimpanzee, bonobo, gorilla, orangutan and gibbon) in a family of ten toll-like receptor genes involved in the innate immune response.

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## 653/W/Poster Board #311

**Positive selection of common variants associated with celiac disease.**
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Celiac disease (CD) is a common (1-2% of Westerns population) food intolerance to dietary proteins of wheat, barley and rye. At present, the genetic background of CD is one of the best elucidated complex diseases as approximately 50% of genetic susceptibility can already be explained by association to both common HLA and non-HLA genetic variants. It is puzzling however why common variants associated with CD are frequent in modern populations, and were not selected out during the evolution. The domestication of wheat, barley and rye started around 10,000 years ago, and since then gluten became the major dietary protein in most populations. Classical CD should have been lethal in the past in grain-consuming societies, whereas latent CD - which is associated with infertility - should have reduced fitness and subsequently led to negative selection of CD. Hence, we hypothesized that some of the common alleles associated with CD are located in the blocks of linkage disequilibrium (LD) which also include genes that have been under positive selection. To test this hypothesis we took advantage of our genome-wide association study in CD comprising 2300 cases and 3000 controls from 4 Caucasian and one North-African Saharawi population. In this study we investigated the HLA locus and the 10 known non-HLA CD loci for the signs of positive selection, using the Extended Haplotype Homozygosity (EHH) and Integrated Haplotype Score (iHS) methods. Apart from the HLA region, we observed strong and consistent signs of positive selection of the CD-associated allele in the SH2B3 loci. Interestingly, this associated variant is predisposing to several autoimmune diseases, as well as to other common diseases including myocardial infarction and hypertension, and is located in an extended block of strong LD. The associated block harbors several good candidates for positive selection during evolution, including immune-related and metabolic genes. Above that, we observed signs of positive selection of the CD-associated variants in the IL12A/SCHIP genes locus. These particular alleles might have been beneficial during the times of infections - the major cause of mortality in the past. We are currently extending our cases and control cohorts and are also including other common variants of several complex traits. The analysis of associated variants in the evolutionary perspective might enhance our understanding of why common diseases are caused by common variants.

## 654/W/Poster Board #312

**Multiple rare variants as a cause of a common phenotype: increased diversity may signal selection.**
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**Background:** Persistence of intestinal lactase into adulthood allows humans to use milk from other mammals as a source of food and water. It has previously been shown that this genetic trait has been subject to convergent evolution and the derived alleles of at least three different single nucleotide polymorphisms (*-13910C>T*, *-13915T>G*, *-14010G>C*) are significantly associated with and probably causal of lactase persistence in different populations. Each of these alleles are present on different extended haplotypes consistent with positive directional selection. The SNPs occur in an intron of a neighbouring gene (*MCM6*) within an 'enhancer' sequence, and modulate lactase transcription *in vitro*. However, in all previous studies, a number of lactase persistent individuals were identified who carry none of the above alleles, and other low frequency single nucleotide polymorphisms have been observed within the enhancer sequence. **Aim:** We investigated a cohort of 107 milk-drinking Somali camel-herders from Ethiopia to establish the frequency of lactase persistence and to characterise patterns of association of *MCM6* intron 13 alleles with the trait. **Results:** The frequency of lactase persistence was found to be 0.24. Milk-drinking was far more common than lactase persistence, providing evidence that the cultural trait can precede the genetic adaptation. Eight polymorphic sites were identified in the enhancer region. Two of these, *-13915*G* and *-13907*G* (a previously reported candidate), were significantly associated with lactase persistence after correction for multiple testing ($p = < 1x10^{-4}$). A new allele, *-14009*G* was also shown to have a borderline association with lactase persistence ($p = 0.056$). However, of greatest note is the novel finding that sequence diversity of the enhancer region is significantly higher in the lactase persistent members of the cohort compared with the non-persistent members of the group ($p = 7.6 \times 10^{-9}$). This contrasts with the widely expected observation of positive selection decreasing diversity by driving up the frequency of a single advantageous allele. By examining diversity of the Y-chromosome, the hypervariable region of the mtDNA, and the allele distributions of 15 autosomal microsatellites, we show that this difference is not due to population substructure, and that increased diversity can be a signal of selection.

## 655/W/Poster Board #313

**Use of 96-plex indexing and Illumina sequencing to analyse the worldwide population genetics of a polymorphism associated with human muscle function.**
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Massively parallel sequencing technologies provide extremely high sequence output, but are challenging to apply to the resequencing of small candidate regions (<1 Mb) in large numbers of samples. Here we describe an index-based approach that permits the pooling of 96 samples in a single Illumina lane, and its application to population genetic analysis of a human gene associated with variation in muscle function.

The human *ACTN3* gene encodes the protein $\alpha$-actinin-3, which is expressed in fast muscle fibres. Remarkably, a common nonsense polymorphism (R577X) in *ACTN3* results in complete deficiency of $\alpha$-actinin-3 in more than one billion humans worldwide. Analysis of human and mouse data suggest that $\alpha$-actinin-3 deficiency decreases muscle size and strength while increasing metabolic efficiency and endurance. These changes appear to have been advantageous during human evolution: 577X displays a genetic signature of recent positive selection in Europeans and East Asians (MacArthur et al. *Nat Genet* **39:**1261-1265).

To further characterise the recent evolutionary history of *ACTN3* we amplified and sequenced 25 kb of genomic DNA around this gene in 1,457 individuals from 57 worldwide populations with Illumina short-read technology. Using enrichment PCR primers that contain unique 8bp indexing tags we are able to load 96 different samples per lane, along with 6 cloned control sequences. All individuals were then sequenced using paired-end 50bp reads. We have developed a custom pipeline for assigning reads to individual samples, mapping reads to the reference genome, calling SNPs, short indels and structural variants and performing basic population genetic analyses.

Here we show that adding several methodological improvements to the standard Illumina pipeline results in very high (>200X per lane) and uniform coverage across pooled samples. We also present detailed analysis of variation within *ACTN3*, including estimates of the age, geographical origin and selection intensity acting on the 577X null allele in a variety of human populations, and integrate functional and population genetic evidence to constrain scenarios for its recent evolutionary history.

(DGM and IK contributed equally.).

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## 656/W/Poster Board #314

**Human-specific insertions and deletions: simply neutral or not?** *C.H. Chen[1], T.J. Chuang[2], B.Y. Liao[1], F.C. Chen[1,3,4].* 1) National health research institutes, Zhunan Town, Taiwan; 2) Genomics Research Center, Academia Sinica, Taipei, 115 Taiwan; 3) Department of Life Science, National Chiao-Tung University, Hsinchu, 300 Taiwan; 4) Department of Dentistry, Chinese Medical University, Taichung, 404 Taiwan.

The number of human-specific insertions and deletions ("HS indels"; with length <100 Bp) is surprisingly large in the human genome, affecting more than 7,000 genes and many noncoding regions. However, the selection pressures that work on HS indels remain unclear. In coding sequences, the indel rate (the length of indels divided by the length of the aligned sequence) is significantly lower than that in pseudogenes, suggesting negative selection on HS indels. Nevertheless, HS indels are most abundant in intergenic and intronic regions. Are all of these indels selectively neutral? Or they actually contribute to human-specific traits? To search for the signatures of positive selection on HS indels, we employ a modified McDonald-Kreitman test, with human-specific substitutions as the neutral reference, to test whether the ratio of fixed-to-polymorphic HS indels significantly exceeds the neutral expectation in sliding windows through the human genome. Although most of the HS indels are selectively neural, we conservatively estimate that ~12MB of the human genome to be potentially positively selected. Moreover, these regions tend to be located in the vicinity of genes. Since the fixed-to-polymorphic indel ratio is significantly higher in noncoding regions than in coding sequences in these positively selection regions, the indel-associated positive selection may have affected the transcriptional or translational regulation, rather than the protein sequences *per se*.

## 657/W/Poster Board #315

**Detecting Natural Selection in the Human Genome from Pilot1 Data in the 1000 Genomes Project.** *P. Hu[1,2], H. Siu[1,2], Z. Hou[1,2], F. Yu[4], Y. Fu[3], J. Jin[1,2], M. Xiong[1,3].* 1) Laboratory of Theoretical Systems Biology and Center for Evolutionary Biology, School of Life Science and Institute for Biomedical Sciences, Fudan University, Shanghai 200433, China; 2) State Key Laboratory of Genetic Engineering and MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai, 200433, China; 3) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77225; 4) Human Genome Sequencing Center, Molecular and Human Genetics Department, Baylor College of Medicine, Houston, TX 77030.

Identifying signatures of natural selection in the human genome is of fundamental implication for the study of population evolution and for the biomedical research. The distribution of selection in genome will provide important functional information. Natural selection modify the level of variability within and between populations and shapes the pattern of genetic variations in the genome. Genetic variation in genome is the raw data for detection of natural selection. The 1000 Genomes Project produces whole genome sequencing data and offers a unique and great opportunity to scan the genome for signature of natural selection. Five statistics: Tajima'D, Fu and Li's F, Achaz's Y, Fay and Wu's H and Zeng et al.'s E (based on comparing the site frequency spectrum within population) and Fst statistic (based on the measure of population subdivision) were applied to Pilot 1 data in 1,000 genome project to scan the entire genome for detection of selection, where 344 chromosomes from ASI, CEU and YRI were sequenced. A total of more than 20 million of variant sites, 4.8 millions common in three populations were identified. We calculated seven statistics in 10 kb and 100 kb windows across the genome for each population and obtained their empirical distributions. Results show that two kinds of windows analyses lead to the similar distributions. The proportional rank of the test statistic in a particular window compared with the overall empirical genomic distribution was taken as empirical P-value for that window. We identified 3,046 candidate selection regions in ASI population, 2,015 selection regions in CEU, and 2,204 selection regions in YRI at 5% empirical significance level in 10 kb by five statistics based on differences in frequency spectrum. Among 457 candidate genes of selection reported from PubMed, we detected 102 selection genes in ASI, 53 selection genes in CEU, and 101 selection genes in YRI and 11 selection genes common in three populations by familiar Tajima D test. By comparison we obtained 3.9 million SNPs and the whole genome's fixation index about 0.10~0.11. By compared with the empirical genome-wide distribution of FST, we identified 5, 278 candidate selection regions at an empirical significance level of 2.5% from each of the 22 autosomal chromosomes. Among 581 identified selection regions by FST which were reported from literatures, we found that 294 selection regions overlap our results.

## 658/W/Poster Board #316

**Genomic Landscape of Positive Natural Selection in North European Populations.** *T. Lappalainen[1,2], E. Salmela[1,2], P.M. Andersen[3], K. Dahlman-Wright[4], P. Sistonen[5], M.-L. Savontaus[6], S. Schreiber[7], P. Lahermo[1], J. Kere[2,4,8].* 1) Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland; 2) Department of Medical Genetics, University of Helsinki, and Folkhälsan Institute of Genetics, Biomedicum Helsinki, Helsinki, Finland; 3) Department of Neurology, Umeå University Hospital, University of Umeå, Umeå, Sweden; 4) Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden; 5) Finnish Red Cross Blood Transfusion Center, Helsinki, Finland; 6) Department of Medical Genetics, University of Turku, Finland; 7) Department of General Internal Medicine, Institute for Clinical Molecular Biology, Christian-Albrechts-University, Kiel, Germany; 8) Clinical Research Centre, Karolinska University Hospital, Huddinge, Sweden.

Analysing genetic variation of human populations to detect loci that have been affected by positive natural selection is important for understanding adaptive history and phenotypic variation in humans. In this study, we analysed recent positive selection in Northern Europe from genome-wide datasets of 250 000 and 500 000 single nucleotide polymorphisms in a total of over 1000 individuals from Great Britain, Northern Germany, Eastern and Western Finland, and Sweden. Coalescent simulations were used to demonstrate that the integrated haplotype score (iHS) and long-range haplotype (LRH) statistics have sufficient power in genome-wide datasets of different sample sizes and SNP densities. Furthermore, the behavior of the FST statistic in closely related populations was characterized by allele frequency simulations. In the analysis of the North European dataset, dozens of regions in the genome showed strong signs of recent positive selection. Most of these regions have not been discovered in previous scans, and many contain genes with interesting functions (e.g. RAB38, INFG, NOS1AP, and APOE). In the putatively selected regions, we observed a statistically significant overrepresentation of genetic association to complex disease, which emphasizes the importance of the analysis of positive selection in understanding the evolution of human disease. Altogether, this study demonstrates the potential of genome-wide datasets to discover loci that lie behind evolutionary adaptation in different human populations.

## 659/W/Poster Board #317

**Signatures of balancing selection at the HLA-G promoter region in Native-American populations.** *M.R. Pincerati[1], E.J.M. Santos[2], D. Labuda[3], A. Ruiz-Linares[4], D. Meyer[1].* 1) Departamento de Genética e Evolução, Universidade de São Paulo, São Paulo, Brazil; 2) Laboratório de Genética Humana e Médica, Departamento de Patologia, Universidade Federal do Pará, Belém, Pará, Brazil; 3) CHU Sainte-Justine, Département de Pédiatrie, Université de Montréal, Montréal, PQ, Canada; 4) The Galton Laboratory, Department of Biology, University College London, London, United Kingdom.

HLA-G is a class Ib HLA gene initially described as being selectively expressed at the maternal-fetal interface. Since it was first described, in cytotrophoblasts, this molecule has attracted attention due to its immunotolerogenic properties. Recent advances show the relevance of HLA-G in physiological and pathological contexts, such as transplantation, autoimmunity, cancer and haematological malignancies. The fact that HLA-G exerts several immunomodulatory effects suggests that its expression is under tight regulation. The singular organization of its promoter region also reflects the unique expression pattern of HLA-G. Evidence of balancing selection at the HLA-G promoter region have previously been found in the African American, European American and Chinese populations. These observations demonstrated the importance of the functions of the HLA-G gene and suggest that its expression may be under strong selective pressures. The aim of the present study was to describe the sequence variation and haplotype structure of the HLA-G promoter region in Native-American populations. We sequenced the 1320 bp region immediately upstream of the HLA-G translation start site in 21 individuals distributed throughout the American continent. We observed high levels of nucleotide variation. In this survey we identified 24 SNPs and 22 different haplotypes, most of them shared with the three major human populations already studied. Nucleotide diversity in the samples was 0.00641, which is about eight times as high as the human genome average. Indeed, a significant departure from the expectation of evolutionary neutrality was observed. A significant positive Tajimas'D value was found, which is consistent with action of balancing selection in this locus. Altogether these features represent evidence that natural selection is influencing polymorphism of the HLA-G promoter region. The fact that we found retention of a substantial amount of allelic diversity, even in populations that have undergone loss of heterozygosity, as is the case of Native-Americans, reinforces the importance of the promoter region of this gene in the human evolutionary history.

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## 660/W/Poster Board #318

**Evidence of Indigenous American specific selection in skin pigmentation genes.** *E. Quillen[1], A.W. Bigham[2], R. Mei[3], M.D. Shriver[1].* 1) Dept Anthropology, Pennsylvania State Univ, University Park, PA; 2) Dept. of Pediatrics, University of Washington, Seattle, WA; 3) Affymetrix, Inc., Santa Clara, CA.

Recent studies of selection in human pigmentation genes have focused on Old World populations, neglecting the evolutionary changes that have occurred in Indigenous American populations since their migration into the Americas. Previous research shows correlations between Indigenous American ancestry and skin pigmentation variation, suggesting a genetic role in the determination of skin pigmentation among these populations. However, few genes contributing to these differences have been described. To identify genes that may have undergone Indigenous American specific changes, this work examines signatures of selection in 82 pigmentation candidate genes by genotyping 88 indigenous individuals from Central and South America using the Affymetrix Genomewide Human SNP Array 6.0. The resulting 906,600 single nucleotide polymorphisms (SNPs) were surveyed for signatures of selection in the Indigenous American populations compared to the HapMap Phase I populations. Evidence of selection was identified using four measures selected for the complementarity of their approaches, including the reduction in heterozygosity (ln*RH*), Locus-Specific Branch Length (LSBL), Tajima's D, and by examination of the haplotype block structure. When computing ln*RH* and LSBL as well as when examining changes in haplotype frequency, the East Asian and European HapMap populations were included because they are the most closely related populations available. These analyses differentiate the selective changes that appear to be shared among East Asian and Indigenous American populations from those that are unique to the Indigenous American populations. For each test, the top 5% of the empirical distribution of results was examined and pigmentation genes falling in this tail of the distribution were considered to show statistically significant evidence of selection. Based on these analyses, 12 genes - *ADAM17, POMC, AP3B1,OPRM1, SILV, OCA2/HERC, PLDN, MYO5A, RAB27A, CYP1A2, ATRN*, and *ASIP* - show evidence of selection unique to the Indigenous American populations. Many of these genes have known functional roles in melanogenesis and suggest potential pathways responsible for the observed differences in skin pigmentation between Indigenous American and Old World populations.

## 661/W/Poster Board #319

**Molecular evolutionary study of the ionotropic glutamate-receptor gene family as schizophrenia susceptibility genes: human-specific non-neutral pattern observed in *GRIN2B* upstream region.** *H. Shibata[1], K. Watanabe[1], K. Tanaka[1], H. Goto[2], S. Mano[3], O. Takenaka[4], Y. Fukumaki[1].* 1) Medical Inst Bioregulation, Kyushu University, Fukuoka, Japan; 2) Center for Comparative Genomics and Bioinformatics, Pennsylvania State University, State College, PA, USA; 3) Graduate School of Natural Science, Nagoya City University, Aichi, Japan; 4) Primate Research Institute, Kyoto University, Aichi, Japan.

Schizophrenia is a common psychiatric disease with relatively strong genetic background ($\lambda_s$ = 10). Typical adolescent onset characterized by loss of sociality suggests severe reduction of fitness. However, the disease prevalence is highly stable to be ~1% in any human populations. We hypothesized that the schizophrenia susceptibility alleles are maintained by non-neutral process such as balancing selection. To test this hypothesis, we resequenced the upstream region (3-5 kb) of fourteen ionotropic glutamate receptor genes: *GRIN1*, *GRIN2A*, *GRIN2B*, *GRIN2C*, *GRIN2D*, *GRIA1*, *GRIA2*, *GRIA3*, *GRIA4*, *GRIK1*, *GRIK2*, *GRIK3*, *GRIK4* and *GRIK5* using 50-72 unrelated humans and 24-50 unrelated chimpanzees as non-human controls. From the neutrality tests for the overall regions, we identified significant negative values of Tajima's *D* in *GRIA2* (-2.14) and *GRIK5* (-1.94) in humans suggesting human-specific positive selection. By window plot analyses, we identified a significant positive value of Tajima's *D* (+2.19) only in humans at the 1.5 kb upstream region of *GRIN2B*. Since population contraction is unlikely for humans, this positive Tajima's *D* is a signature of balancing selection specific to human lineage. The region harbors a common SNP, rs1019385 of which the most significant association with schizophrenia has been shown by meta-analysis (Allen et al 2008). By coalescent simulation we dated the divergence of two common haplogroups in the region to be 1.3 MYA that is much older than the genome average TMRCA, 0.8 MYA. Therefore, we conclude that the *GRIN2B* upstream region has been subject to balancing selection associated with schizophrenia as well as human brain function.

## 662/W/Poster Board #320

**Genotype and allele of the rs1799983 (Glu298Asp) polymorphism in the endothelial nitric oxide synthase gene are associated with high altitude adaptation in Quechua population.** *P. Wang[1], A. Ha[2], KK. Kidd[3], MS. Koehle[1], JL. Rupert[1].* 1) School of Human Kinetics, University of British Columbia, Vancouver, BC, Canada; 2) Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada; 3) Department of Genetics, the Yale University School of Medicine, New Haven, CT, USA.

PURPOSE: Human high altitude adaptation involves a number of physiological and anatomical changes that serve to maximize the uptake, transport and delivery of oxygen. There is substantial evidence that Andean populations are genetically adapted to life at high altitude. Nitric oxide (NO)-dependent endothelial function plays an important role in vascular homeostasis and the exhaled NO level of highlanders has been reported to be 25% - 200% greater than that of lowlanders prompting the postulation that changes in NO mediated hypoxia responses play in evolutionary adaptation to high altitude. Endothelial-derived nitric oxide synthase (eNOS) is the main source of NO in blood vessels. Several polymorphisms in eNOS have been shown to affect the expression, enzyme activity, and concentration of exhaled NO. Our study investigated possible associations between variants of the rs1799983 (Glu298Asp) polymorphism of eNOS and high altitude adaptation. METHODS: Seventy-eight highlanders (Quechua) and fourty-nine lowlanders (Maya) were recruited. DNA was collected from buccal cells. The genotype and allele frequencies were compared between highlander and lowlander groups by Chi-Square analysis. RESULTS: Positive associations were found between variants and genotypes of the rs1799983 (Glu298Asp) polymorphism in eNOS and high altitude adaptation. Both the G allele and GG genotype of the polymorphism were over-represented in Quechua highlanders ($p = 0.01$ for allele frequency; $p = 0.03$ for genotype frequency). CONCLUSIONS: Our data suggest that the G allele and GG genotype may have played a role in high altitude adaptation in the Quechua population.

## 663/W/Poster Board #321

**Ancestry variation along the genome in Latin American populations and implications for recent natural selection.** *S. Wang[1], M. Burley[2], P. Sabeti[1], A. Ruiz-Linares[2].* 1) FAS Center for Systems Biology, Harvard University, Cambridge, MA; 2) Department of Genetics, Evolution and Environment, University College London, London.

Latin American populations stem from the admixture starting about 500 years ago of Europeans, Africans and Native Americans. Extreme deviation in ancestry estimates at certain genome locations (relative to the genome-wide average) could reflect the action of recent natural selection. We evaluated the distribution of ancestry estimates along the genome using 678 microsatellite markers in 249 individuals sampled from 13 admixed populations across Latin America. We found a significant deviation in ancestry at two genomic locations with more than four times standard deviations from the genome-wide mean: an excess of European ancestry at 14q32 (Z-score = 4.14), and an excess of African ancestry at 6p22 (Z-score = 4.71). These deviations in ancestry were observed in the analysis of the combined dataset as well as in most of the individual populations examined. We showed that our findings are robust to the Native American ancestry populations used. We discussed the implications for recent natural selection in the context of the unique history of the New World, as well as the possibility of artifacts.

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## 664/W/Poster Board #322

**A powerful extended homozygosity score test to detect positive selection in genome-wide scans.** *M. ZHONG[1], K. LANGE[2], J. PAPP[2], R. Fan[1,3].* 1) Department of Statistics, Texas A&M University, College Station, TX; 2) Department of Human Genetics, David Geffen School of Medicine, UCLA, Los Angeles, CA; 3) Department of Epidemiology, MD Anderson Cancer Center, University of Texas, Houston, TX.

Extended stretches of homozygosity serve as a surrogate indicator of recent positive selection. We developed, evaluated and applied three new tests, extended genotype-based homozygosity test (EGHT), hidden Markov model test (HMMT) and extended haplotype-based homozygosity test (EHHT) to detect excess homozygosity in genome-wide scans. All three assume random mating and Hardy-Weinberg equilibrium but different level of linkage disequilibrium (LD) under null hypothesis. The EGHT postulates linkage equilibrium, the HMMT allows pair-wise LD but no higher order interactions, and the EHHT allows arbitrary LD. In essence, we started from a measure of extent of homozygosity, calculated its mean and variance via different routes under the corresponding null hypotheses and formulated test statistic based on central limit theorem. Comparing to other haplotype-based methods which lack of clear distribution, the test statistic is asymptotically normal and that makes analysis and application more straightforward. The assumption of multi-locus LD endows the EHHT with a conservative nature and it is confirmed by our preliminary simulation study. Therefore, we focused our consequent study on the EHHT. By running coalescent simulations, we were able to demonstrate that the EHHT leads to appropriate false positive rates under various demographic models in population genetics. We also showed that the EHHT has higher or similar power comparing with the existing popular methods. We applied the EHHT method to HapMap phase II data and replicated previous findings of candidate regions under positive selection. Moreover, by applying multiple filtering criteria including high EHHT scores and population differentiations, we identified 15 new candidate regions which may undergo recent selection and deserve further investigation.

## 665/W/Poster Board #323

**A genome-wide scan for highly polymorphic loci suggests that balancing selection has acted on reproductive and metabolic processes.** *A. Fujimoto[1,2], M. Kato[1,3], N. Hosono[1], M. Kubo[1], Y. Nakamura[1], T. Tsunoda[1].* 1) Ctr Genomic Med, Riken, Japan; 2) Data Analysis Fusion Team, Computational Science Research Program, RIKEN, Japan; 3) Cold Spring Harbor Laboratory, USA.

Besides positive selection, balancing selection is one of the central interests of evolutionary genetics. To identify loci that have been influenced by balancing selection, we scanned for highly variable loci using resequencing data from exonic and promoter regions of 24 Japanese individuals released by the JSNP project. To normalize the mutation rate, we utilized the ratio of number of SNPs per individual to divergence between human and macaca as a measure of polymorphism of each locus. We found the *OVCH1* gene, which is expressed on the egg surface, to have high polymorphism. We also resequenced the *OVCH1* region using HapMap-CHB+JPT individuals and observed an excess of intermediate allele frequencies. We also observed that genes with GO (gene ontology) terms of "heat generation", "negative regulation of blood pressure", and "thymic T cell selection" tend to have high polymorphism. These results suggest that several genes with metabolic and reproductive functions evolved by balancing selection.

## 666/W/Poster Board #324

**Identifying genes under selection using generalized linear mixed models.** *K.E. Eilertson[1], J. Booth[2], C.D. Bustamante[2].* 1) Statistics, Cornell University, Ithaca, NY; 2) Biological Statistics and Computational Biology, Cornell University, Ithaca, NY.

Of significant interest to geneticists is identifying genes under selection and quantifying the selection force. We present an approach for identifying genes under selection using polymorphism and divergence data from synonymous and nonsynonymous sites within genes. Generalized linear mixed models are used to identify the effect of the relationship between polymorphic/fixed mutations and synonymous/nonsynonymous sites on the expected number of mutations. The estimated effects can then be used not only to estimate the selection coefficient, $\gamma$, but also the mutation rate, $\theta$, the proportion of non-lethal mutations, *f*, and the time to the most recent common ancestor, $\tau$, both genome-wide and gene by gene. The model is fit in both the Bayesian and frequentist settings using Markov Chain Monte Carlo Methods in WinBUGS and the lme4 package in R. The proposed methodology was designed to analyze several thousand genes between two species. Using simulated data we compare our method to existing methods for detecting genes under selection, the MK statistic, MKprf and MKprfk. The simulations showed our method to be a significant improvement in both power and false positive rates. We then apply these methods to human v chimp data as well as D. simulans v D. melanogaster . Our method found many human genes under selection that are involved in neurological and cardiological functions, along with DNA replication and repair, and development.

## 667/W/Poster Board #325

**Involvement of the Modifier Gene of a Human Mendelian Disorder in a Negative Selection Process.** *I. Jéru[1], H. Hayrapetyan[2], P. Duquesnoy[1], E. Cochet[1], J.L. Serre[3], J. Feingold[1], G. Grateau[1], T. Sarkisian[2], M. Jeanpierre[4], S. Amselem[1].* 1) INSERM U.933, Université Paris 6 Pierre et Marie Curie, Hôpital Armand-Trousseau, Paris, France; 2) Center of Medical Genetics and Primary Health Care, National Academy of Sciences, Yerevan, Armenia; 3) Equipe Structure-Fonction, EA 2493, Université de Versailles-Saint Quentin en Yvelines, Versailles, France; 4) INSERM U.567, Faculté de médecine, site Cochin, Paris, France.

Identification of modifier genes and characterization of their effects represent major challenges in human genetics. SAA1 is one of the few modifiers identified in humans. This gene is known to influence the risk of renal amyloidosis (RA) in patients with familial Mediterranean fever (FMF), a Mendelian autoinflammatory disorder associated with mutations in MEFV. Indeed, the SAA1 alpha homozygous genotype and the p.Met694Val homozygous genotype at the MEFV locus are two main risk factors for RA. Here, we investigated Armenian FMF patients and controls from two neighboring countries: Armenia, where RA is frequent (24%), and Karabakh, where RA is rare (2.5%). Sequencing of MEFV revealed similar frequencies of p.Met694Val homozygotes in the two groups of patients. However, a major deficit of SAA1 alpha homozygotes was found among Karabakhian patients (4%) as compared to Armenian patients (24%) (p=5.10-5). Most importantly, we observed deviations from Hardy-Weinberg equilibrium (HWE) in the two groups of patients, and unexpectedly, in opposite directions, whereas, in the two control populations, genotype distributions at this locus were similar and complied with (HWE). The excess of SAA1 alpha homozygotes among Armenian patients is readily explained by the recruitment of patients with severe phenotypes. In contrast, a population-based study revealed that the deficit of alpha/alpha among Karabakhian patients results from a negative selection against carriers of this genotype. This study, which provides new insights into the role of SAA1 in the pathophysiology of FMF, represents the first example of deviations from HWE and selection involving the modifier gene of a Mendelian disorder.

## 668/W/Poster Board #326

**Patterns of correlation between genetic ancestry and facial features suggest selection on females is driving differentiation.** *D.K. Liberton[1], K.A. Matthes[1], R. Pereira[2], T. Frudakis[3], D.A. Puts[1], M.D. Shriver[1].* 1) Department of Anthropology, Pennsylvania State University, University Park, PA; 2) Catholic University of Brazil, Brasilia, Brazil; 3) DNAPrint Genomics, Sarasota, FL.

Human facial features show extensive variation within and among populations. By investigating the relationship between dimorphism in facial features and genetic ancestry in different populations, we can explore the roles of sexual and natural selection on the human face. We measured sexual dimorphism in facial traits while controlling for the effects of overall size differences and then tested for interactions between sex and genetic ancestry. The study sample consists of 254 subjects (n=170 females, n=84 males), ages 18-35, showing West African and European genetic ancestry sampled in the United States and Brazil. Maximum likelihood genetic ancestry estimates were determined from 176 ancestry informative markers (AIMs), which allowed for the proportional estimation of genetic ancestry from four parental populations (West African, European, East Asian, and Native American). Three-dimensional photographs of faces were acquired using the 3dMDface imaging system (Atlanta, GA). 22 standard anthropometric landmarks were placed on each image and XYZ coordinates were collected. All 231 possible pairwise inter-landmark distances were calculated and then log transformed. Using the pairwise distances, we tested whether some distances were larger in one sex than the other, having taken size into account, in a) African Americans sampled in the United States, b) Brazilians sampled in Brazil, and c) the combined African American and Brazilian sample. We found that several pairwise distances differed between the sexes. For example, the distance from the brow to nasal bridge was found to be more than 5% larger in females than males. We then tested for an interaction between sex and genetic ancestry by testing for differences in the slopes of the ancestry association between males and females. Although the pattern differed slightly between samples, after Bonferroni correction many correlations were the found to be same in both sexes. However, females in all three samples had many additional significant correlations that were not seen in males, while males had very few correlations that were not found in females. The results of these analyses suggest that selection on females is driving the differentiation in facial features among populations.

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## 669/W/Poster Board #327

**Selection Mapping to Identify West Nile Virus Susceptibility Loci.** *A.W. Bigham, M.J. Bamshad.* Department of Pediatrics, University of Washington, Seattle, WA.

The most common strategy for identifying disease susceptibility alleles is association mapping. This strategy relies on choosing candidate genes based on a priori information about disease pathogenesis, effects in animal models, etc. When such information is unavailable or too many candidate genes exist to screen cost-effectively, this approach is problematic. Consequently, there is keen interest in developing more efficient strategies to find and prioritize candidate alleles. Alleles that influence phenotypic change in a particular population must be functionally different than wild-type alleles, and thus they are likely to have been the target of natural selection. Here, we applied and evaluated the selection mapping approach to identify West Nile Virus (WNV) susceptibility candidate loci in a panel of immune response genes using 31 African, 31 European, and 31 East Asian individuals. Twenty-nine innate and adaptive immune genes were sequenced in the three population samples to detect evidence of local adaptation. We applied three tests that detect departures from neutrality including Tajima's *D*, Fu and Li's *D**, and *F** to assess population specific patterns of variation. Next, in order to prioritize alleles to be tested for association with WNV disease, single nucleotide polymorphism (SNP) - specific $F_{ST}$ values were computed for each allele. At a majority of the loci analyzed, Africans showed evidence of putative balancing selection. For the East Asians and Europeans, evidence of both balancing selection and directional selection was detected. We were especially interested in identifying candidate loci in Europeans because this population will make up the majority of both cases and controls in future association studies. Evidence of positive selection was found for a group of related genes, *OAS1*, *OAS2*, and *OAS3* in Europeans. Moreover, SNPs in *OAS1* and *OAS3* exhibit SNP-specific $F_{ST}$ values falling in the top 5% of the empirical distribution generated from this dataset. Interestingly, significant associations between WNV pathogenesis and SNPs in *OAS1* have been found previously. In summary, we have identified selection nominated candidate genes for WNV susceptibility studies. The results of this study indicate that using the selection mapping approach to identify susceptibility loci can be a powerful tool to rank and prioritize candidate gene lists, but that it may miss important susceptibility loci.

## 670/W/Poster Board #328

**Birth of New Exons in Primates.** *L. Lin[1], S. Shen[2], J. Peng[1], S. Sato[1], A. Tye[1], J. Cai[3], B. Davidson[1,4,5], Y. Xing[1,6].* 1) Internal Medicine, University of Iowa, Iowa City, IA; 2) Department of Biostatistics, University of Iowa, Iowa City, IA; 3) Department of Biology, Stanford University, Stanford, CA; 4) Department of Molecular Physiology and Biophysics, University of Iowa, Iowa City, IA; 5) Department of Neurology, University of Iowa, Iowa City, IA; 6) Department of Biomedical Engineering, University of Iowa, Iowa City, IA.

Transposable elements (TEs) are major sources of new exons in higher eukaryotes. Almost half of the human genome is derived from transposable elements, and many types of transposable elements have the potential to exonize. We conducted a large-scale analysis of human exons derived from two classes of TEs - the primate-specific Alu retrotransposons, and mammalian-wide interspersed repeats (MIRs). Using exon array data of 330 Alu-derived exons and 328 MIR-derived exons, as well as RT-PCR analysis of 77 exons in 10 tissues, we identified numerous Alu and MIR exons with constitutive or tissue-specific splicing patterns. Interestingly, exon array data suggest substantially higher splicing activities of MIR exons as compared to Alu exons. This appears to be a universal difference between exons derived from young and old transposable elements, as it is also observed when comparing Alu exons to exons derived from LINE1 and LINE2, two other groups of old transposable elements. In several human genes (e.g. SEPN1, TTLL6), the tissue-specific splicing of TE-derived exons results from a human-specific splicing change after the divergence of humans and chimpanzees. Together, this study significantly expands current knowledge about functions and evolution of new exons. Our data imply that with sufficient evolutionary time, numerous new exons could evolve beyond the evolutionary intermediate state and contribute functional novelties to modern mammalian genomes.

## 671/W/Poster Board #329

**The Utility of Information Content Analysis in combination with Evolutionary Analysis in Genomic Applications.** *G. Wyckoff[1], M. Yang[1], A. Solidar[2].* 1) Div Molec Biol & Biochemistry, Univ Missouri, Kansas City, Kansas City, MO; 2) Bioinformatica, LLC Oakland, CA.

A common problem in molecular comparative genomics is the identification of genes that are under positive, adaptive selection. Such genes are likely to be crucial for speciation, species differentiation, and functional specialization. We propose a novel approach to help distinguish between different evolutionary trajectories in comparative molecular genomic studies. Information theory allows measurement of the gain or loss of entropy within a sequence when married to evolutionary methodologies, and combined with evolutionary approaches may allow us to distinguish between genes and genetic elements that are under positive selection from those that are evolving neutrally or under constraint. We here show the results from three different analyses performed using the VaSSA software from Bioinformatica, LLC. First, we examined the difference in information content between 500 coding genes and carefully aligned pseudogene homologs of recent derivation. Secondly, we examined single nucleotide polymorphism (SNP) data from humans and rodents. Lastly, we examined several known rapidly-evolving genes and show their information content profile and how it differs from several genes with more constrained functions. The methodology presented here has utility for the analysis not only of coding region data, but also non-coding regions and other genomic segments. We are able to show that we can successfully identify many known genes and SNPs that are clinically relevant, and present an integrated method for testing for SNP function in large, poorly annotated genomic regions. We'd like to acknowledge Bioinformatica, LLC and the NIH for funding of this work.

## 672/W/Poster Board #330

**Evolution of MCPH1 in catarrhine and platyrrhine primates.** *M. Raveendran[1], J. Rogers[1,2].* 1) HGSC, Baylor College of Medicine, Houston, TX; 2) Southwest National Primate Research Center, San Antonio, TX.

Primary microcephaly (MCPH) is a neurodevelopmental disorder characterized by smaller-than-normal brain size with architecturally normal brain and nonprogressive mental retardation in humans. MCPH is genetically heterogeneous. Mutations in seven different loci (MCPH1-MCPH7) can lead to microcephaly. Cross-species comparisons of one gene (microcephalin, MCPH1) have shown that this locus exhibits adaptive sequence changes that may be involved in the significant brain enlargement from lesser apes to great apes and humans. We sequenced 6379 bp covering exons 2-14 (2507 bp, 834 amino acid) and the flanking intron segments of the MCPH1 gene from 48 individuals from five species of Old World (catarrhine) and New World (platyrrhine) monkeys (40 Papio hamadryas from three subspecies, and two animals each from Macaca nemestrina, M. fascicularis, Callithrix jacchus and Saimiri boliviensis). Our analysis identified 24 amino acid changes that distinguish the two New World monkeys from all Old World monkeys and apes, and 81 amino acid changes differing between the two New World monkeys. We found 22 amino acid changes that distinguish baboons from all macaques and various changes that are unique to each Old World monkey species and subspecies. These results indicate that the rapid rate of MCPH1 evolution observed across the human, great ape and lesser ape lineages is also true for Old World and New World monkeys. This gene shows rapid amino acid evolution in primate lineages that have not exhibited the rapid expansion of brain size observed in human evolution.

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## 673/W/Poster Board #331

**Cruciform-forming Inverted Repeats Appear to have Mediated Many of the Microinversions that Distinguish the Human and Chimpanzee Genomes.** *H. Kehrer-Sawatzki[1], J. Kolb[1], N.A. Chuzhanova[2], J. Högel[1], K.M. Vasquez[3], D.N. Cooper[4], A. Bacolla[3].* 1) Human Genetics, University of Ulm, Ulm, Germany; 2) School of Computing, Engineering and Physical Sciences, University of Central Lancashire, Preston UK,; 3) Department of Carcinogenesis, University of Texas M.D. Anderson Cancer Center, Smithville, Texas, USA; 4) Institute of Medical Genetics, School of Medicine, Cardiff University, Cardiff, UK.

Submicroscopic inversions have contributed significantly to the genomic divergence between humans and chimpanzees over evolutionary time. Those microinversions which are flanked by segmental duplications (SDs) are presumed to have originated via non-allelic homologous recombination between SDs arranged in inverted orientation. However, the nature of the mechanisms underlying those inversions which are not flanked by SDs remains unclear. We have investigated 35 such inversions, ranging in size from 51-nt to 22056-nt, with a view to characterizing the DNA sequences in the breakpoint-flanking regions. Using the macaque genome as an outgroup, we determined the lineage specificity of these inversions and noted that the majority (N=31; 89%) were associated with deletions (of between 1-nt and 6754-nt) immediately adjacent to one or both inversion breakpoints. Overrepresentations of both direct and inverted repeats, ≥6-nt in length and capable of non-B DNA structure formation, were noted in the vicinity of breakpoint junctions suggesting that these repeats could have contributed to double strand breakage. Inverted repeats capable of cruciform structure formation were also found to be a common feature of the inversion breakpoint-flanking regions, consistent with these inversions having originated through the resolution of Holliday junction-like cruciforms. Sequences capable of non-B DNA structure formation have previously been implicated in promoting gross deletions and translocations causing human genetic disease. We conclude that non-B DNA forming sequences may also have promoted the occurrence of mutations in an evolutionary context, giving rise to at least some of the inversion/deletions which now serve to distinguish the human and chimpanzee genomes.

## 674/W/Poster Board #332

**Empirical measurement of gene conversion rate in primates.** *R. Assis, A.S. Kondrashov.* Bioinformatics, Univ Michigan, Ann Arbor, MI.

Gene conversion prevents divergence of duplicate genes via recombination. Here, we examine the properties of gene conversion by using a comparative genomics approach on noncoding sequences of human, chimpanzee, and orangutan. We explore the minimum and maximum lengths of and distances between gene conversion tracts. For the first time, we are able to measure the rate of gene conversion in noncoding sequences.

## 675/W/Poster Board #333

**Ferritin L RNA Sequences on Pairs of Human Chromosome Pairs Reflect Genome Duplication.** *R. Lebo[1], K. Bosnick[2].* 1) Dept Pathology, Akron Children's Hosp, Akron, OH; 2) Hathaway Brown, Cleveland, OH.

The 6,000,000,000 basepair human genome and the other similar size mammalian genomes are hypothesized to be the product of two genome duplications between primitive fish and mammals. Given that the genome duplicated twice at different times in ancestoral organisms predicts that pairs of similar size chromosomes arose during the most recent genome duplication after prior changes made the first pair of duplicated chromosomes different in size. The most recent duplication of the human genome was proposed to be reflected in the current 46 human chromosomes by the similar size and banding patterns of human chromosome pairs of similar shaped pairs of "homeologous" chromosomes (Comings, Nature, 1972). This hypothesis was further tested by studying the sequences and distribution of the shorter ferritin L RNA that inserted into multiple locations throughout the genome at different times. Typically these nonfunctional sequences would have escaped selective pressure while serving as a means to determine how long ago these sequences inserted and whether duplication occurred when similar size chromosomes doubled in number. The previously published relationship, function, and expression of the human aldolase A, B, C, and pseudogene supports two genome duplications in human homeologous chromosome pair 9 and 10 and chromosome pair 16 and 17 (Tolan et al, 1987). Studies of similar ferritin L (ferritin L; OMIM #134790) gene messages inserted into multiple other chromosome locations further supports genome duplication. The ferritin L gene had been mapped with the most similar sequences onto three chromosomes (Lebo et al, 1985), but only the gene on chromosome 19 is active (Worwood et al, 1985). The most identical inserted messages from this active gene have been found on chromosomes 19, 20, and X, confirming the chromosome sorting results. This study found a ferritin L mRNA most likely inserted into the short arm of an ancestral chromosome 19/20 that replicated and resulted in the current duplicated copies on homeologous chromosome pair 19 and 20 to suggest the similar size chromosomes 19 and 20 share a common ancestral chromosome. Furthermore, ferritin L messages that previously inserted at additional genomic sites with no negative functional consequences now serve as additional markers. The results of analyzing all these sequence homologies support the ancestral replication of other similar size human chromosomes.

## 676/W/Poster Board #334

**Rickettsia prowazekii shares homology with human sequences that have implications in pathogenesis and vaccine development.** *J. Dong.* Molec Diagnostics/Pathology, Univ Texas Med Branch, Galveston, TX.

Rickettsiae are obligately intracellular gram-negative, arthropod-transmitted bacteria that cause life-threatening rickettsioses such as Rocky Mountain spotted fever, typhus fever, Mediterranean spotted fever, and murine typhus. Rickettsiae are classified into two groups; typhus group (TG), which includes R. prowazekii and R. typhi, and the spotted fever group (SFG), which includes R. rickettsii, R. conorii, and R. sibirica. Rickettsiae grow initially in undetermined cell type(s) in the bite site and spread to regional lymph nodes where they multiply prior to spreading to the blood stream. The main pathology is widespread endothelial injury resulting in increased vascular permeability and microvascular leakage. Despite the availability of effective antibiotic therapy, rickettsial infection has a high mortality rate. Purpose: It is known that during evolution, nucleotide and protein sequences that have essential cellular functions are conserved. The significance of homologous sequences in disease pathogenesis and vaccine safety has not been firmly determined. The purpose of the study is to identify homology between R. prowazekii and human sequences that may be important in the pathogenesis and vaccine development of R. prowazekii. Methods: The genome of R. prowazekii contains 834 protein-coding genes. We performed sequence comparison of Rickettsia prowazekii (strain Madrid E, locus NC_000963) with human sequences using NCBI BLAST search. Summary of Results: We identified regions of matching sequences in several human proteins, supporting the notion that the genome sequence of R. prowazekii has a mitochondrial connection. The chief culprit antigens of circulating antimitochondrial antibodies (AMA) identified in primary biliary cirrhosis patients share homology with R. prowazekii. Some of the proteins identified are predicted to harbor strong cytotoxic T lymphocyte (CTL) epitopes.

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### 677/W/Poster Board #335

**Coevolution causes allelic association between physically unlinked gamete receptor genes.** *R.V. Rohlfs[1], W.J. Swanson[1], B.S. Weir[2].* 1) Genome Sciences, University of Washington, Seattle, WA; 2) Biostatistics, University of Washington, Seattle, WA.

Coevolving interacting genes undergo complementary mutations to maintain their interaction. If coevolving genes are polymorphic, different allele combinations may interact differently, conferring varying degrees of fitness. This differential fitness would result in selection for allele matching. In a population, excess matched alleles in a gene pair could be observed as linkage disequilibrium (LD)-like allelic association. Allelic association is usually thought to be a result of physical linkage or population structure however, intense selection for allele matching may also maintain allelic association.

If coevolving genes are physically unlinked, traditional gametic LD is not an appropriate measure of allelic association. We propose both standard composite linkage disequilibrium (CLD) and novel genotype association (GA), as measures for allelic association. Using a simple selective model, we calculated the power of these tests. Our results indicate the tests can feasibly detect allelic association when there is intense selective pressure. We apply CLD and GA tests to the putatively coevolving gamete recognition genes ZP3 and ZP3R. There is evidence that these unlinked polymorphic genes interact and are under positive selection, making them candidates for coevolution-induced allelic association.

We observe unusual allelic association between ZP3 and ZP3R as compared to random unlinked gene pairs. Since we compare ZP3-ZP3R to other gene pairs in the same individuals, the ZP3-ZP3R association can not be attributed to population structure. Our results support a biological instance of selection for allele matching between unlinked loci causing allelic association in a population. The observation of this selection is surprising since it requires an unusually intense selective coefficient, but that is reasonable in fertilization. This study shows that selection for allele matching can drive allelic association in a modern human population and that selection can be detected using CLD and GA tests. We are currently applying the CLD and GA tests genome-wide to estimate the number of loci associated due to selection for allele matching and to identify classes of coevolving genes.

### 678/W/Poster Board #336

**The presence of a purine rich 6-mer is likely correlated with the occurrence of recombination events.** *H.R. Johnston[1], D.J. Cutler[2].* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205; 2) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322.

We have previously demonstrated that apparent recombination hotspots could be plausibly explained not by elevated rates of recombination, but relatively uniform recombination rates combined with bottlenecks in human demographic history. Under this model recombination "hotspots" are not generally regions of increased recombination "rate", but are instead regions of the genome that are unusually old, and have therefore had more time to accumulate recombination events in their history. Here we identify a purine rich 6-mer likely associated with the occurrence of recombination in humans.

To do this, we analyze two complimentary datasets: the apparent recombination "hotspots" identified by McVean et al, from population data, and the regions of known recombination identified by Coop et al. from large pedigrees. We analyze each dataset independently, and find the same pattern in both data sets. Recombination "hotspots" and regions of known recombination event both show an excess of purine rich 6-mers, and especially G-rich 6-mers over randomly chosen regions, matched to overall GC content. This pattern is highly statistically significant, representing an approximately 10-20% excess of G-rich 6-mers over random regions. When the top 25 motifs are analyzed by MEME, a motif finding tool, a very strong and simple motif is found: (A/G)GG(A/G)AG.

To differentiate whether or not this motif is a merely a statistical artifact of regions of high recombination, or a motif associated with the initiation of recombination, we ask for the location of motif within the "hotspots" and windows of known recombination. We show that the 10-20% excess of this motif is strongly associated with center of the regions, consistent with the motif being a recombination initiation signal.

The implication of this work is that the large-scale differences in recombination rate between regions of the genome could well be due to increased or decreased levels of the motif on a macro scale. Apparent "hotspots" themselves, however, seldom have more than a 10-20% increase in this motif above random sequence. Notably, alpha-satellite DNA, associated with centromeric regions with virtually no recombination, is completely free of this motif.

### 679/W/Poster Board #337

**Effect of natural selection on North Asian mitochondrial haplogroup variation.** *M. Derenko[1], B. Malyarchuk[1], T. Grzybowski[2], G. Denisova[1], U. Rogalla[2], M. Perkova[1], I. Dambueva[3], I. Zakharov[4].* 1) Genetics Laboratory, Institute of Biological Problems of the North, Russian Academy of Sciences, Magadan, Russian Federation; 2) Forensic Medicine Institute, the Ludwik Rydygier Medical College, the Nicolaus Copernicus University in Torun, Bydgoszcz, Poland; 3) Institute of General and Experimental Biology, Russian Academy of Sciences, Ulan-Ude, Russian Federation; 4) Animal Comparative Genetics Laboratory, Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russian Federation.

The human mtDNA exhibits striking, region-specific sequence variation. The regional distribution of mtDNA haplogroups have attributed either to genetic drift assisted by purifying selection (Elson et al., 2004; Kivisild et al., 2006; Ingman, Gyllensten, 2007) or to an adaptation to different climates (Mishmar et al., 2003; Ruiz-Pesini et al., 2004). In an attempt to study the mode of selection in mtDNA variation in human populations we sequenced and analyzed 211 complete mtDNA sequences belonging to haplogroups A, C and D accounting in total for 49.3% of mtDNA lineages in North Asia. The North Asian haplogroups A, C and D showed a highly significant deviation from the standard neutral model as well as a bell-shaped distribution of pairwise differences consistent with rapid population expansion. To determine the overall importance of selection in shaping human mtDNA variation we calculated Ka/Ks ratio both for aggregated mtDNAs and for 13 protein-encoding genes within particular haplogroups (A, C and D). We have found a prevalence of Ks over Ka within haplogroups A, C and D indicating the influence of negative selection on mtDNA during evolution. Consistent with some previous reports we have found the Ka/Ks ratio for the ATP6 gene to be the highest among the North Asian sequences suggesting thereby that this gene has been subject to positive selection. We have also observed a set of genes with a somewhat higher Ka/Ks ratio relative to other mitochondrial genes - CO2 for haplogroup A, ND3 and ND4 for haplogroup C. Meanwhile the other approach taking into account the difference in NS/S ratios between the haplogroup-associated and private substitutions (Elson et al., 2004) shows the significant departures from neutrality only for haplogroup D and its subhaplogroup D4. Furthermore single gene analysis reveals the relatively strong influence of negative selection only in CYTb gene within haplogroup D (p=0.011, NI=14.1). In general, our results indicate that there is an evidence for both gene-specific and lineage-specific variation in selection acting on North Asian mtDNAs. This study was supported by Russian Foundation for Basic Research grant 07-04-00445 and by Far-East Branch of the Russian Academy of Sciences grants 09-III-A-06-220 and 09-I-P-23-10.

### 680/W/Poster Board #338

**Constructing the initial map of transmission distortion based on high resolution haplotype data.** *L. Deng[1,2], D. Zhang[2,3], E. Richards[4], Y. Wang[5], J. Fang[5], F. Long[5], X. Tang[1].* 1) Faculty of Basic Medical College, Nanchang University, Nanchang, China; 2) Beijing Inst Genomics, Beijing, China; 3) Graduate School of the Chinese Academy of Sciences, Beijing, China; 4) Department of Biology, College of Life Sciences, Brigham Young University, Provo UT 84602, USA; 5) The laboratory center of Medicine, Nanchang University, Nanchang, China.

Transmission distortion (TD) is defined as a significant departure from Mendelian predictions of genes or chromosomes to offspring. While many biological processes have been implicated, there is still much to be understood about TD in humans. Here we present our findings from a genome-wide scan for evidences of TD using haplotype data of 60 trio families from the International HapMap Project. Fisher's exact test was applied to assess the extent of TD in 629,958 SNPs across the autosomes. Based on the empirical distribution of PFisher and further permutation tests, we identified 1,205 outlier loci and 224 candidate genes with TD (137 in Africa and 90 in Europe samples). Most of candidate genes (221/224) were observed in only one of the two populations, indicating an ethnicity-related property of TD. Using the PANTHER gene ontology database, we found 19 categories of biological processes with an enrichment of candidate genes. In particular, the "protein phosphorylation" category contained the largest number of candidates in both HapMap samples. Further analysis uncovered an intriguing non-synonymous change in PPP1R12B, a gene related to protein phosphorylation that appears to influence the allele transmission from male parents in the Africa population. Our findings also indicate enrichment for selective signals in genes with TD relative to genomic background and provide new clues for our understanding of TD in humans.

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## 681/W/Poster Board #339

**DIVERGENOME: a bioinformatics tool to assist the analysis of genetic variation.** *W.C.S. Magalhães[1], M. Rodrigues[1], B. Araujo[1], M.L. Iannini[1], A.A. Faria-Campos[2], E. Tarazona-Santos[1].* 1) Departamentos de Biologia Geral e Bioquímica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brazil; 2) Departamento de Ciências da Computação, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Brazil.

We developed a bioinformatic tool (DIVERGENOME) to assist investigators in data storage and analysis for population genetics and genetic epidemiology studies. The platform contains two components. The first component, DIVERGENOMEdb, is a relational database developed using the open source database management system MySQL. It allows to safely storage individual genotypes for three different types of data: contigs (resulted from re-sequencing projects), SNPs/INDELs and microsatellites. Genotypes may be linked to a description of protocols used to generate these data. Individuals may be linked to populations, as well as to individual phenotypic information that may be collected in genetic epidemiology studies, using different kinds of variables: binary, numerical or categorical. The access to DIVERGENOMEdb is controlled by a hierarchical system of passwords that include different privileges for administrators, principal investigators (PIs) and researchers linked to a specific PI. The second component of the platform, DIVERGENOMEtools, is a set of informatics tools (developed in PERL) that allow an easy preparation of input files for commonly used population genetics and genetic epidemiology software, departing from output files obtained from previous analyses. These programs include: PHASE, Structure, Arlequin, Haploview, DNAsp, Haplostats, as well as tools developed for R environment. Moreover, a web interface allows investigators to interact with DIVERGENOMEdb and DIVERGENOMEtools. Currently, DIVERGENOME is being used to assist studies of candidate genes studies about genetic susceptibility to gastric cancer in Latin American populations; population genetics studies from admixed populations of the Minas Gerais State (Brazil), and public data from the HapMap, SNP500Cancer and the HGDP projects (which have been used in ours projects for comparative purposes). We also use DIVERGENOME to storage and facilitate analyses of simulated databases generated under different evolutionary scenarios using coalescent simulations. Financial Support: Brazilian agencies CAPES-Ministry of Education, FAPEMIG and CNPq.

## 682/W/Poster Board #340

**CD4 INTRAGENIC SNPS ASSOCIATE WITH HIV-2 PLASMA VIRAL LOAD AND CD4 COUNTS IN A COHORT FROM GUINEA-BISSAU, WEST AFRICA.** *G. Sirugo[1,2,3], D.R. Velez[4,2], M.F. Schim van der Loeff[6], S.M. Williams[5], T.L. Edwards[4], W.K. Scott[4], C. Bisseye[1], A. Tacconelli[2], E. Brunetti[2], G. Novelli[2,3], P. Aaby[7], S. Kaye[8], A. Jaye[1], H.C. Whittle[1], A.V. Hill[8], B.J. Hennig[9].* 1) MRC, The Gambia; 2) Unita' di Genetica Medica, Ospedale S. Pietro FBF, Rome, I; 3) Dipartimento di Biopatologia e Diagnostica per Immagini, Tor Vergata University School of Medicine, Rome, I; 4) University of Miami, Miller School of Medicine, USA; 5) Center for Human Genetic Research, Vanderbilt University, USA; 6) Academic Medical Center, Amsterdam, NL; 7) Bandim Health Project, INDEPTH Network, Guinea-Bissau; 8) UCL, London, UK; 9) LSHTM, London, UK.

**Background**: HIV infection rates are variable across Africa with the highest prevalence of HIV-1 in Eastern and Southern Africa and the highest prevalence of HIV-2 in West Africa, particularly in Guinea-Bissau where it exceeds 5% in the adult population vs. 1-2% in other West African countries. We investigated single nucleotide polymorphisms (SNPs) in two genes encoding molecules critical in HIV pathogenesis and disease progression: CD4 and DC-SIGN (encoded by CD209). **Methods**: The study comprised of 29 HIV-1, 143 HIV-2, 30 dually infected individuals and 194 HIV-uninfected controls recruited from a rural area in Guinea-Bissau. We genotyped fourteen CD4 and four DC-SIGN intragenic SNPs. HIV infection, viral load, and CD4 count (for HIV-2), were the outcome measures. Genomic variation was analyzed for single markers and haplotypes by logistic regression and multi-marker analysis by multifactor dimensionality reduction (MDR) for dichotomous outcomes (comparing HIV groups (1, 2, and dual infection) to the control group), and with parametric and nonparametric linear and haplotype type trend regression for continuous outcomes (CD4 count). **Results**: HIV-1 infected subjects were younger than other comparison groups, but no other demographic variable showed differences between groups. Our analyses showed associations of four CD4 single SNPs with 'HIV infection', with the most significant observed for CD4 SNP rs2255301with the model TT v CC&CT (OR=2.50 CI 1.34-4.69, p=0.004). We also saw a haplotype association in DC-SIGN (CD209) (rs8105483- rs2287886-rs4804803, global p=0.03). Within the HIV-2 group we observed single marker (rs11575097 p=0.02) and haplotype (rs8105483-rs2287886, global p=0.02) associations of DC-SIGN SNPs with CD4 counts and a more significant association of plasma viral load with CD4 variants (rs11575097-rs10849523, global p=0.004). **Conclusions**: These results suggest that CD4 gene variation has an effect on HIV-2 infection dynamics. Replication studies in well characterized HIV-1 African cohorts will be warranted to confirm the biological relevance of this finding in HIV-1 infection and progression to AIDS.

## 683/W/Poster Board #341

**The spectrum and frequency of MEFV mutations in newborns from Tbilisi, Georgia.** *C. Oberkanins[1], B. Rauscher[1], I. Korinteli[2], M. Korinteli[2], G. Kriegshaeuser[1], K. Pagava[2].* 1) ViennaLab Diagnostics, Vienna, Austria; 2) Department of Pediatrics and Adolescent Medicine, Tbilisi State Medical University, Georgia.

Familial Mediterranean Fever (FMF) is a hereditary inflammatory disorder caused by mutations in the MEFV gene. Carrier rates are known to be particularly high among Sephardic Jews, Turks, Armenians and Arab populations. The spectrum and frequency of MEFV mutations in Georgia has not at all been investigated so far. Multiplex PCR and reverse-hybridization teststrips (FMF StripAssay) were applied to simultaneously analyze twelve common MEFV mutations in DNA samples from dried blood on filter cards, which had been obtained from 202 unselected newborns at various hospitals in Tbilisi, Georgia. We found 30 samples to be heterozygous and 1 to be compound heterozygous or complex (two mutations in cis). The carrier rate of MEFV mutations (15.3%) was remarkable, although lower than data reported from neighbouring Turkey and Armenia (approx. 20%). The most frequently observed variants were E148Q (15x), M680I G/C (5x) and M694V (4x). Five other MEFV mutations were found at lower incidence (V726A, A744S, R761H: 2x each; P369S, F479L: 1x each). Although low in number, newborns of parents with Armenian roots had a substantially higher carrier rate of MEFV mutations compared to the Georgian population overall. Our data indicate that MEFV mutations, including severe ones such as M680I and M694V, are not uncommon in the Georgian population. Based on these new findings, the awareness for FMF and the availability of appropriate testing should be further promoted in Georgia. (oberkanins@viennalab.-co.at).

## 684/W/Poster Board #342

**Detecting epistatic interactions between human alleles through non-monotonic LD decay.** *A.A. Shpunt[1,2], G.V Kryukov[1], S.R. Sunyaev[1].* 1) Division of Genetics, BWH, Harvard Medical School, Boston, MA; 2) Massachusetts Institute of Technology, Boston, MA.

Importance of epistasis for the evolutionary dynamics of alleles in the human population and for genetics of complex phenotypes remains a controversy. We developed a statistical strategy to search for epistatic selection. Our method is based on detecting non-monotonic decay of linkage disequilibrium (LD). Lewontin and Kojima (1960) demonstrated that epistatic selection can maintain LD if the strength of selection is comparable with recombination rate between the two polymorphic sites. Computer simulations suggest that this effect leads to a non-monotonic LD decay. We designed a statistic to detect non-monotonic LD decay and applied it to SNPs from the HapMap dataset. We compared functionally pre-selected pairs of SNPs such as SNPs in the same gene, SNPs in neighboring co-expressed or co-functioning genes, putative regulatory SNP paired with a coding SNP. We used random SNP pairs separated by the same distance as control. Our analysis suggests potential importance of epistatic interactions in the human genome. We further investigated robustness of our analysis to parameters and to various evolutionary and demographic scenarios using forward simulations.

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## 685/W/Poster Board #343

**Evaluation of population-specific reference haplotypes for imputing untyped genotypes.** *K.-K. Kim[1], H.-H. Won[2,3], M.-J. Kim[2], S. Kim[2], K.-A. Lee[4], J.-W. Kim[1].* 1) Department of Laboratory Medicine and Genetics, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea; 2) Samsung Biomedical Research Institute, Seoul, Korea; 3) Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Korea; 4) Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, Korea.

Genome-wide association studies using high density SNP data have reported a number of significant risk alleles affecting disease susceptibilities. However, low genotype relative risk requires a large number of samples to detect association with the disease. Conventional meta analysis of data from different studies is one way to apply the association test more effectively. Another efficient strategy is to genotype a larger number of samples with SNP chips that include fewer SNPs and to impute untyped SNPs or missing genotypes. Recent several association studies showed that imputation could successfully estimate untyped SNPs based on the international haplotype data. Because haplotype structures of a particular population differ greatly from those of other populations, the evaluation of population-specific haplotype references is necessary. We constructed Korean haplotypes based on 90 Korean normal subjects selected from cohort samples of the Korean HapMap using fastPHASE. For comparison of imputation accuracy, we used reference haplotypes of East Asian (CHB+JPT), Caucasian (CEU), and African (YRI) populations from the International HapMap. For a genotype data set, 498 samples were randomly selected from Korean cohort data (KARE, Korean Association Research). To evaluate the accuracy of imputation, we randomly selected a part of the genotype data set for untyped SNPs, used the IMPUTE program to impute the untyped SNPs, and compared imputed genotypes of the SNPs with their real genotypes. We performed imputation on untyped SNPs using reference haplotypes from the four populations. Imputation performance using the CHB+JPT (ethnically close population) haplotypes for imputing the Korean genotype data was superior to those using other ethnic reference haplotypes (CEU and YRI). Moreover, imputation based on the Korean haplotypes was more accurate than that of the CHB+JPT haplotypes. The comparison of the Korean and CHB+JPT haplotypes also showed that minor allele frequency, heterozygosity, and linkage disequilibrium partially influenced the difference in imputation accuracy. These results suggest the need for constructing specific sub-population haplotypes for imputing untyped genotypes of the corresponding population.

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## 686/W/Poster Board #344

**Metabonome Quantitative Trait Linkage Mapping in Organ Extracts using Nuclear Magnetic Resonance Profiling.** *J.-B. CAZIER[1], M.E. DUMAS[2], S. WILDER[3], T. TSANG[4], Y. WANG[4,5], J. NICHOLSON[4], D. GAUGUIER[1].* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OxfordShire, United Kingdom; 2) Centre de RMN à Très Hauts Champs, Ecole Normale Supérieure de Lyon, Villeurbanne, France; 3) European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SD, United Kingdom; 4) Department of Biomolecular Medicine, Imperial College London, South Kensington Campus, London, United Kingdom; 5) State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Centre for Magnetic Resonance, Wuhan Institute of Physics and Mathematics, The Chinese Academy of Sciences, Wuhan, PR China.

Linkage analysis has long been the key method to localize genomic regions associated with a disease phenotype using pedigree information. Lately this method has seen some revival with the integration of a large quantity of newly available molecular phenotypes derived from gene transcription or metabolic profiling. We describe here our method to perform metabonomic Quantitative Trait Linkage (mQTL) in model organism using the 1H Nuclear Magnetic Resonance (NMR) profile of organic sample as a trait. The complexity of the observed signal requires a careful tuning of the analysis to allow true signals to be revealed. Although NMR spectra are very stable, they can vary greatly between tissues. In a previous study of plasma tissue in F2-cross between diabetic and control rats we successfully identified, and validated, the association of benzoate, a gut microbial metabolite, with the deletion of a uridine disphosphate glucuronosyltransferase. We have extended this method in the same rat F2-cross to the analysis of other tissues, such as the aqueous and lipid extracts of adipose tissue. We identified a new locus (LOD>9) on chromosome 4 linked to a specific shift of 2.65ppm in the NMR spectra, which corresponds to candidate metabolites that we can now attempt to validate. In conclusion mQTL analysis of organ extracts provides a very powerful tool to identify new loci and pathways associated with a disease variable, but it requires extra careful care to minimize the noise in the large amount of data. Furthermore this method can help to pinpoint the pathway associated with the locus by identification of the affected metabolite.

## 687/W/Poster Board #345

**The role of CRISPLD2 during Zebrafish Development.** *B. Chiquet[1,2], E. Swindell[1], L. DeVault[1], M. Warman[3], Y. Nakamura[4], J. Hecht[1].* 1) Dept Pediatrics, Univ Texas Med Sch, Houston, Houston, TX; 2) Univ Texas Dental Branch, Houston TX; 3) Dept Genetics, Howard Hughes Medical Institute, Boston, MA; 4) Institute of Medical Science, Univ of Tokyo, Tokyo, Japan.

Craniofacial development is a highly regulated process that involves the complex orchestration of genetic and environmental stimuli, including interaction of cell growth, growth factors and receptors, the convergence and fusion of the facial and palatal processes, apoptosis, and adequate nutrient supply. Disruption of any of these factors can predispose birth anomalies, such as non-syndromic cleft lip with or without cleft palate (NSCLP). NSCLP affects 1 out of 700 live births, or 4000 newborns each year in the United States. Treatment of NSCLP includes surgical, dental and speech therapies, creating a significant healthcare burden on families of affected children. Therefore, research has focused on identifying etiological factors of NSCLP and understanding how these factors contribute to the clefting phenotype. Recently we identified a novel gene, cysteine-rich secretory protein LCCL domain containing 2 (CRISPLD2), which is associated with NSCLP and expressed during murine craniofacial development. However, the in vivo biological function is unknown. To help understand how CRISPLD2 might function during normal development, a zebrafish model was used. In situ hybridizations show that CRISPLD2 is present at all stages of zebrafish embryogenesis and that CRISPLD2 localizes in the head of developing zebrafish. Knockdown of CRISPLD2 using CRISPLD2-specific anti-sense oligonucleotide morpholinos caused a dose-dependent lethality. Morpholino concentrations that allow development to progress past the blastula stage cause abnormal development by one-day post fertilization. Our data shows that embryos with a knockdown of CRISPLD2 have both a lower survival rate and higher percentage of abnormal phenotypes, such as delayed differentiation and head/tail truncation, compared to uninjected and control morpholino injected embryos. On-going studies are determining whether the expression pattern of other genes that regulate craniofacial development (i.e., Dlx2 and Sox9) are altered in CRISPLD2 zebrafish morphants. Together, these results show that CRISPLD2 plays a critical role during zebrafish craniofacial development and will provide information about how perturbation of CRISPLD2 contributes to NSCLP etiology.

## 688/W/Poster Board #346

**A novel mouse model reveals a critical role for *SOX7* in the formation of diaphragmatic hernias and cardiovascular malformations associated with recurrent 8p23.1 deletions.** *M. Wat[1], Y. Chen[1, 3], M. Garcia[2], M. Dickinson[2], B. Lee[1, 3], D.A. Scott[1].* 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Molecular Physiology & Biophysics, Baylor College of Medicine, Houston, TX; 3) Howard Hughes Medical Institute.

Recurrent interstitial deletions of a region of 8p23.1 flanked by low copy repeats are associated with a spectrum of anomalies that can include congenital diaphragmatic hernia (CDH) and cardiovascular malformations (CVMs). Haploinsufficiency of *GATA4*, a retinoid responsive transcription factor, has been hypothesized to be responsible for these birth defects based, in part, on the fact that *Gata4*$^{+/-}$ mice have anterior CDH and heart defects. However, no clearly causative *GATA4* mutations have been identified in patients with CDH, and the spectrum of heart defects associated with 8p23.1 deletions is more severe than that associated with *GATA4* mutations. This led us to hypothesize that haploinsufficiency of another gene may also contribute to this phenotype. *SOX7* is another, retinoid responsive, transcription factor which lies adjacent to *GATA4* in the CDH/CVM critical region on 8p23.1. *SOX7* was a particularly interesting candidate gene for CDH and CVM development since it was known to upregulate *Gata4* expression in retinoic acid-treated F9 cells and human embryonic stem cells. In situ hybridization studies in mice at E13.5 and E16.5 confirmed that *Sox7* was expressed in the developing diaphragm and heart. To further explore the role of *Sox7* in diaphragm and heart development, we created *Sox7*$^{+/-}$ mice. Approximately 15% of these mice have retrosternal CDH in which a portion of the liver and gallbladder have herniated into the thoracic cavity and are encased in a thin membrane. This type of retrosternal sac hernia is identical to that seen in *Gata4*$^{+/-}$ mice and in some patients with 8p23.1 deletions. Although cardiovascular anomalies were not identified in *Sox7*$^{+/-}$ mice, *Sox7*$^{-/-}$ mice die in utero around E10.5, a time period critical for heart development. *Sox7*$^{-/-}$ mutants have enlarged pericardial sacs and are smaller and developmentally delayed suggesting that heterozygosity of *SOX7* likely contributes to the CVMs seen in patients with 8p23.1 deletions. This work clearly demonstrates how a careful analysis of human phenotypes combined with animal modeling can be an effectively means to identify genes that contribute to the complex phenotypes associated with recurrent genomic disorders.

## 689/W/Poster Board #347

**FAF1 in Cleft Lip and Palate Development.** *M. Ghassibe[1], L. Desmyter[1], T. Langenberg[2,3], F. Claes[2,3], O. Boute[4], B. Bayet[5], Ph. Pellerin[6], P. Brouillard[1], N. Revencu[1], R. Vanwijck[5], P. Carmeliet[2,3], M. Vikkula[1].* 1) Laboratory of Human Genetics (GEHU), de Duve Institute, Université catholique de Louvain, Brussels, Belgium; 2) Department for Transgene Technology and Gene Therapy, VIB, Leuven, Belgium; 3) Center for Transgene Technology and Gene Therapy (CTG), K.U.Leuven, Leuven, Belgium; 4) Centre de Génétique, CHU de Lille, Lille, France; 5) Centre Labiopalatin, Service de Chirurgie Plastique, Cliniques universitaires Saint-Luc, Brussels, Belgium; 6) Service de Chirurgie Plastique et Reconstructive, CHU de Lille, Lille, France.

Cleft lip and palate is a congenital anomaly of complex etiology. Predisposition is governed by numerous genetic loci, in combination with environmental factors. Clefts have an incidence of 1/700 births. We recently identified a translocation in a cleft palate only (CPO) and Pierre Robin sequence (PRS) family. The translocation disrupts the FAF1 gene, which has not been implicated in clefts before. We also showed that FAF1 is associated with CPO and PRS in a cohort of 1500 individuals. In order to replicate the association, we have conducted transmission disequilibrium test in five independent cohorts of different ethnic backgrounds. The same FAF1 variant as in our first study was genotyped. Overall, replications yielded positive results in the CPO/PRS subgroup. In parallel, we showed that zFaf1 is expressed in the pharyngeal cartilages of the zebrafish larvae, where its knock-down results in orofacial defects. Proliferation coupled to apoptosis assays, as well as in situ hybridization of different craniofacial markers have shown that whereas early proliferation and migration of neural crest cells towards the cranial region is normal, later morphogenic processes are altered. We have also generated a mouse line defective for Faf1. Although the heterozygous mice are viable, a more detailed study of their facial features, bone structures, as well as the generation of null mice will help us to better characterize the molecular function and role of Faf1 in cleft development. (miikka.vikkula@uclouvain.be).

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## 690/W/Poster Board #348

**Tackling the complexity of the genotype-phenotype relationship in the Down syndrome with the mouse AnEUploidy Zoo: a resource of new models to study aneuplodies involving human chromosome 21.** *Y. Herault[1,6], P. Lopes Pereira[1], L. Magnol[1], I. Sahun[2], A. Duchon[1,6], P. Prandini[3], E. Dalloneau[1], M. Raveau[1,6], V. Nalesso[1,6], J.-C. Bizot[4], B. Chadefaux-Vekemans[5], S. Deutsch[3], C. Borel[3], F. Trovero[5], V. Brault[1,6], S.E. Antonarakis[3], M. Dierssen[2], The AnEUploidy consortium (www.aneuploidy.eu).* 1) MORPHEM, CNRS INTRAGENE, Orleans, France; 2) Genes and Disease Program, Center for Genomic Regulation, Barcelona, Spain; 3) Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland; 4) Key-Obs S.A, Orléans, France; 5) Service de Biochimie Métabolique, Hôpital Necker-Enfants Malades, Faculté de Médecine, Université Paris Descartes, France; 6) ICS/IGBMC, Illlkirch, France.

50 Years after the discovery of the Trisomy 21, the underlying genetic basic of the Down syndrome (DS), the study of the complex genotypes - phenotypes relationship in DS is still a challenge. Currently it is accepted that interactions between “dosage sensitive” genes along the HSA21 are responsible for the complex feature of the pathology. Orthologues of genes on HSA21 exist in the same order separated on mouse chromosomes (MMU) 16, 17 and 10. Most of the exisiting mouse models for DS are trisomic for part of the MMU16 homologous region. These models exhibit many of the characteristics of DS, but not all the mechanisms have been described up to now. In order to complement these models and to decipher the gene interactions generating the DS phenotype, we created new partial trisomies and monosomies for different regions on MMU10, 16 and 17 that are homologous to HSA21 by using chromosomal engineering technic. Now we succeeded in getting a complete series of mouse trisomic models and monosomic models covering the region of the mouse genome homologous to HSA21. These models are listed below: New segmental trisomy, Chr., Modification (interval); Ts1Yah, MMU17, Dup(Abcg1-U2af1); Ts2Yah, MMU16, Dup(Stch-App); Ts3Yah, MMU10, Dup(Cstb-Prmt2); New Segmental Monosomy, Chr., Modification (interval); Ms1Yah, MMU10, Del(Col6a1-Prmt2); Ms2Yah, MMU17, Del(Abcg1-U2af1); Ms3Yah, MMU16, Del(Stch-App); Ms4Yah, MMU10, Del(Cstb-Prmt2); Ms5Yah, MMU16, Del(App-Runx1); Ms6Yah, MMU16, Del(Stch-Runx1); Using such a series we are now oriented toward deciphering the role of each region in the induction of DS phenotypes, their interactions and at term the signal pathways that are affected, with the hope that this would lead to development of new therapeutical approaches. We will report here the phenotypic analysis of the new models and the results obtained on cognition, learning and memory and gene expression in brain of such models. Then we will propose hypothesis to explain the physiopathology of the disease showing that genetic interactions can balance both negative and positive contributions, highlighting the complexity of the genetic codes in DS.

## 691/W/Poster Board #349

**A genome-wide association study identifies two novel loci that regulate canine body size.** *J.J. Schoenebeck[1], N.B. Sutter[2,4], B.M. vonHoldt[3], P. Quignon[1], J. Allen[2,4], A.R. Boyko[4], L. Li[4], R.K. Wayne[3], C.D. Bustamante[4], E.A. Ostrander[1].* 1) Cancer Genetics Branch, National Human Genome Research Institute, Bethesda, MD; 2) College of Veterinary Medicine, Cornell University, Ithaca, NY; 3) Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA; 4) Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY.

Purebred dogs display phenotypic diversity that is unparalleled by any other terrestrial species: size, shape, posture, coat color and texture, as well as behavior can vary profoundly breed to breed. CanMap is an ongoing effort to identify genes important in controlling fixed traits in domestic dogs. To date we have genotyped 1000 domestic dogs from 80 breeds and 400 wild canids using the Affymetrix V2 SNP chip, generating a data set of 50,000 SNPs per dog. This body of data lays the foundation for many mapping studies, including investigations into domestication, population structure, and mapping breed-defining traits such as leg length and width, skull shape, and fur patterning. One of the most striking examples of intraspecies morphological diversity is dog body size, which can vary greater than 40-fold across animals of distinct breeds. We showed previously that variation at the *IGF1* locus plays an important role in determining the body size of small and toy breed dogs. Association testing of CanMap data using breed mass averages and principal components analysis reveals numerous, highly significant quantitative trait loci (QTLs), including associations to *STC2*, a secreted glycoprotein known to inhibit postnatal growth ($P$ = 4.42x10-26) and *SMAD2*, a transcription factor critical for TGF-beta signaling ($P$ = 5.48x10-25). Fine mapping of the *STC2* and *SMAD2* loci (chromosomes 4 and 7, respectively) validated the original associations produced from CanMap analysis. Interbreed haplotype comparisons suggest that small and toy breeds experienced selective sweeps at both loci, implying strong, artificial selection of genetic variants that restrict growth. To identify causal mutations, we are currently fine mapping both *STC2* and *SMAD2* loci. Intriguingly, within the *SMAD2* locus we identified a 10 kb deletion that segregates with small and toy breed dogs. The deletion spans a multispecies conserved sequence (MCS) located downstream of *SMAD2*, raising the possibility that in the absence of this MCS, *SMAD2* transcription is downregulated. Characterization of the functional significance of this deletion, as well as other polymorphisms within the critical intervals of *SMAD2* and *STC2* are planned.

## 692/W/Poster Board #350

**Imputation of Ancestry in Inbred Strain Crosses.** *J.J. Zhou[1], J.S. Sinsheimer[1,2,3], K. Lange[1,2].* 1) Department of Biomathematics, University of California, Los Angeles, CA; 2) Department of Human Genetics, University of California, Los Angeles, CA; 3) Department of Biostatistics, University of California, Los Angeles, CA.

Association studies with inbred strains can assist in mapping quantitative trait loci (QTL) for human traits. A major confounding factor with inbred crosses is polygenic background, but recent theory makes it possible to accurately capture this complication as a random effect in a mixed effects statistical model (Bauman *et al* 2008). Another potential difficulty with inbred crosses is their reduced genetic diversity compared to natural animal populations. Multiple crosses, for example, the eight-way cross announced by Complex Trait Consortium (Churchill *et al* 2004, Chesler *et al* 2008), circumvent these difficulties. In our latest expansion of theory for QTL association mapping with inbred strains, we directly impute local strain origins by choosing the most probable strain. Strain origins can then serve as mean effects in a multivariate normal model for testing association between trait levels and strain origin along the genome. Imputation is a combinatorial process that locally assigns the maternal and paternal strain origin of each animal using prior pedigree information. Without some kind of smoothing, imputation is likely to be ill defined or jump erratically from one strain to another as we travel along the genome of an animal. In practice, we expect to see long stretches where strain origin is constant. This can be arranged by penalizing strain changes from one marker to the next. A dynamic programming algorithm solves the strain imputation process in one quick pass through the genome of an animal.

Simulation was performed by mimicking the 8 way Complex Trait Consortium. Eight inbred strains of mice were chosen as founders. The mating design is rigid for the first 3 generations and random mating for by siblings' mating. By using more than 1000 markers we recover strain origins with 99% accuracy. We also compared our results with the software HAPPY (Mott *et al* 2000) on their published data for 1016 markers. Our method gave more accurate results than HAPPY.

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## 693/W/Poster Board #351

**Developmental elevation of neutrophils is associated with increased Serpina-1 expression in a rat model of innate airway hyperresponsiveness.** *N. Carpe[1,2], I. Mandeville[2], T. Bao[2,3], J. Chu[4], B.A. Raby[4], S.T. Weiss[4], F. Kaplan[1,2,3,4,5].* 1) Dept of Human Genetics, McGill University; 2) Montreal Children's Hospital Research Institute; 3) Dept of Biology, McGill University; 4) Channing Laboratory, Brigham and Womens Hospital, Harvard Medical School; 5) Dept of Pediatrics, McGill University.

Rationale: An growing body of evidence suggests that a significant proportion of asthma in both children and adults, including mild to moderate asthma, is associated with neutrophilic airway inflammation. Yet the role of neutrophil mediated inflammation in asthma pathophysiology remains unclear. We are exploring the origins of innate airway hyperresponsiveness (AHR) in developing lung of the Fisher rat. Atopic (Brown Norway) and normoresponsive (Lewis) rats serve as controls. We hypothesized that neutrophil-mediated inflammation may contribute to the Fisher respiratory phenotype. Methods: We assessed bronchoalveolar lavage (BAL) cell differentials in postnatal lungs of naïve Fisher, BN and Lewis rats from postnatal day (PN)1-14. Pregnant dams and adult rats were sensitized and challenged with ovalbumin (OVA) using the inExpose aerosol inhalation system. BAL fluid was collected from pups of sensitized dams and adult rats to analyze inflammatory cell profiles. Illumina genome wide profiling was used to compare global gene expression patterns in naïve PN rat lung. Lung mRNA levels of Serpina-1(alpha-1-antitrypsin), were validated by real-time [RT]-PCR. Results: Fisher rats displayed a significantly greater number of neutrophils in BAL fluid than BN and Lewis rats from PN1-14 ($P<0.05$). Following a 3 day acute OVA exposure, adult Fisher rats had significantly elevated neutrophils in BAL fluid relative to Lewis rats. Increased BAL neutrophils were also observed in lungs of Fisher rat pups compared to Lewis pups born to dams challenged with OVA or saline during pregnancy. OVA exposure enhanced this effect. Serpina-1expression was elevated in adult Fisher rat and in PN7 rat pups of naïve and OVA challenged mothers. Linear regression analysis of Illumina expression profiles (n=4 per strain per time point, with interaction terms age x strain) identified 4 additional genes displaying significantly different expression trajectories over time that were unique to Fisher rats. Two of these (Defb3, FCN) are expressed in neutrophils and produce proteins previously reported to have a role in innate immunity. Conclusions: The Fisher rat exhibits innate neutrophilic airway inflammation that is enhanced by OVA challenge *in utero* and in adulthood. These findings suggest that the Fisher rat can serve as a useful model to elucidate developmental mechanisms involved in neutrophil mediated inflammation in asthma.

## 694/W/Poster Board #352

**Investigating the role of Fragile X related proteins in mammalian circadian behaviors.** *J.G. Lumaban, D.L. Nelson.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Fragile X syndrome, the most common form of inherited mental retardation, results from the absence of the fragile X mental retardation 1 (FMR1) gene product FMRP. FMR1 has two paralogs in vertebrates: fragile X related gene 1 and 2 (FXR1 and FXR2); it has been postulated that the presence of FXR1P and FXR2P could partially compensate for the loss of FMRP in Fragile X syndrome. One of the behavioral symptoms observed in Fragile X patients is the increased occurrence of sleep disorders. Studies on animal models revealed that Fmr1/Fxr2 double knockout (KO) and Fmr1 KO/Fxr2 heterozygote mice exhibit a complete loss of rhythmic activity in a light:dark (LD) cycle, and that Fmr1 or Fxr2 KO mice display a shorter free-running period of locomotor activity in total darkness (DD). Furthermore, the cyclical patterns of abundance of several core clock component messenger RNAs are altered in the livers - which contain a major peripheral clock - of Fmr1/Fxr2 double KO mice, while the overall clock gene expression in the central clock appears unchanged between animals of different genotypes. These findings suggest that Fmrp and Fxr2p are acting downstream of the central clock to control rhythm in mice. Current efforts are aimed at determining the relevance of this role to sleep and other behavioral alterations observed in fragile X patients. Locomotor assays after restricted feeding are being employed to test whether the Fmr1/Fxr2 double KO mice are able to entrain to food, even when they cannot entrain to light. Electroencephalography studies are also in progress to analyze brain electrical activity of Fmr1/Fxr2 double KO mice and determine whether their sleep/wake cycles are significantly altered compared to the WT mice. Results of these assays will help to determine the nature of circadian defect. The close association between the circadian system and the timing of sleep and wakefulness together with the typical disturbances of circadian behavior and sleep in Fragile X syndrome opens up a new perspective for the investigation and treatment of patients suffering from this disorder.

## 695/W/Poster Board #353

**Modifier Genes Buffer Cardiac Developmental Pathways From The Effects of Nkx2-5 Mutation.** *J. Winston, J. Erlich, C. Green, A. Aluko, P. Jay.* Dept of Pediatrics, Washington Univ, St Louis, MO.

Mutations of the transcription factor NKX2-5 cause pleiotropic heart defects with incomplete penetrance. In this study we assess the role of cryptic genetic variants to phenotypic variability. *Nkx2-5*$^{+/-}$ mice in the inbred strain background C57Bl/6 frequently have atrial and ventricular septal defects (ASD, VSD). Outcrosses were performed to FVB/N and A/J to assess the effect of genetic background variation on phenotype. Our analysis of >3000 *Nkx2-5*$^{+/-}$ hearts from five F2 crosses reveals the profound influence of genetic modifiers on disease presentation and possibly human epidemiologic patterns. All three strains carry susceptibility alleles for ASD and VSD. Relative to the other two strains, C57Bl/6 carries polymorphisms that confer greater susceptibility to muscular VSD, and A/J for ASD and atrioventricular septal defects. Genome-wide linkage analysis on 200 *Nkx2-5*$^{+/-}$ mice each with VSD+ or normal hearts from the C57Bl/6 X FVB/N intercross yielded main effect and interacting loci throughout the mouse genome that correlate with risk. Significant main effect loci (LOD > 3.0) were mapped to chromosomes 4, 8, 10 and 19. A second linkage analysis on 100 cases and controls from the C57Bl/6 X A/J intercross yielded shared and unique modifiers with the previous scan. Similar analyses for ASD yielded loci shared with VSD on chromosomes 8 and 10, which indicates that some polymorphisms may affect the development of multiple cardiac structures. Interestingly, the loci generally contain no known cardiac developmental genes. Alleles of modifier genes can either buffer perturbations on cardiac development or direct the manifestation of a defect in response to an insult such as *Nkx2-5* haploinsufficiency. Our unbiased genetic analysis of modifiers of the *Nkx2-5* mutant phenotype elucidates the genetic architecture of cardiac developmental pathways.

## 696/W/Poster Board #354

**Fine-mapping and genetic architecture underlying obesity-related phenotypes.** *G.L. Fawcett[1,5], J.P. Jarvis[2], C.C. Roseman[3], B. Wang[5], J.B. Wolf[4], J.M. Cheverud[5].* 1) Dept of Mol. & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Genetics, University of Pennsylvania, Philadelphia, PA; 3) Department of Anthropology, University of Illionois at Urbana-Champaign, Urbana-Champaign, IL; 4) Faculty of Life Sciences, The University of Manchester, Manchester, UK; 5) Department of Anatomy and Neurobiology, Washington University in St. Louis, St. Louis, MO.

Obesity is one of the most prevalent diseases in developed countries with heritability estimated between 30-90%. While some causes of obesity are monogenic (melanocortin-4 receptor, leptin, leptin receptor), these are rare and most obesity in human populations is caused by many genes of relatively small (less than 10% of heritable variance explained) effect. Current focus in genome-wide association studies is on identifying loci, but most of these studies either lack the statistical power or the computational wherewithal to effectively study the genetic architecture of the heritable variation underlying obesity. Understanding epistatic effects and pleiotropy will be critical to effective drug therapy design. We present the fine-mapping ($F_{9/10}$ combined generations) of previously identified obesity-related loci in the LG,SM murine model of obesity. In comparison to the $F_2$ and $F_{2/3}$ populations, we observed narrowed QTL confidence regions (~5-fold). More interestingly, most of the $F_2$ and $F_{2/3}$ QTL peaks resolved into very narrow multiple $F_{9/10}$ QTL peaks and revealed new QTLs that may have been previously masked due to opposite gene effects at closely linked loci. Pleiotropic effects may be important in species evolutionary history and in considering side-effects for drug design. We observed significantly less pleiotropy in the $F_{9/10}$ population than had been reported previously with this model. This drop in pleiotropy is likely due to increased resolution of effects, which presents a more accurate depiction of the architecture. Epistatic interactions were primarily novel, although several strong epistatic interactions were observed to replicate from the $F_2$ population. We propose that increased consideration of the genetic architecture underlying complex traits in human studies will facilitate translational applications to human health.

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## 697/W/Poster Board #355

**A novel glaucoma locus identified in Dandie Dinmont Terrier dog breed.** *S.J. Ahonen[1,2,3], L. Hansen[4], G. Johnson[4], H. Lohi[1,2,3].* 1) Department of Medical Genetics, University of Helsinki, Finland; 2) Department of Molecular Genetics, the Folkhälsan Institute of Genetics, Finland; 3) Department of Basic Veterinary Sciences, University of Helsinki, Finland; 4) Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, US.

Hereditary glaucoma is a common eye disease both in dogs and in humans. In both species glaucoma is the second leading cause of blindness after cataracts. Unlike in many cataracts, the blindness in glaucoma is irreversible and cannot be restored. In humans, glaucoma is considered a complex disease with multiple susceptibility genes combined with environmental factors. Glaucoma is a disease where the intraocular pressure has raised, damaging the optic nerve and causing loss of retinal ganglion cells. In primary glaucoma there is no obvious reason for glaucoma development and it is considered to be hereditary. Glaucoma can also be secondary occurring as a result of some other cause, for example, diabetic retinopathy, uveitis, lens luxation, cancer and trauma. Although several genes and loci have been found in human there are no known glaucoma genes in dogs. Glaucoma has been described in several dog breeds including Dandie Dinmont Terrier (DDT), Basset Hound, Beagle and Chow Chow. In DDT primary glaucoma seems to develop slowly affecting middle-aged and elderly dogs and there are several abnormalities associated with the drainage or iridocorneal angle. We have established a large pedigree including 26 affected DDTs. To map the glaucoma locus 21 affected and 21 controls were genotyped with Illumina canine 22K SNP array. The average onset of glaucoma in affected dogs was 7.07 years. Control dogs selected for the analysis were over 10 years and confirmed healthy at older age by veterinary ophthalmologists. Analysis of the genotyping results by PLINK software revealed a single locus with a genome-widely significant association ($P_{raw}$= $1.6*10^{-7}$, $P_{genome}$ =0.00116) at CFA8. As expected for a small breed, the associated gene-rich region spans several mega bases and ongoing fine-mapping of the region with additional samples and markers aim to narrow down the region. The most prominent candidate genes are being sequenced in parallel. The identified locus maps to a syntenic region in human that has been associated with human glaucoma but there are no known glaucoma genes in the region in either species. Further studies are likely to reveal a novel glaucoma mutation in this breed and will facilitate the identification of the corresponding human gene. This study will also establish an important new glaucoma model for therapeutic studies while the breed will benefit from the upcoming genetic test.

## 698/W/Poster Board #356

**Genome-wide linkage and association mapping of hip dysplasia using dog as a model organism.** *K. Zhao[1], Z. Zhang[2], L. Zhu[3], L. Corey[4], G. Lust[4], C.D. Bustamante[1], S. Harris[5], R. Todhunter[6].* 1) Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY, 14853; 2) Institute for Genomic Diversity, Cornell University, Ithaca, NY, 14853; 3) Dept of Statistics, Oklahoma State University, Stillwater OK,74078; 4) Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, 14853; 5) Waltham PetCare, Waltham-on-the-Wold, Leicestershire LE14 4RT, UK; 6) Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca NY, 14853.

Canine hip dysplasia (CHD) is a heritable complex disease characterized by hip instability that results in secondary osteoarthritis, lameness, and physical disability. Human Hip Dysplasia (HD) and CHD share common phenotypic characteristics, thus the study in dogs would also shed light on the genes and pathways involved in humans. The unique genetic structure of long haplotype within breeds and short haplotype across breeds and ability to manipulate the pedigrees make dog an idea model organism for studying the genetic basis of this disease. Platforms used to date for canine complex disease mapping have mainly used microsatellite markers and linkage or sib pair analysis. Here, we present the first genome-wide linkage and association mapping analysis by means of a 22K Illumina SNP platform. The sample of 366 dogs consisted of a crossbred pedigree formed by crossing and backcrossing dysplastic Labrador retriever/greyhounds, and six other pure breeds (Labrador Retriever, Bernese Mountain Dog, German Shepherd, Newfoundland, Golden Retriever, Rottweiler). We confirmed and narrowed many QTL for CHD and hip osteoarthritis identified by traditional microsatellite linkage mapping and identified additional QTL. This study demonstrated both the feasibility and the challenges of utilizing genome-wide SNP data for the identification of the genetic contribution to complex diseases using dog as a model organism. Power simulation studies also showed the importance of controlling genetic relatedness in association mapping when combining pedigree and unrelated samples.

## 699/W/Poster Board #357

**Transgenic rescue of neurobehavior in Ube3a KO mice.** *T. Kishino[1], L. Rayburn[2], J. Wagstaff[3].* 1) Center for Frontier Life Science, Nagasaki University, Nagasaki, Japan; 2) Department of Pediatrics, James G. Cannon Research Center, Carolinas Medical Center, Charlotte, N.C; 3) Genetics Division, Department of Pediatrics, Levine Children's Hospital at Carolinas Medical Center, Charlotte, N.C. deceased 4/08.

Angelman syndrome is a neurodevelopmental disorder caused by a defect of the maternally expressed imprinted gene, *UBE3A*. The mouse models of AS with targeted disruption of *Ube3a* reveal that *Ube3a* is imprinted only in neurons and the UBE3A protein may function in synaptic morphology and/or plasticity. Maternal chromosomal duplications of 15q11-13, where *UBE3A* is located, are the most frequent cytogenetic abnormalities found in autism, suggesting that dup(15) phenotypes result from over-expression of the contiguous loci including maternally expressed *UBE3A*. To address the role of UBE3A in neurons, we generated transgenic *UBE3A* mice expressing a full length UBE3A cDNA under control of the rat enolase 2, gamma, neuronal (*Eno2*) promoter. Transgene expression in neurons was clearly demonstrated by RT-PCR and Western Blot. *UBE3A*-overexpressing transgenic mice showed normal phenotype. Neurobehavioral phenotypes of AS model mice with maternal *Ube3a* deletion were partially rescued by transgene expression in neurons. We present detailed phenotypic data of the transgenic mice and rescued phenotypes.

## 700/W/Poster Board #358

**Identification of Novel Loci for Canine Steroid Responsive Meningitis-Arteritis.** *P.J. Jokinen[1], M. Wilbe[2], K. Truvé[2], E.H. Seppälä[1], E. Karlsson[3,4], T. Biagi[3], A. Hughes[5], D. Bannasch[5], K. Ahlgren[6], O. Kämpe[6], G. Andersson[2], H. Hansson-Hamlin[2], K. Lindblad-Toh[3,7], H. Lohi[1].* 1) Department of Basic Veterinary Sciences, Department of Medical Genetics, Program in Molecular Medicine, University of Helsinki and Folkhälsan Institute of Genetics, Helsinki, Finland; 2) Swedish University of Agricultural Sciences, Uppsala, Sweden; 3) Broad Institute of Harvard and Massachusetts Institute of Technology (MIT), Cambridge, USA; 4) FAS Center for Systems Biology, Harvard University, Cambridge, USA; 5) School of Veterinary Medicine, University of California, Davis, USA; 6) Department of medical sciences, Uppsala University, University hospital, Uppsala, Sweden Uppsala; 7) Department of Medical Biochemistry and Microbiology, University, Uppsala, Sweden.

Dog breeds form homogeneous genetic isolates that originate from few ancestors and provide therefore feasible models to map genes in complex phenotypes. We have established a large sample collection of Nordic and North American Nova Scotia Duck Tolling Retrievers (NSDTRs) that have genetic predisposition for different type of autoimmune disorders including immune-mediated rheumatic disease (IMRD), steroid-responsive meningitis-arteritis (SRMA) and hypoadrenocorticism (Addison's disease). To identify the genetic risk factors for SRMA we performed a genome wide association (GWA) study with 44 SRMA cases and 57 healthy controls from Finland and Sweden. We identified a region with multiple associated SNPs on CFA32 (praw=7.10 x 10-6 and pgenome=0.04). By examining Finnish and Swedish SRMA dogs separately associations were found for a single SNP on CFA30 (praw=6.7 x 10-6 and pgenome=0.03) and again on CFA32 (praw=2.1 x 10-4 and pgenome=0.69), respectively. To narrow down and validate the loci we used additional NSDTRs and also SRMA cases from other breeds such as Boxers and Petite Basset Griffon Vendeen in fine-mapping. Our results further supported the association on CFA32 for a protective haplotype (praw=2.4*10-7, OR=0.3) while the association on the Finnish cases weakened on CFA30 (praw=1.2*10-4, OR=0.2). However, this locus has a 69 kb-haplotype consisting of three SNP's that contains a strong candidate gene (RORA) and the association is also supported by a shared haplotype with the other breeds. Combining GWA data from IMRD and Addison's disease in a parallel study with SRMA we found shared loci on CFA32 (praw=4.4*10-6 and OR=3.0), CFA8 (praw=3.2*10-7 and OR=2.6) and CFA3 (praw=4.4*10-6 and OR=3.0). In summary, we have successfully discovered four candidate loci for canine SRMA in a small case-control material, three of which are shared with other complex autoimmune diseases. Meningitis is also known to be a symptom in human systemic autoimmune conditions such as SLE. Re-sequencing of the associated regions is likely to unravel causative variants that may have important implications for the understanding of the immune-mediated diseases in both species.

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## 701/W/Poster Board #359

**Genome-wide association mapping identifies multiple loci for a canine SLE-related disease complex.** *M. Wilbe[1], P. Jokinen[2], K. Truvé[1], E. Seppala[2], E. Karlsson[3,4], T. Biagi[3], A. Hughes[5], D. Bannasch[5], K. Ahlgren[6], O. Kämpe[6], G. Andersson[1], H. Hansson-Hamlin[7], H. Lohi[2], K. Lindblad-Toh[3,8].* 1) Department of Animal Breeding and Gentics, Swedish University of Agricultural Sciences, Uppsala, Sweden; 2) Department of Basic Veterinary Sciences, Department of Medical Genetics, Program in Molecular Medicine, University of Helsinki and Folkhälsan Institute of Genetics, Helsinki, Finland; 3) Broad Institute of Harvard and Massachusetts Institute of Technology (MIT), Cambridge, USA; 4) FAS Center for Systems Biology, Harvard University, Cambridge, USA; 5) School of Veterinary Medicine, University of California, Davis, USA; 6) Department of medical sciences, Uppsala University, University hospital, Uppsala, Sweden; 7) Department of Clinical Sciences, Swedish University of Agricultural Science, Uppsala, Sweden; 8) Department of Medical Biochemistry and Microbiology, University, Uppsala, Sweden.

The dog is an outstanding model for human disease based on the many similar disease phenotypes, a similar gene set and shared environment with humans. The unique canine breed structure also makes the mapping of both monogenic and complex genetic diseases more efficient. The dog has gone through two major bottlenecks that affect their haplotype structure; the domestication from the wolf population (resulting in short haplotypes) and the breed creation (resulting in long haplotypes within breeds). We take advantage of the long haplotypes within a breed for genome-wide association (GWA) mapping and the short haplotypes shared across breeds in for fine-mapping. In this proof-of-principle study we mapped the first canine complex trait, a systemic lupus erythematosus (SLE)-related disease complex in the canine breed Nova Scotia duck tolling retriever. These dogs share many symptoms with human SLE patients. All affected dogs show polyarthritis of a non-erosive character, 70 % display antinuclear antibodies (ANA). Additional symptoms occurring are muscle pain, skin, liver and kidney problems and fever. MHC class II is also a risk factor for SLE in both dogs and humans. GWA of 81 cases and 57 controls revealed six highly associated loci, and fine-mapping in twice as many dogs validated the results with twice as strong p-values. The ANA-positive disease was validated particularly strongly as a complex trait with three loci, on chromosomes 3, 11 and 24, showing p-values of $10^{-11}$ to $10^{-13}$ and odds ratios of 4.5-8. All associated regions contain strong candidate genes based on biological function. Interestingly, four of these genes (*PTPN3*, *HOMER2*, *DAPP1* and *PPP3CA*) have all been defined to influence nuclear factor of activated T-cells (NFAT) activities, which might be a major novel SLE-related pathway. Re-sequencing of associated regions and expression studies of candidate genes in the dog are ongoing to identify causative mutations. The corresponding regions in human populations affected by SLE are currently being examined, both by data mining of human GWA data and by additional genotyping, to detect genetic risk factors that are common to canine and human disease. We conclude that the dog is a highly efficient model for comparative complex trait mapping.

## 702/W/Poster Board #360

**Activin signaling: effects on body composition and mitochondrial energy metabolism.** *C.W. Brown[1,2], J.J. Shen[3], J.C. Bournat[1], L. Huang[1], A. Chattopadhyay[1], Z. Li[1], C. Shaw[1], B.H. Graham[1], L. Li[4].* 1) Dept Molec/Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 3) Children's Hospital Central California, Madera, CA; 4) Dept of Pediatrics, M.D. Anderson Cancer Center, Houston, TX.

Activin beta A and activin beta B (encoded by *Inhba* and *Inhbb* genes, respectively) are closely related TGF beta superfamily members that participate in a variety of biological processes. These include maintaining body composition, adiposity, and energy metabolism. We previously generated mice with an insertion allele at the *Inhba* locus, *Inhba*$^{BK}$. The *Inhba*$^{BK}$ allele results in the misexpression of activin beta B in locations where activin beta A is normally produced and does not adversely affect the expression level or processing of the protein, yet renders the gene product functionally hypomorphic in this context. Homozygous (*Inhba*$^{BK/BK}$) and hemizygous (*Inhba*$^{BK/-}$) mice are smaller and leaner than their wild-type littermates in an allele dose-dependent fashion, and many tissues (liver, spleen, white adipose) are disproportionately small relative to total body weight. To determine the mechanisms that contribute to these phenomena, we have investigated the metabolic consequences of the mutation. Although the growth of *Inhba*$^{BK}$ mice is improved by providing a calorie-rich diet, diet-induced obesity, fatty liver, and insulin resistance (hallmarks of chronic caloric excess) do not develop, despite greater caloric intake than wild-type controls. Physiologic, molecular, and biochemical analyses all revealed characteristics that are commonly associated with increased mitochondrial energy metabolism, with a corresponding up-regulation of several genes in metabolically active tissues that reflect enhanced mitochondrial biogenesis and function. Oxygen consumption, an indirect measure of the metabolic rate, was markedly increased in *Inhba*$^{BK/BK}$ mice, and polarographic analysis of liver mitochondria revealed an increase in ADP independent oxygen consumption, consistent with constitutive uncoupling of the inner mitochondrial membrane. Preliminary expression microarray data from the livers of these mice suggest prominent roles for a variety of biological processes and pathways in the hypermetabolic phenotype. These findings establish a functional relationship between activin signaling and mitochondrial energy metabolism, provide clues as to how these processes are linked, and further support the rationale to target this signaling pathway for the medical treatment of cachexia, obesity, and diabetes.

## 703/W/Poster Board #361

**Genetic Regulation of Chronic Pain Responses After Sciatic Nerve Injury.** *R. Levitt[1], E. Fu[1], Y. Zhang[1], Q. Li[1], R. Morris[2].* 1) Department of Anesthesiology, The University of Miami, Miami, FL 33136; 2) Department of Human Genetics, The University of Miami, Miami, FL 331336.

Neuropathic pain (NP) is a complex heritable disorder. We utilized the well described chronic constriction injury of the sciatic nerve (CCI) model to evaluate NP behaviors using inbred mice (C57BL6/J, DBA2/J, A/J, NZW/LacJ, SJL/J, AKR/J, 129X1/SvJ, CAST/EiJ, n=8 each). Thresholds for withdrawal to mechanical pain (von Frey filaments applied to the affected hindpaw) were measured on day 7 and 14 after CCI. Thresholds after CCI as compared with baseline indicates A/J and NZW/LacJ are the two least sensitive strains and AKR/J is most sensitive (see Table 1). Based on these data, our estimates of heritability exceed 0.5 with an estimated 2.5 effective factors underlying the measured strain differences (assuming independent equal and additive gene effects). We report here for the first time that mechanical hypersensitivity after CCI is heritable and strain differences are likely to be determined by a small number of genes. These data establish the feasibility of mapping susceptibility genes underlying NP in the CCI model. Mapping neuropathic pain susceptibility genes may facilitate an understanding of mechanism and better diagnostic and therapeutic approaches.

| Day After CCI | | Strain Pairs | | P Value |
|---|---|---|---|---|
| Day 7 | | A/J vs. AKR/J | | 0.00186 |
| Day 7 | | NZW/LacJ vs. AKR/J | | 0.00016 |
| Day 14 | | A/J vs. AKR/J | | 0.00016 |
| Day 14 | | NZW/LacJ vs. AKR/J | | 0.00016 |

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## 704/W/Poster Board #362

**LOXL1 null mouse phenotype suggests that loss of lysyl oxidase like 1 function contributes to exfoliation glaucoma.** *J.L. Wiggs[1], L.R. Pasquale[1], B. Pawlyk[1], X. Xu[1], E. Connolly[1], I. Kim[1], J.W. Miller[1], D. Rhee[1], R. Haddadin[1], C. Grosskreutz[1], T. Chen[1], B.J. Fan[1], J.L. Haines[2], T. Li[1].* 1) Dept Ophthalmology, Harvard Med Sch, MEEI, Boston, MA; 2) Center for Human Genetics Rearch, Vanderbilt University Medical Center, Nashville, TN.

Exfoliation syndrome is a common condition that is a major risk factor for open angle glaucoma. The condition is characterized clinically by the deposition of fibrillar material systemically and throughout the eye, compromise of the blood aqueous barrier in the ocular anterior segment and changes in the ocular lens. Approximately 50% of individuals with this condition develop a high intraocular pressure form of glaucoma causing blindness due to permanent damage to the optic nerve. Exofoliation glaucoma is inherited as a complex trait, and three SNPs in the LOXL1 gene coding for lysyl oxidase like 1 have been shown to be significantly associated with exfoliation syndrome in human populations throughout the world. Two of the associated SNPs are missense changes which could contribute to disease through a gain of function mechanism or could be associated with a loss of function of the LOXL1 protein. The purpose of this study is to evaluate the phenotype of a LOXL1 knockout (null) mouse to help define the pathogenic disease mechanism. Twenty LOXL1 null mice (ages 3-8 months) and 20 control mice (DBA/2J) were characterized for the following phenotypic traits: ocular histology, intraocular pressure (rebound tonometer), and iris fluorescein angiography. Compared with controls null mice demonstrated a pronounced vesiculation of the anterior lens, and also a compromise of the blood aqueous barrier in the ocular anterior segment (shown by iris angiography). Other phenotypic measures were normal including intraocular pressure. These results show that the LOXL1 null mouse replicates the lens and blood aqueous barrier abnormalities observed in human exfoliation syndrome, suggseting that a loss of LOXL1 protein function is the underlying genetic mechanism responsible for the condition. Certain aspects of the human phenotype, including increased intraocular pressure, are not apparent in the LOXL1 null mouse suggesting that other factors may also contribute to this disease. Supported by NEI grants: R21EY019161 (Li); R01EY013882 (Wiggs); P30EY014104 (Wiggs).

## 705/W/Poster Board #363

**Detection of Bax and Bak proapoptotic determinants polymorphisms in Iranian Ataxia -Telangiectasia patients.** *M. Sanati[1], A. Isaian[2], T. Dork -Bousset[3], N. Bogdanova[3], M. Houshmand[1], M. Movahadi[2].* 1) Medical Genetics department, National Institute for Genetic Engineering and Bio, Tehran, Tehran, Iran; 2) Tehran Medical science University , Medical School Dept.of Immunology and Allergy Tehran- Iran; 3) Medical School of Hannover, Molecular Gynaecology Research Group ,Hannover Germany.

AT is an autosomal recessive disorder That is characterized by early onset progressive cerebellar ataxia,oculocutaneous telangiectasia bronchopulmonary disease and lymphoid tumors,immunodeficiency and chromosomal instability. The defective gene is ATM (AT mutated) was localized to chromosome 11q22-23.AT patients are in the risk of cancer. and it is the second cause of death in this syndrome.40% of cancers are non-Hogkin's lymphoma,25% are leukemia.25% are associated with solid tumors and 10% are Hogkin's lymphoma.The risk for malignancy in AT is 38%. In addition apoptosis is critical not only for tissue homoestasis but also in the pathogenesis of a variety of disease including cancer . The major goal of this study was to determine the role and alternations of Bax ,Bak proapoptotic determinants and in general ,mitochondrial apoptotic pathway in developing of cancer in AT patients.Bax mutation are commonly formed in lymphoma and leukemia ,and Bak gene mutations in different types of cancer are detected . For the first time we studied 50 AT patients and 50 healthy control for Bax and Bak genes alternations.We performed sequencing analysis of entire coding region of Bak gene (exons 2-6) and bax gene( exons 1-7).Our observation suggest that Bak gene polymorphisms were detected in region of c342C to T in exon 2 and Intronic site of exon6 IVS6-35T to C in Bak gene .In Bax gene we detected polymorphism in exon 3 IVS3+14A to G as homozygote and heterozygote. In conclusion we suggest that in AT patients in addition of ATM gene mutation ,the loss or mutation of Bax and Bak genes are associated with shorter overall survival or developing and acceralation of lymphoma.

## 706/W/Poster Board #364

**Candidate genes for presbycusis from mouse to man.** *D.L. Newman[1], L.A. Birdsall[1], D. Cunningham[1], C.A. Hu[2], R.D. Frisina[3].* 1) Department of Biological Sciences, Rochester Institute of Technology, Rochester, NY; 2) Department of Biochemistry & Molecular Biology, Univeristy of New Mexico School of Medicine, Albuquerque, NM; 3) Department of Otolaryngology, University of Rochester School of Medicine, Rochester, NY.

Presbycusis is a major public health problem in the U.S. People with this disorder suffer from gradual hearing loss, beginning at high frequencies in middle age and progressing to lower frequencies in old age, and compounded by a loss of the ability to understand speech in the presence of background noise. Glutamate is the primary excitatory neurotransmitter, and serotonin is a modulatory neurotransmitter in the central/peripheral auditory system. The CBA mouse is a model for human presbycusis that has been used to search for genes that are up- or down-regulated in the auditory system as it ages. Glutamate-related genes and serotonin-related genes were examined specifically. Glutamate pathway gene *Pycs* mRNA level decreased with age in the auditory midbrain (inferior colliculus, IC), while Serotonin 2B receptor mRNA was upregulated with both age and hearing ability in the IC. We examined the human homologues of these two genes in a population of ~600 aged Caucasians from the Rochester, NY area. Nine nonsynonymous SNPs in *PYCS* and six noncoding tagging SNPs in *HTR2B* were genotyped in this population. Little or no association was observed with any SNP or haplotype. We conclude that although these genes may play a role in hearing ability, there is no evidence for our population carrying polymorphisms in these genes that contribute to presbycusis susceptibility.

## 707/W/Poster Board #365

**IL-6, IL-10, IFN-$\gamma$, TGF-$\beta$1 and TNF$\alpha$ Gene Polymorphisms as Risk Factors for Aggressive Periodontitis: A Brief report.** *K. Erciyas[1], S. Pehlivan[2], T. Sever[2], M. Igci[2], A. Arslan[2], R. Orbak[3].* 1) Gaziantep University, Faculty of Dentistry, Department of Periodontology, Gaziantep, Turkey, DDs, PhD; 2) Gaziantep University, Faculty of Medicine, Department of Medical Biology and Genetics, Gaziantep, Turkey, PhD; 3) Ataturk University, Faculty of Dentistry, Department of Periodontology, Erzurum, Turkey, DDs, PhD.

The aim of this study was to investigate links between cytokine genetic variants and aggressive periodontitis. Twelve aggressive periodontitis patients and 30 healthy controls without periodontitis were included in the study. We studied the polymorphisms of IL-6, IL-10, IFN-$\gamma$, TGF-$\beta$1 and TNF$\alpha$ genes, using the PCR-SSP method. Significant differences were not determined for IL-6, IL-10, IFN-$\gamma$, TGF-$\beta$1(C25/G25) and TNF$\alpha$ cytokine polymorphism from the genotype distribution and allele frequency between aggressive periodontitis and healthy control groups. In contrast, significant differences were determined for TGF-$\beta$1(T10/C10) polymorphism from the genotype distribution (TC) between aggressive periodontitis and healthy control groups (p:0.032).

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## 708/W/Poster Board #366

**In silico analysis of genes in the genome regions covered by quantitative trait loci for essential hypertension in rat model.** *W. Gu[1], J. Zhu[2], Q. Xiong[1,3], A. Postlethwaite[4], Y. Wang[5].* 1) Dept Orthopedic Surgery, Univ Tennessee-HSC, Memphis, 38163, TN; 2) Rust College, Holly Spring, Mississippi, 38635, USA; 3) Department of Computer Science and Technology, Southwest University, Chongqing 400715, P.R.China; 4) Department of Medicine, University of Tennessee Health Science Center, Memphis, TN 38163, USA; 5) Beijing Tiantan Hospital, Capital University of Medical Sciences, Beijing, 100050 PR China.

Introduction: Hypertension in humans is a quantitative trait and controlled by many genes. Currently, more than 300 hundred Quantitative trait loci (QTL) for blood pressure and hypertension have been reported in Rat genome. In this study, we used our recently developed software named PGMapper (http://www.genediscovery.org/pgmapper/index.jsp) to systematically examine the genes of essential hypertension in the genomic regions for QTL across whole rat genome. Materials and Methods: We first selected blood pressure as a trait to pick up all possible QTL from Rat Genome Database (RGD, http://rgd.mcw.edu/). We then selected all QTL that have a LOD score >2.8. If two QTL are overlap and connected, we analyze them independently. If one QTL is located in the other, we just use the QTL with the larger gnome size. We used flanking markers to search genes for QTL that are fine-mapped and well-defined. We next used PGmapper software to search candidate genes. The key words are “hypertension, hypertensive, and blood pressure”. At least one abstract of the report was read to determine the candidacy of a gene. In most of the cases, more than one abstracts was read to confirm the importance of a gene. A gene is considered to be a hypotension-associated gene if it was associated with hypotension in at least one of the following studies: 1) functional studies such as knockouts, transgenics, mutagenesis, RNA interference, etc.; 2) association studies; 3) clinical studies. Results: A total of 62 hypotension QTL cover 2,015,062,129 base pairs (bp) of genomic sequence, which is roughly 73% of the total rat genome. Every autosome and the X chromosome, except chromosome 19 and Y, contains at least one hypotension QTL. The genomic size of those QTL ranges from 5873857 bps to 167769667 bps. Within the 2,015,062,129 bp genomic sequences, a total of 20,266 genes have been located. The number of genes in a QTL region is from 79 to 1398. The average gene density throughout the whole rat genome except ChrY is about one gene per 99,918 bp. Within the total of 2,015,062,129 bp genomic sequences representing hypotension QTL, there is about one gene per 99,430 bp, similar to the whole genome. Among total of 20266 genes, 160 was selected as candidate genes for hypotension, about 127 genes per candidate. Only one of those chromosomes, Chr 15, does not contain obviously recognizable candidate genes.

## 709/W/Poster Board #367

**QuTie - Rare Variant Analysis Tool for Quantitative Traits.** *R. Lawrence[1], A. Morris[1], E. Zeggini[2].* 1) Wellcome Trust Centre for Human Genetics, Oxford, OX3 7BN, UK; 2) Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA, UK.

We have recently developed rare variant analysis software (CCRAVAT - Case-Control RAre Variant Analysis Tool) for case-control genome-wide association studies (GWAS). The program analyses rare variants by using a collapsing method, which allows the analysis of low minor allele frequency (MAF) single nucelotide polymorphisms (SNPs) by pooling rare variants within defined regions and treating them as a single “super” locus. Genes (usually extended by 50kb either end) or sliding windows of defined sequence length are used to define regions for collapsing multiple rare variants into single loci. We have now also addressed the lack of analytical frameworks for the association analysis of quantitative trait information with rare variants genome-wide. We have developed new analysis software (QuTie) that uses the same collapsing method as CCRAVAT to convert multiple rare variants into single loci. Individuals are grouped into those that contain rare variants within a defined region and those that do not contain rare variants. Using linear regression and student’s t-tests we are then able to test the difference between the two means to determine if any regions demonstrate significant cumulative rare variant associations with the phenotype of interest. QuTie uses PED and MAP files (as used in genetic applications such as PLINK) to analyse rare variants. Summary statistics, including regression and student’s t-test derived p-values, are produced for all genes or windows of defined sequence that contain SNPs with MAFs less than a certain threshold (usually 1-5%). The script also runs permutations on any regions that pass significance thresholds. QuTie normally takes 10-16 hours to run in a GWAS of approximately 500,000 SNPs and 2,000-5,000 individuals. Permutations can add significantly to running time. QuTie can produce a Manhattan plot in addition to lists and summaries of regions genome-wide. Lists of SNPs that are within regions producing p-values that pass specified threshold are also produced to allow the user to verify SNP quality. Also, histograms showing distributions of QTs from individuals with and without rare variants in specified significant regions are produced so the user can check normality of QT distributions. In summary, we have developed software for the analysis of rare variants and quantitative traits. Our program can be applied to genome-wide scans and resequencing data.

## 710/W/Poster Board #368

**Sequencing of highly conserved non-coding regions (HCNRs) surrounding the IRX gene family in families with Kyphoscoliosis.** *C.M. Justice[1], K. Swindle[2], P. Cruz[3], B. Maskeri[3], J. Mullikin[3], N.H. Miller[2], A.F. Wilson[1].* 1) Genometrics Section, IDRB, National Human Genome Research Institute, National Institutes of Health, Baltimore, Maryland; 2) University of Colorado, The Children’s Hospital, Denver, Colorado; 3) NISC,National Human Genome Research Institute, National Institutes of Health, Rockville, Maryland.

Idiopathic scoliosis (IS) affects 2 to 3% of children or adolescents and is defined by a structural lateral curvature of the spine ≥ 10° in individuals which are otherwise phenotypically normal. When patients have a thoracic curve ≥ 40º in conjunction with a scoliotic curve ≥ 10º, the condition is referred to as kyphoscoliosis. The occurrence of kyphoscoliosis is quite rare. Miller et al.[2006] analyzed 7 families in which at least two members had kyphoscoliosis. These 7 families were comprised of 53 individuals, 16 of which had kyphoscoliosis. Genome-wide model-independent linkage analysis identified areas suggestive of linkage on 2q22, 5p13, 13q and 17q11 and analyses of single nucleotide polymorphism (SNP) markers narrowed the region on 5p to 3.5 Mb. This region contains only three genes, IRX1, IRX2 and IRX4, with the largest peak corresponding to the location of IRX1. The Iroquois gene family (IRX) is comprised of six genes which code for homeoproteins grouped in two clusters: IRX1, IRX2 and IRX4 on chromosome 5, and IRX3, IRX5 and IRX6 on chromosome 16 [Peters et al., 2000]. Both clusters are located in regions that are considered to be gene deserts [Nobrega et al., 2003]. Because of the lack of other genes in the candidate region on 5p, IRX1, IRX2 and IRX4 were selected for sequencing. The exons from IRX1, IRX2 and IRX4 were previously sequenced in all 53 individuals in the kyphoscoliosis subset. Sequencing results identified two unpublished synonymous SNPs on IRX4 which did not appear to be significant, but no functional sequence changes were identified. In this study the highly conserved non-coding regions (HNCRs) 500 kb upstream and downstream from IRX1, IRX2 and IRX4 were sequenced in 46 individuals. Selection of these conserved elements was based on the PhastCons Placental Mammal Conserved Elements, 28-way Multiz Aligment, which were limited to LOD scores above 100 within that window. All the exons of a nearby predicted gene, BC035019 (mRNA) were also sequenced. Polyphred 6.11 was used to do the genotype calls. Sequence results from HNCRs include single base pair changes, insertion/deletion sequences and copy number variants. At least one 20 bp homozygous deletion was identified in an individual with kyphoscoliosis in a HNCRs 390 kb upstream from IRX2. The conserved non-coding DNA sequences in this region may be integral to gene transcriptional regulation during embryogenesis.

## 711/W/Poster Board #369

**A polymorphism in the IL1B gene is associated with vertebral fracture independent of bone mineral density.** *X. Wu[1], V. Kondragunta[1], L. Lui[2], K. Stone[2], K. Kornman[1], N. Aziz[1].* 1) Interleukin Genetics, Inc., Waltham, MA; 2) Research Institute, California Pacific Medical Center, San Francisco, CA.

Osteoporosis is a highly prevalent disease that results in loss of bone mass and increased risk of bone fracture. Loss of bone mass is known to be a critical determinant for fracture, however, other factors such as suboptimal microarchitecture of bone tissue have also been proposed to be essential components in developing fractures. Moreover, the genes responsible for low bone mass and bone fracture are not fully understood and in a large pedigree study of 50 Caucasian families the genetic correlation between these two phenotypes was reported to be low. To address the question whether BMD and bone fracture share common genetic determinants, we conducted a genetic analysis to determine if 12 polymorphisms in selected genes (IL1A, IL1B, IL1RN, IL6, ESR1, VDR, and Col1A1) thought to be involved in bone metabolism are associated with these two clinical phenotypes of osteoporosis. DNA from 2500 subjects selected from the Study of Osteoporosis (SOF) cohort were genotyped in this retrospective case-cohort analysis. Vertebral fracture (VF) was assessed at baseline and at follow-up after 3.7 years. Both prevalent and incident VF cases were combined as VF cases for analysis. BMD was measured at the hip and dichotomized into 2 classes with low BMD defined as t score <-1 and normal BMD with t score greater than -1. Association between these SNPs and VF or low BMD was determined by logistic regression analysis with adjustment for age, BMI, estrogen use and other covariates. IL1B rs16944 genotype T/T (OR=1.44, p=0.04) was determined to be associated with VF but not with low BMD. In contrast, ESR1 rs9340799 allele A (OR=1.53, p=0.01) was associated with low BMD but not with VF. Interestingly, IL1B rs16944 was associated with VF even after adjustment for BMD, suggesting that this inflammatory cytokine is associated with VF independent of BMD. Although it is known that one of the causes leading to vertebral fracture may be due to low BMD, our genetic analysis suggests that other mechanisms may come into play in these two distinct phenotypes of osteoporosis - vertebral fracture and low bone mass.

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## 712/W/Poster Board #370

**Genetic variation in NOS2A is associated with risk for anencephaly.** *A. Ashley-Koch, C. Potocky, K. Dunlap, A. Trott, J.M. Rusnak, C. Haynes, H. Cope, K. Soldano, S. Gregory.* Center for Human Genetics, Duke University Medical Center, Durham, NC.

Anencephaly, the failure of formation of the brain and skull, is one of the most common and severe forms of neural tube defects (NTDs). Little is known about the genetic causes of anencephaly, including whether or not anencephaly shares the same genetic risk factors which contribute to other types of NTD. The nitric oxide (NO) pathway has been implicated in the process of neural tube closure, particularly with respect to folate-homocysteine homeostasis (Weil et al., 2004). A previous report demonstrated genetic association between NOS3 and risk for spina bifida (Brown et al, 2004). To explore the possibility that genetic variation in the NO pathway is also crucial to anencephaly risk, we performed a haplotype tagging SNP analysis of the three NO synthases (NOS1, NOS2A and NOS3) in our data set of 86 anencephalic families and 22 families with other cranial NTDs. Three affection models were considered for analysis: anencephaly only, all cranial NTDs, and any NTD regardless of lesion location. Using the pedigree disequilibrium test (PDT; Martin et al., 2000), these phenotypes were analyzed for association with 16 SNPs in NOS1, 16 SNPs in NOS2A and 7 SNPs in NOS3. Contrary to the report by Brown et al (2004), we did not observe any evidence for association with NOS3, including the G894T coding variant associated in their population. Similarly, little evidence for association was observed in NOS1, where only one SNP provided nominal evidence for association (rs11611788, p=0.03 narrow phenotype). The best evidence for association in our data set was observed in NOS2A where five SNPs showed nominal evidence for association with one or more of the phenotype models. Importantly, a nonsynonymous SNP (rs2297518) was nominally associated with all phenotype models, the most significant association occurring with any type NTD (p=0.02). These data provide continued support for the involvement of the nitric oxide pathway in neural tube closure. We are currently testing these genes in our larger collection of NTD families with other NTD lesions (n>1000 families), including spina bifida, to discern whether specific NO genes contribute to specific forms of NTDs.

## 713/W/Poster Board #371

**Frequency report of the 5HTTLPR polymorphism of the SLC6A4 gene in Mexican-mestizo population.** *V. Peralta Leal[1], E. Leal Ugarte[1], JP. Meza Espinoza[1], J. Durán González[2], M. Gutiérrez Angulo[3], G. Castañeda Cisneros[4], MA. Llanas Lira[1].* 1) Genética, Universidad Autónoma de Tamaulipas, Matamoros, Tamaulipas, Mexico; 2) Biological Sciences Dept. University of Texas at Brownsville/Texas Southmost College (UTB/TSC); 3) Universidad de Guadalajara CU ALTOS; 4) Servicio Neurocirugía/Unidad Médica de alta especialidad (UMAE)/Centro Médico nacional de Occidente (CMNO)/instituto Mexicano del Seguro Social (IMSS).

Introduction.The serotonin system is considered as one of the main systems involved in the development of diverse psychiatric and personality traits (Blair IP et al., 2005). The human gene that codifies for the serotonin transporter (5HTT) is denominated SLC6A4 (Solute to carrier family 6, member 4) and it is located at the chromosome 17q12.2. Two functional polymorphisms in the promoter region of the gene SLC6A4 has been described (5HTTLPR Longitudinal Polymorphism Repeat), located at 5' and before the first exon, those LPR are not located in the coding section of the gene and do not alter the structure of the codified protein 5HTT, but they modifies the regulation of the transcription. The LPR polymorphisms consists in an insertion (allele "l") and a deletion (allele "s") of 44 pb, (Paredes B et al., 2008). Through the behavioral perspective has been associated the presence of the s allele with a greater susceptibility to develop anxiety related personality traits and fear conditioned response (Graff A et al., 2004). Material and methods. 60 individuals of Mexican population were analyzed (39 males and 21 females). Genomic DNA was isolated from peripheral blood, and DNA fragments were amplified by PCR, the products were visualized by electrophoresis in 6% polyacrylamide gels and stained with silver nitrate. Results. Distribution of genotypes in our sample was in Hardy-Weinberg equilibrium, 21 (36%) subjects had the s/s genotype, 26 (44%) had l/s, and 12 (20%) were l/l. Frequency of the long allele was lower with 42% versus 58% of the short LPR. No difference in allele or genotype frequencies was found between sex groups. Discussion. Most of the studies all over the Word report high frequency of the l allele, but similar frequencies to this study have been reported before in Japanese and Korean populations by Nakamura ET et al., and Ho Joo et al., The authors suggest that the high frequency of the s allele in Japanese population is related with the interpersonal sensitivity and emotional restrains regarded in their culture.

## 714/W/Poster Board #372

**Schizophrenia susceptible genes are associated with cranial volume variations in Chinese population.** *B. Su[1], J. Wang[1,2], X. Luo[1,2], Y. Li[3], H. Diao[4], X. Shi[3].* 1) Kunming Inst Zoology, Chinese Academy Sci, Kunming, China; 2) Graduate School of Chinese Academy of Sciences, Beijing, China; 3) Biological Resources and Environmental Science College, Qujing Normal University, Kunming, China; 4) Yunnan Mental Health Hospital, Kunming, China.

Schizophrenia is a severe mental illness and affects about 1% of world populations. Cognitive dysfunction and structural brain abnormalities in schizophrenia have been extensively studied and smaller brain volumes of schizophrenia patients were consistently reported. Though genetic studies indicated that more than a hundred genes were associated with schizophrenia, the connections between the genetic makeup and the observed structural brain abnormalities is not well characterized. To examine whether the schizophrenia susceptible genes are involved in brain development and if the disease-associated sequence variations in these genes are correlated with the variation of brain volume in healthy populations. A total of 22 single nucleotide polymorphisms (SNPs) from 10 schizophrenia susceptible genes were genotyped for the 1,013 human subjects. Among the 10 schizophrenia susceptible genes tested, six of them show significant association with brain volume under at least one of the three genetic models in at least one sex. The combined effect of the nominally significant SNPs could explain about 2% of the cranial volume variation observed in the population. Additionally, we also identified two pairs of SNPs (rs951439 and rs35753505 in males; rs2020917 and rs2270335 in females) showing sex specific epistatic association with cranial volume. We demonstrate that a proportion of the schizophrenia susceptible genes are likely involved in brain development by showing a significant correlation with cranial volume in healthy human subjects, providing a possible genetic explanation to the brain volume abnormality of schizophrenia patients. Our data provide further evidence that schizophrenia is likely a neurodevelopmental disorder.

## 715/W/Poster Board #373

**Lung cancer and candidate DNA repair genes: Evidence of significant association with MSH5 and potential interaction between smoking and RAD54B.** *R. Kazma[1,2], E. Génin[2,3], M.-C. Babron[4], P. Brennan[2,3], R.J. Hung[5], H. Krokan[6], A. Metsapalu[7], J.K. Field[8], M. Lathrop[9,10], A. Sarasin[11], S. Benhamou[2,3,11], the ILCCO consortium.* 1) Univ Paris-Sud, Faculté de Médecine, Le Kremlin Bicêtre, France; 2) INSERM UMR-S946, Paris, France; 3) Univ Paris-Diderot, Paris, France; 4) International Agency for Reasearch on Cancer (IARC), Lyon, France; 5) University of California at Berkeley, Berkeley, California, USA; 6) Norwegian University of Science and Technology, Trondheim, Norway; 7) Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia; 8) University of Liverpool Cancer Research Center, Liverpool, UK; 9) Centre National de Génotypage, Commissariat à l'Energie Atomique, Evry, France; 10) Fondation Jean Dausset - CEPH, Paris, France; 11) CNRS FRE2939, Institut Gustave Roussy, Villejuif, France.

Although lung cancer is typically caused by smoking, inherited factors [that can be assessed by single-nucleotide polymorphisms (SNPs)] could contribute to individual susceptibility to tobacco carcinogens. DNA repair genes are good candidates for such susceptibility because of their critical role in maintaining genome integrity. Furthermore, SNPs in these genes could modify the effect of smoking on lung cancer risk.

To assess the role of SNPs in DNA repair genes on lung cancer risk, and their interaction with smoking exposure, we pooled individual data from 6 recent genome-wide association studies for a total of 3,416 lung cancer cases and 4,374 controls, all of European descent. Genotypes for 1,760 SNPs on selected 233 DNA repair genes were available and analyzed assuming an additive model and using unconditional logistic regression, controlling for sex, age and study.

We found a significant increase in risk for lung cancer associated with the rs3131379 on MSH5 gene ($p = 3.57\ 10^{-5}$) with an odds ratio per allele of 1.30 (95% confidence interval (CI): 1.15-1.47).

The interaction analyses yielded potential differences in cancer risk associated with RAD54B rs2930961 polymorphism between never- and ever-smokers but came with a non-significant p-value after correction for multiple testing ($p = 2.31\ 10^{-4}$) for an interaction coefficient estimate of 1.54 (95% CI: 0.78-2.95). Compared to the T/T genotype, the odds-ratios were 0.79 (95% CI: 0.59-1.05) for the T/C genotype and 0.43 (95% CI: 0.25-0.73) for the C/C genotype among never-smokers, and 1.10 (95% CI: 0.98-1.25) and 1.13 (95% CI: 0.94-1.36) respectively, among ever-smokers.

Our results confirmed a previously reported association with the MSH5 gene with a similar risk estimate. This gene encodes a member of the mutS family of proteins that are involved in DNA mismatch repair and in mitotic or meiotic recombination processes. RAD54B, a DNA-dependent ATPase, is similarly involved in mitotic and meiotic homologous repair. Homozygous mutations of this gene are often found in various cancers. These results disclose an association between recombination repair pathways and lung cancer.

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## 716/W/Poster Board #374

**Serum asymmetric dimethylarginine levels are associated with diabetic retinopathy and influenced by sequence variation in the *DDAH1* and *DDAH2* genes.** *S. Abhary[1], N. Kasmeridis[2], K.P Burdon[1], A. Kuot[1], M.J. Whiting[3], Y. Yew[4], N. Petrovsky[2], J.E. Craig[1].* 1) Ophthalmology, Flinders University and Flinders Medical Centre, Adelaide, South Australia, South Australia, Australia; 2) Endocrinology, Flinders University and Flinders Medical Centre, Bedford Park, SA, 5042, South Australia, AUSTRALIA; 3) Chemical Pathology Laboratory, SA Pathology, Bedford Park, SA, 5042, AUSTRALIA; 4) Department of Medical Biochemistry, Flinders University, Bedford Park, SA, 5042.

Objective: Asymmetric dimethylarginine (ADMA) is present at varying levels in serum, and as a substrate for nitric oxide synthase influences nitric oxide production. Dimethylarginine dimethylaminohydrolase (DDAH) is an ADMA degrading enzyme and has two isoforms: DDAH1 and DDAH2. ADMA levels are known to be associated with type 2 diabetes mellitus (T2DM), and some small studies have suggested a correlation with diabetic complications. We aimed to determine: 1) whether serum ADMA levels are correlated with severe diabetic retinopathy (DR) in a large cohort of individuals with type 2 diabetes mellitus, and 2) whether ADMA serum levels are influenced by common polymorphisms in the *DDAH1* and *DDAH2* genes. Methods: A total of 343 participants with T2DM were recruited, ophthalmological assessment performed and peripheral blood obtained for serum and genetic analysis. ADMA concentrations were determined by mass spectroscopy of serum and tested for association with DR. Twenty six tag SNPs in the *DDAH1* and 10 in the *DDAH2* genes were genotyped in all subjects and tested for association with serum ADMA levels. Results: Two hundred and twenty five participants were found to have no DR, 59 had proliferative diabetic retinopathy (PDR), 82 had clinically significant macular edema (CSME), and 27 had severe non-proliferative DR (NPDR). Blinding DR was defined as severe NPDR, PDR or CSME. After adjustment for relevant covariates, the presence of blinding DR, PDR and CSME were associated with significantly increased serum levels of ADMA ($p<0.001$). Several SNPs and haplotypes in the *DDAH1* gene were associated with ADMA levels. Under the additive model, rs669173 (p=1.35E-06) and rs7521189 (p=2.31E-05) and the AATTT haplotype consisting of SNPs rs12132677, rs7521189, rs17388437, rs11161614 and rs553257 (p=1.52E-4) were the most significantly associated with serum ADMA. The rs3131383 SNP (p=0.0049 under a dominant model) and TGCCCAGGAG haplotype consisting of SNPs rs805287, rs6916278, rs805285, rs15574, rs805294, rs805293, rs9267551, rs2272592, rs3131383 and rs3131382 (p=0.0024) in the *DDAH2* gene were also significantly associated with serum ADMA levels. These associations remained significant after Bonferroni correction. Conclusion: Severe forms of DR causing visual impairment were associated with elevated serum concentrations of ADMA, which are in turn determined by genetic variation in the *DDAH1* and *DDAH2* genes.

## 717/W/Poster Board #375

**Nicotinic acetylcholine receptor polymorphism interacts with smoking history to affect lung function decline.** *L. Akhabir[1], X. Zhang[1], J. Connett[2], N. Anthonisen[3], M. Lathrop[4], W. Cookson[5], P.D. Parè[1], A.J. Sandford[1].* 1) The James Hogg iCAPTURE Centre, vancouver, British Columbia, Canada; 2) Division of Biostatistics, University of Minnesota, USA; 3) Faculty of Medicine, University of Manitoba, Canada; 4) Centre National de Génotypage, Institut Génomique, France; 5) National Heart & Lung Institute, Imperial College London, UK.

Single nucleotide polymorphisms (SNPs) in the nicotinic acetylcholine receptor gene cluster have been associated with lung cancer, nicotine addiction, cocaine and alcohol dependence as well as smoking behavior. In particular, the SNP rs16969968 in the CHRNA5 gene was the most significant signal in genome-wide association study data, and its association with nicotine dependence and cancer was consistently replicated. Chronic obstructive pulmonary disease (COPD) is a complex trait caused by a combination of environmental risk factors and suspected genetic factors. Cigarette smoking is by far the most important environmental element in the pathogenesis of COPD. A genetic variant that affects the nicotinic acetylcholine receptor function is thus an important target for investigation in relation to COPD phenotypes. We genotyped 1600 COPD patients' samples for the rs16969968 SNP using the TaqMan genotyping platform. This dataset is part of the Lung Health Study (LHS) cohort, and is comprised of patients in both extremes of lung function decline as measured by FEV1 (slow or fast decline) during the five years of the study. Analysis was performed using Wilcoxon test, Chi Square test and multiple regression models with analysis of gene-environment interaction when appropriate. In agreement with previous results, the rs16969968G allele was associated with amount of cigarette smoking during the LHS (p=0.027). In contrast, this SNP was not associated with cigarette smoking history before the study. After adjusting for smoking, the GG genotype was significantly associated with lung function decline (p=0.0180). Moreover, the rs16969968 SNP significantly modified the effect of smoking on lung function decline (p=0.044). The GG genotype was associated with a stronger relationship between cigarette smoking and lung function decline We successfully replicated the rs16969968 association with cigarette smoking and found a novel gene by environment interaction, namely rs16969968 GG genotype-by-smoking history affecting lung function decline in a COPD cohort. This SNP seems to be associated with level of addiction to nicotine in COPD patients, since after an anti-smoking intervention patients with the GG genotype smoked less than those with GT/TT genotypes. Future directions for this project are to genotype the entire LHS cohort (~4800 samples), and to further investigate other available phenotypes; e.g. successful smoking cessation.

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### 718/W/Poster Board #376

**An investigation of stress response and DNA repair pathways identifies additional risk loci for multiple sclerosis.** *L.F. Barcellos[1], F.B.S. Briggs[1], S.E. Bartlett[2], B.A. Goldstein[3], International Multiple Sclerosis Genetic Consortium.* 1) Division of Epidemiology, School of Public Health, University of California, Berkeley; Berkeley, CA; 2) Ernest Gallo Clinic and Research Center University of California, San Francisco; San Francisco, CA; 3) Division of Biostatistics, School of Public Health, University of California, Berkeley; Berkeley, CA.

Multiple sclerosis (MS) is a complex inflammatory and demyelinating autoimmune disease (AD) of the CNS with a prominent genetic component. The primary genetic risk factor is the HLA-DRB1*1501 (DR2) allele. Additional risk loci with modest effects have been identified using genome wide association and meta-analytical approaches; however, much of the remaining genetic contribution to MS has not been elucidated. Here, we investigated variation in several genes from two candidate pathways relevant to MS pathogenesis: stress response and DNA repair. Previous studies of psychological stress in MS have shown associations with both increased disease risk and greater risk for relapse and exacerbation. A potential role for DNA repair in MS has also been described. A recent gene expression study of MS lymphocytes reported aberrant expression of several DNA repair genes. Further, a role for DNA repair has been implicated in several ADs including autoimmune nephritis, SSc, SLE, and RA. Univariate testing was performed using genotypes from 1,343 MS cases and 1,379 healthy controls of European ancestry. A total of 354 SNPs within 10 genes related to stress response and 485 SNPs within 72 genes related to DNA repair pathways; including BER, NER, NHEJ, and HRR, were investigated. Results meeting our criteria for significance are described here. A significant association with decreased risk was observed for multiple intronic SNPs within CRHR1 on 17q21 (OR=0.7, p=3 x 10-3). Several intronic SNPs within OPRD1 on 1p35 (OR=0.75, p=3 x 10-3) were associated with decreased risk for MS in DR2 positive individuals (N= 702 cases, 345 controls), while intronic SNPs within OPRK1 on 8q11 (OR=1.5, p=3 x 10-4) were associated with increased risk of MS in DR2 negative individuals (N=638 cases, 1,030 controls). Amongst the DNA repair genes, SNP variants within GTF2H4 and XAB2 were significantly associated with MS (OR=0.7, p=7 x 10-8; OR=1.3, p=9 x 10-4). In DR2 positive individuals, a coding variant in XAB2 on 19p13 (OR=1.5, p= 1 x 10-3) was associated with increased risk, while GTF2H4 on chr 6 (OR= 0.7 p=6 x 10-5) was strongly associated with decreased risk of MS in DR2 negative individuals. Using a human protein interaction database software tool, STRING, we were able to construct a subnetwork for each pathway that included our final candidates. Collectively, our results provide strong evidence for the involvement in MS, of variation within genes from these important candidate pathways.

### 719/W/Poster Board #377

**Gender Specific Associations Between Lipid Related Candidate Genes and Carotid Plaque.** *A. Beecham[1], DM. Yanuck[2], H. Gardener[2], S. Slifer[1], SH. Blanton[1], L. Wang[1], RL. Sacco[2], T. Rundek[2].* 1) Institute for Human Genomics, Univ Miami, Miami, FL; 2) Department of Neurology, Univ Miami, Miami, FL.

Introduction: Carotid plaque is a distinct phenotype of atherosclerosis and an important subclinical marker of stroke. Sex differences have been identified in stroke risk and in the distribution of determinants of carotid atherosclerosis; however, the sex specific genetic contribution to carotid plaque is unknown. We sought to examine the sex specific associations between SNPs in candidate genes involved in lipid metabolism and several carotid plaque phenotypes (presence of plaque, multiple, thick, irregular, and calcified plaque). Methods: Two-hundred eighty-seven Dominicans from the Genetic Determinants of Subclinical Carotid Disease study were examined for carotid plaque by high-resolution ultrasound. Data was divided by sex (167 females; 120 males) and multiple logistic regression was used to test for association between 64 SNPs implicated in lipid metabolism and plaque phenotypes, controlling for age and smoking. To test for a SNP*sex interaction, an overall analysis in both females and males was done, controlling for age, smoking, sex, and the SNP. Two SNPs in the OLR1 gene (rs11053646 and rs11053639) were chosen, based on linkage disequilibrium (LD) structure and direction of effects, for haplotype analysis (haplo.stats). Results: Variations in five distinct genes (MTP, OLR1, LPL, APOE, LIPC) may have sex specific roles (p< 0.1 for SNP*sex interaction). In particular, rs11053646 in the OLR1 gene demonstrated a sex specific effect for all plaque phenotypes. Analysis of haplotypes identified an OLR1 2-SNP haplotype (rs11053639 and rs11053646; A-C) associated with an increased risk for irregular (p=3.00E-05), thick plaque (p=0.003), and plaque presence (p= 0.004) in women only. The G-G haplotype showed a protective effect for thick (p=0.039) and irregular plaques (p=0.047) in men but only for irregular (p=0.031) in women. Conclusions: We provide evidence suggesting that variations in lipid metabolism genes have sex-specific roles in carotid plaque burden. Differences in common carotid risk factors between men and women could play a role in gene*environment interactions and may be the subject of further study. Although the mechanism is unknown, variations in lipid metabolism genes may respond differently to sex-hormones and therefore affect lipid metabolism and atherosclerosis in a sex dependent manner.

### 720/W/Poster Board #378

**Genetic variants of the DDR1 gene are associated with vitiligo in a brazilian population.** *C.C.S Castro[1,3], L.M. Nascimento[1], G. Walker[2], E. Nogoceke[2], R.I. Werneck[1], M.T. Mira[1].* 1) Graduate Program in Health Sciences, Pontifical Catholic University of Parana, Curitiba, Parana, Brazil; 2) F. Hoffmann-La Roche Ltd, Basel, Switzerland; 3) Department of Dermatology, Pontifical Catholic University of Parana, Curitiba, Parana, Brazil.

Vitiligo is an acquired, systemic, chronic disease characterized by maculae devoid of melanic pigment and identifiable melanocytes. Adhesion of melanocytes to the basement membrane by integrin CCN3 is mediated through collagen IV receptor DDR1. We hypothesize that genetic variants of the DDR1 gene are associated with the occurrence of vitiligo. To test this hypothesis, we genotyped 10 Tag SNPs spanning DDR1 and the neighbouring gene GTF2H4 in 639 individuals distributed in 213 family trios composed by an affected child and both parents. Associated markers were then genotyped in an additional sample of 134 unrelated individuals with vitiligo and 134 unrelated controls from the same geographic area. Allele “A” of DDR1 intragenic tag SNP rs2267641 was associated with an increase risk for vitiligo on both family-based and case-control population sample (P=0.008, OR=4.00; 95% C.I. = 1.44 - 11.58 and P=0.04, OR=6.00; 95% C.I. = 1.73 - 52.33, respectively). In addition, we observed an age-dependent enrichment of rs4618569 “G” allele and rs2267641 “A” allele in affected individuals younger than 25 years (P=0.008; OR=7.04; 95% CI = 1.66-29.86 and P= 0.01; OR=4.26; 95% CI = 1.28-14.16, respectively). In conclusion, we propose the DDR1 as a new susceptibility gene for vitiligo, possibly implicating a defective cell adhesion on vitiligo pathogenesis.

### 721/W/Poster Board #379

**Fine mapping of Hirschsprung’s disease loci in 9q31.** *S.S. Cherny[1], C.S.M. Tang[1], Y. Sribudiani[4], X.P. Miao[2], M.T. So[2], P.C. Sham[1,3], P.K.H. Tam[2], M.M. Garcia-Barceló[2], R. Hofstra[4].* 1) Psychiatry, The University of Hong Kong, Pokfulam, Hong Kong; 2) Surgery, The University of Hong Kong, Pokfulam, Hong Kong; 3) Genome Research Centre, The University of Hong Kong, Pokfulam, Hong Kong; 4) Genetics, University Medical Center Groningen, Groningen, The Netherlands.

Hirschsprung’s disease (HSCR) is a congenital disorder in which there is an absence of ganglion cells in variable portions of the lower digestive tract, according to which patients are classified. The *RET* gene is the largest risk factor in HSCR, although reduced penetrance of *RET* mutations, absense of *RET* mutations in some patients, and variable expression of the HSCR phenotype indicate that more than one gene is involved. A *RET*-dependent modifier which segregates in families harboring no or hypomorphic *RET* mutations was mapped to 9q31. Fine mapping of the region performed on 142 Dutch trios by genotyping 370 tag-SNPs spanning approximately 7 Mb (from 108.5-115.5 Mb) of 9q31 on an Illumina GoldenGate platform identified two different 9q31 HSCR-associated regions in which genes with biological plausibility lie.

Since evaluation of an association in a population of different origin from that of the initial finding increases the association confidence and, since linkage disequilibrium (LD) differences across populations can be used to narrow the regions of interest, we genotyped 181 Chinese HSCR patients and 179 controls for 38 tag-SNPs chosen from the CHB population spanning the 9q31 regions of interest. In addition, we made use of genotype data for the 9q31 region obtained from a genome-wide association study recently conducted in Chinese HSCR patients.

Only one of the two 9q31 HSCR-associated regions identified in the Dutch population was associated in Chinese ($p = 0.021$), although the most associated SNPs within the region differed, probably due to differences in LD and/or different genotyping densities. Importantly, the associated region encompasses *IKBKAP* and *CTNNAL1*, which have been linked to neurodevelopmental disorders.

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## 722/W/Poster Board #380

**Comprehensive resequencing of F2 gene in Systemic Lupus Erythematosus.** *F.Y. Demirci[1], Y. Wang[1], A.H. Kao[2], E.Y. Rhew[3], R. Ramsey-Goldman[3], S. Manzi[2], M.I. Kamboh[1].* 1) Dept of Human Genetics, Univ of Pittsburgh, Pittsburgh, PA; 2) Lupus Center of Excellence, Univ of Pittsburgh, Pittsburgh, PA; 3) Div of Rheumatology, Northwestern Univ, Chicago, IL.

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease that primarily targets women of child-bearing age. SLE women are also at high risk of premature cardiovascular disease. Prothrombin (F2), a key enzyme in blood coagulation, is one of major antigens recognized by antiphospholipid antibodies that are associated with vaso-occlusive events in SLE patients. Prior studies have implicated *F2* variants in thrombosis and cardiovascular disease in non-SLE individuals. The aim of this study was to comprehensively evaluate the role of *F2* genetic variation in SLE risk and related features. We resequenced approximately 22-kb-long genomic fragment that harbors the entire F2 gene and flanking regions in a total of 285 individuals (115 White SLE patients & 105 White normal controls and 40 Black SLE patients & 25 Black normal controls). A total of 217 sequence variants (153 in Whites and 123 in Blacks) were identified. In each ethnic group, more than 2/3 of identified variants were relatively uncommon with ≤5% minor allele frequency (MAF). We identified a large number of novel variants that were not previously reported in publicly available databases. *F2* sequence variants were mostly single nucleotide substitutions, however, a small number of indels were also observed. Only 6.5% of all identified variants were located in exons. The vast majority of rare variants (MAF<1% in each ethnic group) were observed in cases only (~68% of rare variants in Whites and ~80% of rare variants in Blacks) and a higher percentage of cases were found to carry rare variants as compared to controls in both ethnic groups. These observations implicate enrichment of *F2* rare variants in SLE patients. The ongoing screening of identified rare variants and common tag SNPs in the large SLE case-control sample (>1,400 individuals) will document the full spectrum of the contribution of *F2* rare and common genetic variation to SLE pathogenesis and related clinical features.

## 723/W/Poster Board #381

***ELMO1* variants and susceptibility to diabetic nephropathy in American Indians.** *J.K. DiStefano[1], M.P. Millis[1], N.J. Young[1], K.A. Yeatts[1], S. Kobes[2], R.G. Nelson[2], W.C. Knowler[2], R.L. Hanson[2].* 1) Diabetes, Cardiovascular and Metabolic Diseases Division, Translational Genomics Research Institute, Phoenix, AZ; 2) Diabetes Epidemiology and Clinical Research Section, NIDDK, NIH, Phoenix, AZ.

Variants in the engulfment and cell motility 1 gene, *ELMO1*, have previously been associated with diabetic kidney disease attributed to type 2 diabetes mellitus in Japanese and African American individuals. The Pima Indians of Arizona have very high rates of diabetic nephropathy (DN), which is strongly dependent on genetic determinants; therefore, the goal of this study was to investigate the role of *ELMO1* polymorphisms in mediating susceptibility to DN in this population. We sequenced 17.4 kb of *ELMO1* corresponding to all exons, exon-intron boundaries, and 3 kb of upstream regulatory region in 36 Pima Indians and identified 19 single nucleotide polymorphisms (SNPs), six of which were novel. Seven SNPs were excluded from further study; four which were in 100% genotypic concordance with other *ELMO1* variants and three with a minor allele frequency <0.05. The remaining 12 SNPs, along with 21 additional markers showing association with DN in the previously reported Japanese and African American studies, were genotyped in 141 individuals with nephropathy and 416 individuals without heavy proteinuria in a family study of 257 sibships. Association was analyzed using a mixed effects logistic regression model with control for age, sex and duration of diabetes and with a random effect to account for sibship. We saw the strongest evidence for association with rs1345365 (odds ratio [OR]= 2.42 per copy of the A allele [1.35-4.32]; P=0.001) and rs10951509 (OR= 2.42 per copy of the A allele [1.31-4.48]; P=0.002), both of which are located within 13 kb of one another in intron 13 and are in strong linkage disequilibrium ($r^2$=0.97). These markers also showed statistically significant evidence for association with DN in the African American study although the association was in the opposite direction (OR=0.82 per copy of the A allele P<0.004); further, three additional markers showing statistically significant evidence for association with DN in the present study were also located in this region (P<0.05). None of the markers from the Japanese study were associated with DN in Pima Indians (P>0.05). These results identify novel *ELMO1* variants and provide additional support for this gene as a susceptibility locus for diabetic kidney disease across multiple populations.

## 724/W/Poster Board #382

**Allele specific gene expression supports *GABRG3* as a candidate gene affecting risk for alcohol dependence.** *H. Edenberg[1,2], X. Xuei[1], L.F. Wetherill[2], T. Foroud[2], Collaborative Study on the Genetics of Alcoholism.* 1) Dept Biochemistry & Molecular Biology, Indiana Univ School Medicine, Indianapolis, IN; 2) Dept. Medical & Molecular Genetics, Indiana Univ School Medicine, Indianapolis, IN.

We have previously reported evidence from family-based association studies that variations in *GABRG3* are associated with the risk for alcohol dependence (Dick et al. 2004, Alcoholism Clin Exp Res 28, 4). Of the 11 single-nucleotide polymorphisms (SNPs) tested in trios, 7 yielded evidence of association (p<0.05) and 3 others were suggestive; those 10 SNPs were in high LD with each other. Additional analysis indicates that the association is with those subjects who have a later age of onset. One of the associated SNPs, rs140679, is a synonymous SNP in the coding region (Thr321). Because we hypothesized that the association could be based upon differences in gene regulation, we analyzed whether the alleles were differentially expressed in samples of individual human brains. Differential allele expression was measured using allelotyping software on the Sequenom MassARRAY System. We obtained data from 19-22 heterozygous individuals in each of 9 brain regions: prefrontal cortex, cingulated cortex, visual cortex, amygdala, thalamus, hippocampus, caudate nucleus, putamen, and cerebellum. In nearly all of the samples, there was differential allelic expression (median p value = $1.4 \times 10^{-4}$), with the T allele overexpressed (average expression ratio T/C = 1.24). The T allele is overtransmitted to affected individuals. These data strengthen the evidence that variations in *GABRG3* affect alcohol dependence, probably by altering the relative expression of the gamma3 subunit of the $GABA_A$ receptor. [Supported by U10AA008401; brain tissues were provided by the Tissue Resource Center, Univ. of Sydney, supported by R01AA12725.].

## 725/W/Poster Board #383

**Identification of rare variants in the NRG1 gene of Hirschsprung's patients.** *M. Garcia-Barcelo[1], W.K. Tang[1], X.P. Miao[1], C. Tang[2], M.T. So[1], Y.Y. Leon[1], P.C. Sham[2,3], S.S. Cherny[2], P.K. Tam[1].* 1) Dept Surgery, Univ Hong Kong, Hong Kong, Hong Kong; 2) Dept Psychiatry, Univ Hong Kong, Hong Kong, Hong Kong; 3) Genome Research Centre, Univ Hong Kong, Hong Kong, Hong Kong.

Hirschsprung's disease (HSCR, aganglionic megacolon), is a congenital disorder characterized by the absence of enteric ganglia in variable portions of the distal intestine. HSCR has a complex pattern of inheritance and presents mainly sporadically. Besides the major HSCR gene, RET, and other implicating genes (e.g. EDNRB), there is evidence that other loci contribute to HSCR. Recently, through a genome-wide association study, we identified NRG1 as a new HSCR contributing locus. Several lines of evidence indicate that in addition to common variants/SNPs, rare variants may contribute substantially to the multifactorial inheritance of complex diseases, and that the genes with disease-associated SNPs are to be considered candidates for the search of deleterious rare variants. We hypothesized that rare NRG1 variants contributing to HSCR may exist. To identify these variants, we have sequenced the 17 exons (including exon/intron boundaries) of NRG1 of 386 HSCR patients and 100 controls on an ABI 3730xl DNA Analyzer. We have identified 7 novel rare variants in 7 patients that cause 4 non-synonymous amino-acid changes, a truncation of the protein, a disruption of the splice site and an alteration of the conserved region intronic region in the exon boundary. The non-synonymous amino-acid substitutions affect highly conserved residues and have not been found in the controls. The four patients bearing NRG1 rare variants have no disease-associated variants in RET or any other HSCR-associated gene. The finding of rare NRG1 variants with an obvious functional effect on the NRG1 protein, suggest that NRG1 is one of the several molecules contributing to the pathology of HSCR. More controls are currently being sequenced.

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## 726/W/Poster Board #384

**ABO blood type is associated with lung function among elderly men of Japanese ancestry in a within sibship association study.** *J. Grove[1,2], E. Tam[2], J. Curb[2].* 1) Biostat Prog, Pub Hlth Sci, Univ Hawaii, Honolulu, HI; 2) John A. Burns School of Medicine, Univ Hawaii, Honolulu, HI.

Research Question: Is ABO histotype associated with lung function among the elderly?

Study Design: Within sibship gene association study based on a birth cohort study of men of Japanese ancestry living in Hawaii (Honolulu Heart Program)

ABO histo-blood type has been reported to be related to the risk of asthma, with presence of type A antigen being associated with lower risk. Reported associations with other aspects of lung function have been inconsistent. ABH antigens are expressed on lung epithelial tissue and differences in antigen type may influence opportunistic bacterial growth and inflammation of the lung's lining. We investigated whether ABO type is associated with normal lung function among participants in the Honolulu Heart Program, a longitudinal study of a birth cohort of men of Japanese ancestry who lived on the island of Oahu in 1965.

Forced expiratory volume in one second (FEV1) and total forced expiratory volume (FVC) were regressed within sibships on age at exam, packs per day smoked, height, bi-achromial diameter, and presence of A antigen, with standard errors estimated using generalized estimating equations. Adjusted for these covariates, the sibling intraclass correlations were 0.25 for FEV1 and 0.24 for FVC. Men with type A antigen had non-significantly greater FEV1 and FVC than their siblings at the first exam (ages 45-67, $p > 0.10$). At a later examination held in 1992-93, when the survivors were elderly (ages 73-93), FEV1 and FVC were very highly significantly greater among men with A antigen than their non-A bearing siblings ($p = 0.0006$ for FEV1, $p = 0.0063$ for FVC, 3098 men with valid measurements); men with A antigen averaged 5.1% greater FEV1 than their siblings without A antigen.

## 727/W/Poster Board #385

**Association of *SPI1* 3' untranslated region polymorphism with systemic lupus erythematosus.** *K. Hikami[1], A. Kawasaki[1], I. Ito[1], M. Koga[1, 2], S. Ito[3], T. Hayashi[3], D. Goto[3], I. Matsumoto[3], A. Tsutsumi[3, 4], Y. Takasaki[5], H. Hashimoto[6], T. Arinami[2], T. Sumida[3], N. Tsuchiya[1].* 1) Mol Genet Epidemiol Lab, Univ of Tsukuba, Tsukuba, Japan; 2) Dept Med Genet, Univ of Tsukuba, Tsukuba, Japan; 3) Div Clin Immunol, Univ of Tsukuba, Tsukuba, Japan; 4) Takikawa Municipal Hospital, Takikawa, Japan; 5) Div Rheumatol, Juntendo Univ, Tokyo, Japan; 6) Juntendo Univ Sch Med, Tokyo, Japan.

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease caused by a complex combination of genetic and environmental factors. In view of the significance of type I interferon (IFN) pathway in the pathogenesis of SLE, 55 IFN-related genes were screened for association with SLE. One of the genes that demonstrated association in the first screening, *SPI1* (also referred to as PU.1), is an Ets family transcription factor that is essential for lymphoid and myeloid development. SPI1 is expressed highly in myeloid cells and B cells, and is known to interact with IRF2, IRF4 and IRF8. In this study, we performed a case-control association study using six tag SNPs in *SPI1* and its promoter, and a SNP located in 17 kb upstream of *SPI1*, previously associated with acute myeloid leukemia, in 400 Japanese patients with SLE and 450 healthy controls. Significant association was detected in an intron 2 SNP rs10769258 ($P = 0.01$, OR = 1.29, 95% CI 1.06-1.57). With respect to the disease subset, the association was observed in the patients with nephritis ($P = 0.007$, OR = 1.40, 95% CI 1.10-1.80) but not in those without nephritis. We next performed resequencing of enhancer, promoter, and all exons in *SPI1* gene to identify functional polymorphisms associated with pathogenesis of SLE. A SNP rs1057233 was identified in *SPI1* 3' untranslated region (3' UTR) that was in linkage disequilibrium with rs10769258. Expression analysis using HapMap database and GENEVAR B cell line expression database showed that the number of risk allele at rs1057233 was strongly correlated with *SPI1* mRNA level ($P = 0.0002$, JPT + CHB data). Furthermore, a reporter assay using luciferase reporter vector demonstrated that the rs1057233 risk allele was associated with higher expression. These results suggested that a functional polymorphism in the 3' UTR of *SPI1* that positively regulates gene expression may be associated with SLE.

## 728/W/Poster Board #386

**Variation in the uric acid transporter gene (*SLC2A9*) is associated with memory performance.** *L.M. Houlihan[1], N.D. Wyatt[2], S.E. Harris[3], A.J. Gow[1], R.E. Marioni[4], J.F. Price[4], J.M. Starr[5], C. Hayward[2], A.F. Wright[2], I.J. Deary[1].* 1) Centre for Cognitive Ageing and Cognitive Epidemiology, Department of Psychology, The University of Edinburgh, 7 George Square, Edinburgh, EH8 9JZ, UK; 2) MRC Human Genetics Unit, The Institute of Genetics and Molecular Medicine, Western General Hospital, Edinburgh, EH4 2XU, UK; 3) Centre for Cognitive Ageing and Cognitive Epidemiology, Medical Genetics Section, The University of Edinburgh, Edinburgh, EH4 2XU, UK; 4) Public Health Sciences Section, Division of Community Health Sciences, The University of Edinburgh, Medical School, Teviot Place, Edinburgh, EH8 9AG; 5) Centre for Cognitive Ageing and Cognitive Epidemiology, Geriatric Medicine Unit, The University of Edinburgh, Royal Victoria, Craigleith Road, Edinburgh, EH4 2DN, UK.

Understanding the basis of human cognitive ageing is important to improve the health and well-being of an expanding elderly population. Serum uric acid levels have been related to cognitive functioning in humans, though the direction of the association is equivocal. SLC2A9 is a urate transporter and its association with serum uric acid levels is well-established. This study tested four *SLC2A9* SNPs for association with cognitive abilities in three cohorts. First, the Lothian Birth Cohort 1921 (LBC1921) were tested on general cognitive ability at age 11 and, at mean ages of 79 (n=520), 83 (n=281) and age 87 (n=177), they completed cognitive ability test batteries. Second, approximately 1,000 Scots, the Lothian Birth Cohort 1936 (LBC1936) were tested on general cognitive ability at age 11 and, at mean age 70, they re-sat the same general cognitive ability test and a battery of diverse cognitive tests. Third, replication is being attempted in another sample of approximately 1,000 elderly Scots aged from 60 and 74 years, namely the Edinburgh Type 2 Diabetes Study (ET2DS) where cognitive testing and serum uric acid levels are also available. *SLC2A9* was associated with logical memory in LBC1921 at age 79, 83 and 87 (all $P$-values < 0.05). Significant associations were detected with *SLC2A9* and a general memory factor in LBC1936 and other individual cognitive ability tests: logical memory, spatial span, verbal paired associates, block design and simple and choice reaction time (best $P$-value = 0.0002). Results of the replication study in ET2DS will also be presented. If *SLC2A9* variants are a proxy for serum uric acid levels, this study would suggest that higher serum uric acid levels are associated with increased performance in memory-related tasks.

## 729/W/Poster Board #387

**Fetal Genetic Risk Factors for Isolated Cleft Lip Only.** *A. Jugessur[1], M. Shi[2], H.K. Gjessing[3,4], R.T. Lie[4,5], A.J. Wilcox[6], C.R. Weinberg[2], K. Christensen[7], A.L. Boyles[6], S. Daack-Hirsch[8], T. Trung Nguyen[5], C. Bille[7], A.C. Lidral[9], J.C. Murray[7,9].* 1) Craniofacial Development, Musculoskeletal Disorders, Murdoch Childrens Research Institute, Royal Children's Hospital, 3052 Parkville, Australia; 2) Biostatistics Branch, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, Durham, NC 27709; 3) Department of Epidemiology (EPAM), Norwegian Institute of Public Health, N-0403 Oslo, Norway; 4) Section for Epidemiology and Medical Statistics, Department of Public Health and Primary Health Care, University of Bergen, N-5018 Bergen, Norway; 5) Medical Birth Registry of Norway, Norwegian Institute of Public Health, N-5018 Bergen, Norway; 6) Epidemiology Branch, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC 27709; 7) Department of Epidemiology, University of Southern Denmark, DK-5000 Odense, Denmark; 8) College of Nursing, University of Iowa, Iowa City, IA 52242; 9) Departments of Pediatrics, Epidemiology and Biological Sciences, University of Iowa, Iowa City, IA 52242.

Cleft lip only (CL) and cleft lip and palate (CLP) are commonly regarded as a single entity and pooled together to form the single group of cleft lip with or without cleft palate (CL/P) prior to analysis. However, recent data suggest that CL may be distinct from CLP and should, when feasible, be analyzed separately. To evaluate the risk of isolated CL from genetic variants carried by the offspring, we analyzed 121 Norwegian and 76 Danish isolated CL trios from two nationwide studies of orofacial clefts in Scandinavia. Genotypes for 1315 SNPs in 334 autosomal candidate genes for clefting were available for analysis in these trios. Two complementary statistical methods (TRIMM and HAPLIN) were used to scan for associations with these genes and to look for consistency in the pattern of associations across the two national samples. TRIMM and HAPLIN both identified strong associations with *FGF12* in both populations, but only TRIMM identified associations with IRF6 and VCL. In contrast, *CX43* was only detected by HAPLIN. Quantile-quantile (QQ) plots of the Fisher-combined p-values from the Norwegian and Danish analyses showed overall association with *IRF6*, *FGF12* and *CX43*. In conclusion, we observed some consistency in the pattern of associations across the two populations, irrespective of the analytic method used. Whereas both *IRF6* and *FGF12* have previously shown strong associations with isolated CL/P, *VCL* and *CX43* appear to be specific for isolated CL and warrant further investigation in other populations.

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## 730/W/Poster Board #388

**A Genome-wide association study in human narcolepsy.** *M. Kawashima[1], J. Hallmayer[1], J. Faraco[1], L. Lin[1], S. Hesselson[2], J. Winkelmann[3], G. Plazzi[4], S. Nevsimalova[5], P. Bourgin[6], S. Hong[7], M. Honda[8], W.T. Longstreth Jr[9], J. Montplaisir[10], D. Kemlink[5], G.A. Rouleau[11], T. Meitinger[3], K. Tokunaga[12], P. Kwok[2], N. Risch[2], E. Mignot[1].* 1) Ctr Narcolepsy Sciences and Department of Psychiatry, Stanford Univ, Palo Alto, CA; 2) Institute for Human Genetics, University of California San Francisco School of Medicine, San Francisco, CA; 3) Institute for Human Genetics, Technische Universität München, Munich, Germany; 4) University of Bologna, Bologna, Italy; 5) Department of Neurology, Charles University in Prague, 1st Faculty of Medicine and General Teaching Hospital, Prague, Czech Republic; 6) Sleep Clinic, Hõpital Civil, Louis Pasteur University, Strasbourg, France; 7) Department of Neuropsychiatry, St. Vincent's Hospital, The Catholic University of Korea, Suwon, Korea; 8) Tokyo Institute of Psychiatry, Setagaya, Japan; 9) Departments of Neurology and Epidemiology, University of Washington, Seattle, Washington; 10) Sleep Disorders Center, Université de Montré al, Montré al, Que'bec, Canada; 11) Center of Excellence in Neuromics, Université de Montré al, Montré al, Canada; 12) Department of Human Genetics, University of Tokyo, Tokyo, Japan.

Narcolepsy with cataplexy is characterized by day time sleepiness and rapid onset into REM sleep. It affects 0.02-0.06% of the population in Caucasians. Narcolepsy is tightly associated with *DQB1*0602* and a loss of hypocretin producing cells in the hypothalamus. An autoimmune basis has long been suspected but remains unproven. We performed a genome-wide association study (GWAS) using the Affymetrix Mapping 500K and 6.0 array sets in Caucasians (1,074 *DQB1*0602* positive controls and 807 *DQB1*0602* positive cases). Four genome-wide significant markers were found and replicated in three additional cohorts (700 Caucasians, 250 Koreans, and 250 African Americans). Three of these markers were located in the T-cell receptor alpha locus, J segment region, on chromosome 14, with highest significance at rs1154155 (allelic odds ratio: 1.83 [1.39-2.42], bonferoni-corrected P = $10^{-5}$) and replicated strongly across ethnic groups. We also tested 14 other markers, all only nominally significant, of higher significance than rs9275523, a marker located in the *HLA-DQ* region. This marker was selected as it likely represents established HLA heterozygote effects in narcolepsy, also demonstrating the well HLA matched nature of our sample. One of these 14 markers is in T-cell receptor beta locus on chromosome 7 (rs2367191). Eight of these markers had non significant but similar direction effects in Caucasians as reported with GWAS. One replicated significantly in Caucasians but showed no effects in the other ethnic groups, although a similar allele frequency was present. Five out of 9 markers also showed same tendency in Koreans and African Americans. Replication in an additional Caucasian sample, and fine typing of the TCR loci effects in underway, but these results strongly suggest autoimmune mechanisms in the cause of narcolepsy.

## 731/W/Poster Board #389

**Common variants in the *IL6R* and *PAI1* genes are associated with PAI-1 levels in pregnant women of European ancestry.** *H. LEE[1], R.M. Freathy[1,2], M. Urbanek[1], L.P. Lowe[1], C. Ackerman[1], T.W. McDade[1], N.J. Cox[3], D.B. Dunger[4], A.R. Dyer[1], A.T. Hattersley[2], B.E. Metzger[1], W.L. Lowe[1], M.G. Hayes[1] for the HAPO Study Cooperative Research Group.* 1) Northwestern University, Chicago, USA; 2) Peninsula Medical School, Exeter, UK; 3) University of Chicago, Chicago, USA; 4) University of Cambridge, Cambridge, UK.

Adipokines control diverse metabolic functions and are implicated in the development of insulin resistance by altering insulin sensitivity in insulin target organs. Our previous study showed a continuous relationship between maternal glucose level in pregnancy and adipokine levels. Therefore, we hypothesized that genetic factors contribute to maternal adipokine levels as well as glucose metabolism during pregnancy and conducted a candidate gene screen to evaluate this hypothesis. The Hyperglycemia and Adverse Pregnancy Outcome is a multicenter study, which examined the impact of maternal glucose levels on fetal outcomes in 25,000 pregnant women from multiple ethnic groups. Of these, 819 mothers of European ancestry (UK and Australia) were selected for the current study. Participants underwent 75-g oral glucose tolerance test (OGTT) between 24 and 32 weeks of gestation. We examined 1536 maternal single nucleotide polymorphisms (SNPs) in 79 candidate loci previously implicated in insulin secretion or sensitivity, fetal growth, or inflammation to determine associations with adipokine levels (adiponectin and PAI-1) at ~28 weeks gestation. Associations were assessed through linear regressions with the single trait or outcome under an additive genetic model adjusting for necessary covariates. Two SNPs in the *IL6R* gene (rs7411976 and rs12090237, both $P$=3.2×$10^{-5}$, $r^2$= 0.9) and rs2227631 in *PAI1* gene ($P$=3.2×$10^{-5}$) were associated with PAI-1 levels. The PAI-1 level in those who carried a rare allele of rs7411976 or rs12090237 was 8 ng/ml lower than in those who did not carry a rare allele (23.7 ng/ml [95% CI: 20.5-27.4] vs. 32.0 ng/ml [95% CI: 31.1-32.8]). The PAI-1 level in rare homozygotes for rs2227631 was 5 ng/ml lower than in common homozygotes (28.2 ng/ml [95% CI: 26.4-30.1] vs. 33.1 ng/ml [95% CI: 31.8-34.6]). The same SNP has previously been associated with PAI-I levels in non-pregnant subjects. No significant associations were found with adiponectin levels. Rs7411976 and rs12090237 in the *IL6R* gene also showed a significant association with 1 hour glucose level at OGTT (both -0.7 mmol/L [95% CI: -1.2- -0.1] per rare allele, $P$<0.05). There was no significant association between rs2227631 and maternal glucose levels. These results suggest that common variants in *IL6R* and *PAI1* have a strong impact on PAI-1 levels in European women with normal pregnancy and that those in *IL6R* may influence maternal glucose levels.

## 732/W/Poster Board #390

**New common IRF6 variant associated with specific risk for cleft lip and palate.** *E.J. Leslie[1], A.L. Petrin[1], M. Marazita[2], M. Cooper[2], F. Rahimov[3], J.C. Murray[1].* 1) University of Iowa, Iowa City, IA; 2) University of Pittsburgh, Pittsburgh, PA; 3) Children's Hospital Boston/Harvard Medical School.

Cleft lip and/or cleft palate (CL/P) is a common birth defect with complex etiology due to multiple genetic and environmental factors. The search for genetic contributors to cleft lip and/or palate has identified several candidate genes and loci including *IRF6*, *FOXE1*, *BMP4*, *MSX1*, and a region on 8q24. *IRF6* is particularly interesting, not only for its contribution to Van der Woude syndrome, but variants in and around *IRF6* have been associated with nonsyndromic CL/P. The common allele of the variant V274I (rs2235371) located in exon 7 of *IRF6* was strongly associated with nonsyndromic CL/P in several populations and recently, Rahimov et. al. (2008) reported a functionally relevant association of rs642961 to cleft lip only (CL) caused by the disruption of an AP-2 binding site upstream of *IRF6*. The rare allele (A) of the rs642961 is in complete linkage disequilibrium with the common allele of V274I (G) explaining the strong association. However, rs642961 seems to play a preferential role in the etiology of CL, raising the hypothesis of a third variant that plays a role in cleft lip and palate (CLP). As a follow-up to this study, we have sequenced 3 highly conserved regions including the 607 bp immediately flanking rs642961 in Philippine cases and controls (673 and 302, respectively) and have identified a previously unreported SNP (G>A) at chromosome position 208055904 (UCSC Browser v206 Build 36.1). The rare allele (A) is in almost complete linkage disequilibrium with the common allele (G) for rs642961, allowing us to exclude any effect of the rare (associated) allele of rs642961. When analyzed individually, neither the rare allele (A) of 208055904 nor the rare allele (A) of rs2235371 is associated with CL/P. Interestingly, when these rare alleles are combined with the common allele (G) of rs642961, the resulting haplotype, AGA (rs2235271, rs642961, 208055904), is significantly associated. When stratified by cleft type, the AGA haplotype is more strongly associated in CLP (p=1.2 x $10^{-9}$) than CL (p=1.072 x $10^{-4}$) supporting the hypothesis of a third variant in the *IRF6* region that plays a preferential role in CLP etiology, at least in a large population from the Philippines. Additional analysis in other populations and functional analysis of this variant should be enable a better characterization of the role of *IRF6* in clefting and help identify other pathway members to investigate.

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## 733/W/Poster Board #391

**Sex specific effect of the APLN gene on the metabolic traits and gene expression levels.** *Y. Li[1], Y. Liao[1], M. Yu[4], S. Juo[1,2,3].* 1) Graduate Institute of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Department of Neurology, Kaohsiung Medical University, Kaohsiung, Taiwan; 3) Center of Excellence for Environmental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 4) Hepatobiliary Division and Department of Preventive Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

**Background and Purpose** Dysregulation of the production of adipokines plays a pivotal role in the development of metabolic syndrome and cardiovascular comorbidity. Apelin, a newly identified adipokine, is up-regulated by insulin and highly expressed in obese subjects. The Apelin gene is located on X chromosome. The purpose of present study is to investigate whether the polymorphisms at the gene encoding apelin confer risks for metabolic syndrome.

**Methods** The study subjects are volunteers recruited from the general population in southern Taiwan. The diagnosis of metabolic syndrome is based on the modified version of ATPIII where a subject of metabolic syndrome needs to have at least 3 of 5 risk components (HDL-C, triglyceride, waist circumference, blood sugar and blood pressure). To reduce the concern of disease uncertainty, the subjects with two risk components were not included in the analysis. Four tagging SNPs at apelin gene were genotyped. Since we found the SNPs had different effects between men and women, we compared the apelin expression levels between in the human adipocytes obtained from women and men.

**Results** A total of 498 cases and 1311 controls were included in the present study. Men accounted for 51.4 % and the average age was 51.1 ± 13.5 years. The four SNPs were not statistically associated with metabolic syndrome. We further evaluated the SNPs vs. each of the five components of metabolic syndrome. SNP 3 appeared to be associated with abdominal obesity (adjusted p = 0.023) and fasting glucose (adjusted p = 0.002) in women. In addition, SNP 4 was associated with abdominal obesity (adjusted p = 0.026) in women. None of the SNPs were significant in men. The cellular studies showed that apelin levels were higher in female adipocytes than male adipocytes when the adipocytes were treated with TNF-$\alpha$.

**Conclusions** The present study demonstrated that apelin polymorphisms may influence abdominal obesity and insulin resistance, this gene may be more influential in the women subjects in terms of metabolic traits.

## 734/W/Poster Board #392

**Study of 390 candidate genes for obesity using data from genome wide meta-analysis of BMI in 32,387 individuals.** *R.J.F. Loos[1], K.S. Vimaleswara[1], C.J. Willer[2], E.K. Speliotes[3,4], S. Li[1], C.M. Lindgren[5], I.M. Heid[6], G.R. Abecasis[7], J.N. Hirschhorn[4,8,9] for the GIANT consortium.* 1) MRC Epididemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, United Kingdom; 2) Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, Michigan 48109, USA; 3) Division of Gastroenterology, Massachusetts General Hospital, Boston, Massachusetts 02114, USA; 4) Metabolism Initiative and Program in Medical and Population Genetics, Broad Institute of Harvard and Massachusetts Institute of Technology, Boston, Massachusetts 02142, USA; 5) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, UK; 6) Institute of Epidemiology, Helmholtz Zentrum München, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany; 7) Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, Michigan 48109, USA; 8) Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA; 9) Program in Genomics and Divisions of Endocrinology and Genetics, Children's Hospital, Boston, Massachusetts 02115, USA.

In the past 15 years, candidate gene and genome-wide linkage studies have proposed many genes for obesity. However, replication in subsequent studies often provided inconsistent results. Limitations of these traditional gene-discovery methods are 1) small sample size (typically with n<1,000) and thus insufficient power to identify the small effects, and 2) poor coverage of the genetic variation in the genes. Recent genome-wide association (GWA) studies have resulted in a rapid increase in the number of loci associated with body mass index (BMI), although for most of these function is poorly understood. The aim of this study is to pursue a targeted association analysis between previously proposed obesity candidate genes and BMI by using the GWA data of the GIANT (Genetic Investigation of ANthropometric Traits) consortium (Willer et al, 2009). The advantage of this approach is that the sample size is large (n=32,387) and that most common genetic variation in genes is captured. We performed a systematic literature search for candidate genes implicated in obesity by animal studies, genome-wide linkage studies, genetic association studies and studies of Mendelian syndromes relevant to human obesity. The GWA encompassing 15 cohorts and 32,387 individuals of European ancestry was examined at ≈2.5 million genotyped or imputed HapMap SNPs. Association between BMI and SNPs was tested assuming an additive effect. Summary statistics of each study were meta-analysed using the inverse variance method. We captured 10kb up- and downstream of all the identified candidate genes. Out of 390 candidate genes, 16 independent loci ($r^2$<0.5) from 13 candidate genes reached a significance of p<$10^{-3}$. Of these, variants in SH2B1 (p=$10^{-11}$) and BDNF (p=$10^{-4}$) were already identified and replicated by the recent GWA studies. The remaining 11 genes are: ADAM12 (risk allele frequency: 20.4%, beta= 0.04 z-score increase per risk allele, p=6x$10^{-5}$), LEPR (98%, 0.13, p=6x$10^{-5}$), CPE (11%, 0.04, p=$10^{-4}$) MCTP2 (85%, 0.05, p=$10^{-4}$), EBF2 (52%, 0.03, p=$10^{-4}$), TCF7L2 (25%, 0.03, p=2x$10^{-4}$), PRKAG2 (54%, 0.03; p=3x$10^{-4}$), TBC1D1 (83%, 0.04; p=4x$10^{-4}$), SCD5 (91%, 0.05; p=4x$10^{-4}$), SCG3 (62%, 0.03; p=4x$10^{-4}$) and ACACA (77%, 0.03; p=9x$10^{-4}$). By taking advantage of large scale GWA meta-analyses, out of 390 previously proposed candidate genes, we identified 16 independent loci in 13 genes (p<$10^{-3}$). The identified loci may help focus replication efforts and the search for underlying biology.

## 735/W/Poster Board #393

**Study of the possible role of MKX in non syndromic clef lip and palate.** *E. Masiero[1], M.A. Mansilla[3], F. Carinci[2], J.C. Murray[3].* 1) Histology, Embriology I, University of Bologna, Bologna, Italy; 2) Department of D.M.C.C.C., Section of Maxillo-facial Surgery, University of Ferrara, Corso Giovecca, ferrara, Italy; 3) Department of Pediatrics University of Iowa, 500 Newton Road 2182 ML Iowa City IA 52242.

Facial clefts are the most common multifactorial birth defects caused by genetic and environmental factors. The aim of this work is to study the possible role of MKX in non syndromic cleft lip with or without cleft palate (NSCL/P) Mohawk (Mkx) is a novel homeobox gene that is highly expressed, among other tissues, in the frontonasal process and palatal mesenchyme during primary and secondary palate development. This gene maps close to the twirler mutation; mouse homozygotes for this mutation present with cleft lip and cleft palate or cleft palate only. In addition Jugessur et al. 2009 found positive association between facial clefts and SNPs in MKX in NS CL/P trios from Norway and Denmark. To pursue the role of MKX in clefting we sequenced the complete coding region and exon/intron boundaries of the MKX gene in DNA samples of 91 NSCL/P cases from the Philippines to look for possible etiologic mutations. We detected one new missense mutation in exon 2 of MKX, a Arginine (R) to Leucine (L) at position 60 of the protein sequence that has not been reported previously. This amino acid is normally highly conserved in the most of the species. Polyphen analysis of the protein structure suggests this mutation is probably damaging, suggesting it may be etiologic in clefting. We will now sequence family members for this case and additional cases and controls in the same region. We propose that mutations in MKX may interfere with the conformation of the protein and can change its functionality, during palate development, that can result in cleft lip/palate.

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## 736/W/Poster Board #394

**Associations of folate and choline metabolism gene polymorphisms with orofacial clefts.** *A. Mostowska[1], K.K. Hozyasz[2], P. Wojcicki[3,4], B. Biedziak[5], M. Dziegelewska[1], J. Misiak[1], M. Holysz[1], P.P. Jagodzinski[1].* 1) Department of Biochemistry and Molecular Biology, University of Medical Sciences in Poznan, Poland; 2) Department of Pediatrics, Institute of Mother and Child in Warsaw, Poland; 3) University Clinic of Medical Academy in Wroclaw, Poland; 4) Department of Plastic Surgery Specialist Medical Center in Polanica Zdroj, Poland; 5) Department of Orthodontics, University of Medical Sciences in Poznan, Poland.

Nonsyndromic cleft lip with or without cleft palate (NCL/P) is a common congenital anomaly in humans. It has been reported that nutritional factors are likely to play a major role in the development of this malformation. Since the mechanism by which folic acid and choline supplementation prevents NCL/P is poorly understood we decided to investigate the relationship between 16 polymorphic variants of 12 genes encoding enzymes involved in metabolism of these nutrients and the risk of facial clefts. SNPs were selected based on validation status, functional relevance and importance, and minor allele frequency >0.1 in Caucasians. Genotyping was carried out either by PCR followed by the appropriate restriction enzyme digestion (PCR-RFLP) or high-resolution melting curve analysis (HRM) in a group of 174 patients with NCL/P and 176 controls. We found that individuals with AA genotype for *BHMT* rs3733890 polymorphism have significantly lower risk of facial clefts. The calculated Odds ratio for homozygotes AA compared to individuals with G allele (AG or GG) was 0.145 (95%CI: 0.042-0.499; p= 0,0005; statistical power=94%). This strong protective association persisted even after correcting for multiple comparisons ($p_{corr}$=0.008). *BHMT* encodes the zinc metalloenzyme that catalyses homocysteine remethylation to methionine using betaine, formed during choline oxidation, as a methyl donor. The expression of *BHMT* is found mainly in human liver, where encoding enzyme is responsible for up to 50% of homocysteine remethylation. Another finding of our study was significant association of *PCYT1A* polymorphism with the risk of NCL/P. Compared with individuals with GG genotype, the A allele carriers had an Odds ratio of 1.891 (95%CI: 1.151-3.107; p=0.011; statistical power=72%). The *PCYT1A* encodes a rate controlling enzyme in the CDP-choline pathway of phosphatidylcholine synthesis. Analysis of the influence of polymorphisms in *CBS*, *MTHFD1*, *MTHFR*, *MTR*, *MTRR*, *TCN2*, *BHMT2*, *CHDH*, *CHKA* as well as *PEMT* on NCL/P recurrence did not reveal any significant correlations. In conclusion, this report demonstrates that choline metabolism may play an important role in etiology of NCL/P. We found that polymorphic variants of *BHMT* and *PCYT1A* might influence the risk of facial clefts in the Polish population. Supported by grant no. 501-01-01124182-08638 from the University of Medical Sciences in Poznan and grant no. N N401 006835 from the Polish Ministry of Science and Higher Education.

## 737/W/Poster Board #395

**The β2 adrenergic receptor gene (ADRB2) polymorphisms and obesity in Oceanic populations.** *I. Naka[1], N. Nishida[2], R. Kimura[3], T. Furusawa[4], K. Natsuhara[5], T. Yamauchi[6], M. Nakazawa[7], Y. Ataka[8], K. Tokunaga[2], T. Ishida[9], T. Inaoka[10], Y. Matsumura[11], R. Ohtsuka[12], N. Tsuchiya[1], J. Ohashi[1].* 1) Doctoral Program in Life System Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Ibaraki, Japan; 2) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; 3) Transdisciplinary Research Organization for Subtropics and Island Studies, University of the Ryukyus, Okinawa, Japan; 4) ASNET Promotion Office, Division for International Relations, The University of Tokyo, Tokyo, Japan; 5) School of Nursing, Fukuoka Prefectural University, Fukuoka, Japan; 6) Department of Health Sciences, Hokkaido University School of Medicine, Hokkaido, Japan; 7) Department of Public Health, Graduate School of Medicine, Gunma University, Gunma, Japan; 8) School of Policy Studies, Kwansei Gakuin University, Hyogo, Japan; 9) Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan; 10) Department of Environmental Sociology, Faculty of Agriculture, Saga University, Saga, Japan; 11) Faculty of Healthcare, Kiryu University, Gunma, Japan; 12) Japan Wildlife Research Center, Tokyo, Japan.

To clarify the genetic factors associated with body mass index (BMI, $kg/m^2$) in Oceanic populations, the associations of two single nucleotide polymorphisms (SNPs) of β2 adrenergic receptor gene (ADRB2), Arg16Gly (rs1042713) and Gln27Glu (rs1042714), and a β3 adrenergic receptor gene (ADRB3) SNP, Trp64Arg (rs4994), with BMI and obesity were examined in 695 adult subjects living in Solomon islands and Tonga. A multiple regression analysis adjusted for age, sex, and population revealed that a copy of 27Glu significantly increased a BMI of 2.3 $kg/m^2$ (P-value = 0.0009). A multiple logisitic regression analysis showed that 16Gly,and 27Glu were weak but significant risk factors for obesity (BMI > 30 $kg/m^2$) (P-value for Arg16Gly = 0.0298 and P-value for Gln27Glu = 0.0347). Trp64Arg showed no association with BMI and obesity. Further conditional logistic regression analysis suggested that Arg16Gly was not an independent risk factor of obesity (P-value for Arg16Gly = 0.1209 and P-value for Gln27Glu = 0.0379). The linkage disequilibrium (LD) analysis revealed that Arg16Gly was in weak LD with Gln27Glu (D' = 0.39 ~ 1 and $r^2$ = 0.01 ~ 0.09 in five Oceanic populations studied), implying that the association of Arg16Gly with obesity may have come from LD with Gln27Glu. Our results suggest that the Gln27Glu polymorphism of ADRB2 plays an important role in the pathogenesis of obesity in Oceanic populations.

## 738/W/Poster Board #396

**The PAX6 promoter in high myopia.** *C.P. Pang[1], T.K. Ng[1], C.Y. Lam[1,2], P.O.S. Tam[1], L.J. Chen[1], S.W.Y. Chiang[1], D.S.P. Fan[1], D.S.C. Lam[1].* 1) Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong S.A.R; 2) Department of Surgery, Hong Kong University, Hong Kong S.A.R.

A myopia locus was reported on chromosome 11p13 and the PAX6 gene located at that region was postulated to be associated with myopia development. PAX6 expression levels are tightly regulated and PAX6 gene dosage effects create a range of ocular phenotypes. This study investigated the association of the PAX6 P1 promoter, the major promoter of PAX6 gene, with high myopia (HM) in a Chinese cohort in Hong Kong. The HM patients had refractive errors -6.00 diopters (D) or below and axial length longer than 26 mm. The control subjects had refractive errors greater than -1.00 D and axial length shorter than 24 mm. The P1 promoter, flanking up to 3.5 kb, of the PAX6 gene was screened in 379 HM patients and 349 controls by polymerase chain reaction (PCR) and direct sequencing. No single nucleotide polymorphisms (SNPs) showed association with HM. Two dinucleotide repeats, (AC)m and (AG)n, located around 1 kb upstream of the transcriptional initiation site, were found to be highly polymorphic. Significant association was observed between these two repeats and HM. Higher repeat numbers were observed in HM patients for both (AC)m (global p = 0.016 and trend p = 0.03) and (AG)n dinucleotide polymorphisms (global p = 0.014 and trend p = 0.014) with 1.327-fold of increased risk for (AG)n repeat (p = 0.014; 95% CI: 1.059 - 1.663). Luciferase-reporter analysis showed elevations of transcription activity with increasing individual (AC)m and (AG)n repeat lengths and the combined (AC)m(AG)n repeat lengths. Our results revealed an association between AC and AG dinucleotide repeat lengths in the PAX6 P1 promoter and HM, indicating the involvement of PAX6 in the pathogenesis of myopia.

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## 739/W/Poster Board #397

**The Sortilin-related receptor (SORL1) influences variation in memory in late-onset Alzheimer Disease.** *C. Reitz[1,2,3], J. Lee[1,2,5], R. Cheng[1,2], E. Rogaeva[4], S. Tokuhiro[4], L. Clark[1,2,6], P. St.George-Hyslop[4], R. Mayeux[1,2,3,5].* 1) G.H Sergievsky Ctr, Columbia Univ, New York, NY; 2) Taub Institute, Columbia University, New York; 3) Department of neurology, Columbia University, New York; 4) Centre for Research in Neurodegenerative Diseases, University of Toronto; 5) Departments of Epidemiology and Biostatistics, School of Public Health Columbia University; 6) Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, NY.

Background and Objective. Recent studies implicated the neuronal sortilin-related receptor gene SORL1 as a susceptibility gene for late-onset Alzheimer disease (AD). We explored whether variation in SORL1 is associated with the memory performance using 2 independent datasets. Methods. We first examined the association between measures of cognitive function and the same 29 single nucleotide polymorphisms (SNPs) used our earlier paper (Rogaeva et al, 2007) in 1,180 individuals from 228 families participating in the Caribbean Hispanic Family Study of Familial Alzheimer disease using family-based association analyses. Then we explored the association of the same 29 SNPs with memory performance in 425 Caribbean Hispanics (212 cases vs. 213 controls) from a multiethnic random sample of Medicare recipients ≥65 years and residing in northern Manhattan. Results. In the family cohort, haplotypes at SNPs 1-4 (p=0.0008), 7-10 (p=0.05) and 14-18 (p=0.003) were significantly associated with measures of memory performance. In the epidemiological cohort, we replicated these findings by showing that haplotypes at SNPs 1-3 were significantly associated with total recall (p=0.02) and Benton recognition (p=0.04), haplotypes at SNPs 15-17 were associated with total recall (p=0.05) and Benton recognition (p=0.03), haplotypes at SNPs 21-23 were associated with total recall (p=0.05), delayed recall (p=0.02) and Benton recognition (p=0.03), and haplotypes at SNPs 27-29 were associated with total recall (p=0.02), delayed recall (p=0.0008) and Benton recognition (p=0.02). However, these SNPs that were significant for memory performance were not associated with abstract reasoning and language function. Conclusions. Genetic variation in SORL1 is specifically associated with memory function.

## 740/W/Poster Board #398

**Association of CRP polymorphisms with obesity severity in Mexican population.** *Y. Saldaña-Alvarez[1], MG. Salas-Martínez[1,2], S. Jiménez-Morales[1], A. Luckie-Duque[3], G. García-Cárdenas[4], H. Vicenteño-Ayala[5], A. Carnevale-Cantoni[6], L. Orozco[1].* 1) Instituto Nacional de Medicina Genómica; 2) Posgrado en Ciencias Genómicas-UACM; 3) Hospital Regional 1° de Octubre-ISSSTE; 4) Clínica de Diagnóstico Automatizado-ISSSTE; 5) Hospital Regional López Mateos-ISSSTE; 6) Coordinación de Medicina Genómica-ISSSTE.

Introduction. Changes in inflammatory status of adipose tissue support a growing evidences that obesity represents a state of chronic low-level inflammation. In this way, some studies has been reported a strong relationship between high circulating level of C-reactive protein (CRP), a systemic pro-inflammatory risk marker and increasing body mass index (BMI). Objective. In this study we examined the relation between polymorphisms located in C-reactive gene (CRP) and BMI, in adult Mexican patients. Methods. We performed a case-control association study in 413 obese cases with a BMI>30 and 263 controls with a BMI <25. Cases and controls were older than 18 years old recruited from two tertiary Hospitals in Mexico City. We analyzed r1205C/T, rs1130864C/T and rs1417938T/A CRP polymorphisms. Genotyping was carried out by 5' exonuclease assay (TaqMan). The association test, Hardy-Weinberg Equilibrium (HWE) and haplotypes were evaluated using EPIDAT, FINETTI and Haploview software, respectively. Results. Genotype distributions were in HWE both in patients and controls. When CRP genotypes and alleles frequencies were compared between cases and controls, no significant differences were observed in the genotype and allelic distribution between obese and no obese. When we stratified by severity rs1130864T and rs1417938A alleles, showed a significantly association with protection to morbid obesity (OR= 0.563, 95% CI 0.382-0.829, p= 0.0034, and OR= 0.585 95% CI: 0.398-0.860, p= 0.006, respectively). Consistently, haplotype analysis reveled a severity obesity protection haplotype (CTA), containing the minor alleles from rs1130864 and rs1417938 polymorphisms (OR= 0.55; 95% CI: 0.36-0.82, p= 0.002). In contrast, we observed that carriers of the rs1205T allele have a high risk to develop severe obesity with a BMI>40 (OR= 2.57; 95% CI: 1.201-5.500, p=0.01). Interestingly, haplotype analysis shown that the polymorphisms rs1130864 and rs1417938 are in high linkage desequilibrium (D' 95), while the rs1205 is harbored in a different haplotype block. Conclusion. Our results suggest that polymorphisms within the CRP gene could be a severity obesity modifier in Mexican population.

## 741/W/Poster Board #399

**Co-segregation of Norrie disease and idiopathic pulmonary hypertension in a family with a microdeletion of the NDP locus at Xp11.4.** *J.F. Staropoli[1], K. Limbo[2], W. Xin[3,4], K. Sims[3,4].* 1) Department of Pathology, Massachusetts General Hospital, Boston, MA; 2) Division of Child Neurology, Huntsville Hospital, Huntsville, Alabama; 3) Neurogenetics DNA Diagnostic Laboratory, Massachusetts General Hospital, CHGR, Boston, Massachusetts; 4) Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts.

Norrie disease is an X-linked congenital retinal vasculopathy that may be accompanied by mental retardation, sensorineural deafness, and other neurological deficits. Idiopathic pulmonary arterial hypertension is a poorly understood, usually adult-onset condition characterized by progressive hypertrophy and narrowing of the small arteries of the lung in the absence of other conditions such as connective tissue diseases, portal hypertension, diet and medicines, and left-sided cardiac anomalies. Here we present a family in which Norrie disease in 3 brothers co-segregated with early-onset idiopathic pulmonary hypertension that led to demise in the first few months or years of life. Molecular analysis of the Norrie disease gene (NDP) and adjacent loci at Xp11.4 was performed by multiplex ligation-dependent probe amplification (MLPA) after failed polymerase chain reaction (PCR) amplification of the 3 exons encoding NDP. DNA from two affected brothers available for testing showed a hemizygous deletion restricted to NDP and two genes immediately centromeric to it: monoamine oxidase-A (MAO-A) and monoamine oxidase-B (MAO-B). These two closely related enzymes metabolize intraneuronal and endothelial pools of biogenic amines including serotonin, dopamine, and norepinephrine. Testing of the mother demonstrated that she is a carrier of the same deletion. Norrie disease is not known to be associated with pulmonary hypertension. Because abnormalities of biogenic amines, particularly serotonin, has been implicated in the pathophysiology of pulmonary hypertension, we propose that presumed MAO deficiency in these patients may represent a novel risk factor for pulmonary hypertension, particularly forms with very early onset.

## 742/W/Poster Board #400

**Association of the *MMP9* gene with cedar pollinosis in the Japanese children.** *Y. Suzuki[1], H. Inoue[1], Y. Mashimo[1], S. Yoneyama[2], S. Horiguchi[2], N. Shimojo[3], Y. Kohno[3], Y. Okamoto[2], A. Hata[1].* 1) Department of Public Health, Chiba University, Chiba, Japan; 2) Department of Otorhinolaryngology, Chiba University, Chiba, Japan; 3) Department of Pediatrics, Chiba University, Chiba, Japan.

**Background:** The matrix metalloprotainase 9 (*MMP9*) gene has been shown to be involved in allergic rhinitis and asthma. It was suggested that SNPs of the *MMP9* gene confer risk for childhood atopic asthma. However there was no report investigating whether the SNPs confer risk for allergic rhinitis.

**Aim:** To investigate whether the SNPs of the *MMP9* gene associate with risk for allergic rhinitis and serum IgE levels.

**Methods:** A total of 670 school children were recruited from two different regions in Japan and genotyped for 2127G/T (SNP1) and 5546G/A (R668Q) (SNP2) of the *MMP9* gene. Serum levels of total and 8 specific IgE were determined. Disease status and other clinical characteristics of the subjects were investigated by a questionnaire. Cedar pollinosis was defined as symptoms evoked in February to May with positive cedar-specific IgE in serum.

**Results:** SNP1 and SNP2 showed a significant association with cedar pollinosis (P=0.002, OR= 2.39, 95%CI=1.38-3.85; P=0.001, OR= 2.33, 95%CI=1.33-3.49, respectively). The frequency of the G-G haplotype was significantly higher in children with cedar pollinosis when compared with the controls (P=0.007). The frequency of the T-A haplotype of the patients was significantly lower than that of the controls (P=0.003). Both SNPs showed a significant association with serum level of cedar-specific IgE (SNP1: P=0.002; SNP2: P=0.001). The similar change in the allele frequencies in the pollinosis patients was observed in the two regions. Association of the two SNPs with mite-positive perennial rhinitis was less evident (SNP1: OR=1.68, 95%CI=0.76-3.04; SNP2: OR=2.20, 95%CI=0.98-3.88). The risk alleles for cedar pollinosis were G in the two SNPs, whereas those for atopic asthma were T (SNP1) and A (SNP2).

**Conclusions:** Our results suggest that the *MMP9* gene confers susceptibility to cedar pollinosis in the Japanese children. The *MMP9* gene is involved in pathogenesis of both cedar pollinosis and atopic asthma. Multifaceted function of this gene may reflect the difference in the risk alleles for cedar pollinosis and mite-sensitive asthma.

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## 743/W/Poster Board #401

**Genetic Variation in MECP2 gene is associated with Childhood-onset Systemic Lupus Erythematosus in the Mexican Mestizo population.** *R. Velázquez-Cruz[1], H. García-Ortiz[1], F. Espinosa-Rosales[2], G. Escamilla-Guerrero[3], V. Baca[4], L. Orozco[1]*. 1) Investigacion, Inst Natl de Medicina Genomica; 2) Servicio de Inmunología, Instituto Nacional de Pediatría; 3) Banco de Sangre, Instituto Nacional de Pediatría; 4) Hospital de Pediatría Centro Médico Nacional Siglo XXI, IMSS. Mexico City, Mexico.

Childhood-onset systemic lupus erythematosus (SLE) is present in about 15-17% of all patients with SLE and represents a unique subgroup of patients for genetics studies because their earlier disease onset and a more severe disease. Methyl-CpG-binding protein 2 (MECP2) gene is located on chromosome Xq28 and encodes a protein that function as a key factor in epigenetic transcriptional regulation of methylation-sensitive genes. Recently, two studies have been reported several polymorphisms in this gene associated with the risk to develop SLE in adult-onset disease. Objetive: To determine if SNPs of the MECP2 gene are associated with the occurrence of childhood-onset Systemic Lupus Erythematosus in the Mexican population. Methods: We performed a case-control study in 287 female childhood-onset SLE patients and 332 female controls recruited from Mexico City. Additionally, we analyzed a 225 family set for transmission disequilibrium test (TDT). Genotyping of seven SNPs (rs2075596, rs3027933, rs17435, rs1734787, rs1734791, rs1791792 and rs2239464) previosly reported on the MECP2 gene, was carried out by the 5'nuclease assay (Taq-Man). Hardy-Weinberg equilibrium was tested using chi-square test. The distributions of polymorphisms were compared between SLE patients and healthy controls by contingency table analysis and Pearson's test. P-values smaller than 0.05 were considered to be significant. Results: Genotype frecuencies of all markers were found in Hardy-Weinberg equilibrium in both cases and controls. When SNPs frequencies were compared between cases and controls, the SNP rs2075596 showed the strongest association in the Mexican Mestizo patients (65% vs 56%, P=0.0016, OR=1.45, 95 % CI 1.16-1.84). This finding was confirmed by analysis of the transmission disequilibrium test (rs2075596, P=0.03). The analysis with the Haploview 3.32 software identified only three haplotypes, as previously reported to other populations. The risk haplotype "ACTCAAA" was observed with a frequency of 63% in SLE patients compared to 54% in controls (OR = 1.46, p = 0.0017). Conclusions: These data show that SNPs on MECP2 are associated to childhood-onset SLE and replicate the previous findigs reported in adulthood-onset SLE.

## 744/W/Poster Board #402

**Genetic Study of the Gamma-Aminobutyric Acid type A receptor Gamma Subunit Gene (GABRG2) with Smoking, Alcohol Use Disorder, and Suicidal Behavior in Schizophrenia Patients.** *C. Zai, A. Tiwari, S. Shaikh, V. de Luca, D. Mueller, J. Kennedy.* Dept Neurogenetics, CAMH, Toronto, ON, Canada.

Schizophrenia is a severe neuropsychiatric disorder characterized by symptoms that include auditory or visual hallucinations, paranoid delusions, and disorganized behaviors. It is also associated with increased rate of smoking, alcohol abuse/dependence, and suicide, thus increasing its morbidity and mortality rate. The GABRG2 gene, mapped on chromosome 5q31.1-33.1, a schizophrenia susceptibility region, has nine exons spanning 85kb. The long isoform has an additional eight amino acids in exon 9 that contains an additional regulatory site phosphorylated by Protein Kinase C (Krishek et al, 1994), and may mediate the effects of ethanol (Cheng et al, 1997). We genotyped 19 single-nucleotide polymorphisms and tagged eight for analysis with smoking status, number of cigarettes per day, presence or absence of alcohol dependence or abuse, suicide attempt, and suicide specifier in our sample of Caucasian chronic schizophrenia patients (n= 208). We included age at assessment and sex as covariates in our analyses. Preliminary results from logistic regression showed rs647625 to be associated with alcohol dependence (p=0.004). Analysis of other phenotypes did not yield significant findings. Our results suggest that GABRG2 may be involved in alcohol dependence in schizophrenia. We will be investigating other GABA receptor genes, including GABBR1, GABRB1, in this continuing study. Further studies in other psychiatric populations are required before we can generalize this observed association.

## 745/W/Poster Board #403

**Can aromatase and 5-alpha reductase genes expressions responsible for idiopathic hirsutism.** *M. Dundar[1], A.O. Caglayan[1,4], N.A. Baysal[2], F. Tanriverdi[2], Y. Ozkul[1], K. Unluhizarci[2], M. Borlu[3], F. Kelestimur[2]*. 1) Department of Medical Genetics, Erciyes University, Kayseri, Turkey; 2) Department of Endocrinology, Erciyes University Medical School, Kayseri, Turkey; 3) Department of Dermatology, Erciyes University Medical School, Kayseri, Turkey; 4) Department of Medical Genetics, Kayseri Education and Research Hospital, Kayseri, Turkey.

Objective: Hirsutism affects between 5% and 10% of women. Hirsutism with normal ovulatory function and normal circulating androgen concentrations called idiopathic hirsutism (IH) and little information is available regarding the pathogenesis. We have previously show that patients with idiopathic hirsutism may be associated with decreased aromatse activity. Aim: We evaluated the expression of mRNA for type 2, 5-alpha-reductase isoenzyme gene (SDR5A2) and aromase gene (CYP19) in dermal papillae cells (DPCs) from the lower abdominal region of the skin and from peripheral blood of women with idiopathic hirsutism and controls. Additionally we aimed to investigate the relationship between insulin resistance and hormon profile with target gene expression among women with IH. Methods: Our study population consisted of 10 untreated idiopathic hirsute patients and 10 normal women. In all patients, follicule stimulating hormon, luteinizing hormone, estriol, free and total serum testosterone, androstenedione, 17-OH progesterone, dehydroepiandrosterone sulfate, sex hormone binding globuline, and HOMA values were measured. The expression of the SRD5A2 and CYP19 genes by Real-Time-PCR using peripheral blood mononuclear cells (PBMCs) and subumblical midline hairs plucked from patients and controls. Results: There was a negative correlation among the CYP19 gene expression in tissue r=-0.818, p=0,004 in relation to the SRD5A gene expression in blood in patients with IH according to the spearman's correlation test. Conclusion: More studies in larger numbers of patients should be performed to investigate the role of type 2, 5-alpha-reductase isoenzyme gene and aromatase gene expression in women with IH. By establishing the pathogenetic mechanisms underlying IH, new therapeutic strategies may play an important role in offering more effective therapies.

## 746/W/Poster Board #404

**A positional candidate approach to identifying genes involved in esophageal atresia/tracheoesophageal fistula.** *H. Zaveri[1], E. de Jong[2], T. Beck[1], J. Williams[1], D. Pearson[1], R. Masand[1], C. Fernandes[1], A. Johnson[1], K. Lally[3], D. Tibboel[2], B. Lee[1], A. Klein[2], D.A. Scott[1]*. 1) Baylor College of Medicine, Houston, TX; 2) Erasmus Medical Center, Rotterdam, The Netherlands; 3) University of Texas Health Science Center, Houston, TX.

The sporadic nature of many common structural birth defects makes a linkage-based approach to identifying underlying genetic factors impractical. Array comparative genome hybridization has been shown to be an effective means of mapping and identify genes that predispose to these defects in an unbiased fashion. The genetic factors responsible for most cases of esophageal atresia/tracheoesophageal fistula (EA/TEF)a life threatening birth defects that affects 1:3,500 live birthshave not been identified. EA/TEF is often associated with other structural birth defects and VACTERL (Vertebral, Anal, Cardiac, Tracheo-Esophageal Fistula, Renal, Limb) association is found in approximately 10% cases. A review of all published cases of EA/TEF revealed 11 chromosomal regions that are recurrently deleted/duplicated in EA/TEF. The pattern of these regions is distinct from the pattern identified in a similar review of congenital diaphragmatic hernia, another sporadic birth defect, making it more likely that each of these regions harbors one or more EA/TEF-related genes. To identify new chromosomal regionsand refine those previously reportedwe screened a cohort of patients with EA/TEF for cryptic deletions/duplication in affected individuals using high density genome-wide array comparative genome hybridization. This screen identified ~40 rare genomic variants some of which involve genes known to play an important role in cell migration, adhesion, differentiation, proliferation, and signaling. Using information from both human patients and mouse models with abnormal esophageal/tracheal development, we selected several candidate genes for further investigation. We are presently resequencing these genes in our patient cohort to identify deleterious alleles which may contribute to these disorders. Although identification of rare de novo sequence changes in these genes would be considered strong evidence for causality, we recognize that the majority of cases may result from a combination of inherited changes affecting important developmental pathways combined with environmental stressors. This suggests that inherited deleterious changes may also contribute to these disorders.

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## 747/W/Poster Board #405

**Deletion of the late cornified envelope genes, *LCE3C and LCE3B*, is a new susceptibility factor for rheumatoid arthritis.** *E. Docampo[1], R. Rabionet[1], E. Riveira[1], G. Escaramís[1], A. Julià[2], S. Marsal[2], J.E. Martín[3], M.A. González-Gay[4], A. Balsa[5], E. Raya[6], J. Martín[3], X. Estivill[1].* 1) Center for Genomic Regulation CRG-UPF, Barcelona, Barcelona, Spain; 2) Grup de Recerca de Reumatologia, Institut de Recerca Hospital Universitari Vall d'Hebron, Barcelona, Spain; 3) Instituto de Parasitología y Biomedicina López-Neyra, CSIC, Granada, Andalucía, Spain; 4) Rheumatology Unit, Hospital Xeral-Calde, Lugo, Galicia, Spain; 5) Rheumatology Unit, Hospital La Paz, Madrid, Spain; 6) Rheumatology Unit, Hospital Virgen de las Nieves, Granada, Andalucía, Spain.

Rheumatoid arthritis (RA) is a complex disorder involving both environmental and genetic factors, being HLA-DRB1 (shared epitope) and PTPNPN22 the most robust associations, but they only explain 30% of the heritability of this disorder. Copy number variants are being identified as risk factors for several complex disorders. Recently, the *LCE3C_LCE3B* deletion was shown to be a copy number variant associated with psoriasis. There is evidence regarding a common genetic risk for both RA and psoriasis. The purpose of this work was to assess whether this genomic variant could also be a risk factor for RA. Two sets of DNA samples (n1=189 and n2=334) from Caucasians subjects of Spanish origin suffering RA, fulfilling 1989 ACR criteria, were collected at 4 Spanish hospitals. Control samples (n1=411 and n2=567) were obtained from blood donors from matched geographical regions. All samples were directly typed for the presence of the *LCE3C_LCE3B* deletion by PCR, and association analysis was performed with the SNPassoc R package. Association of *LCE3C_LCE3B* deletion with risk to RA was observed in the first dataset (p=0.0055; OR=1.66 (2.38-1.16)), and replicated in an independent case-control set (p=0.027; OR=1.34 (1.04-1.74)). Joint analysis (adjusted by population) showed a total p=0.0012 (1.53 (1.96-1.21)). Since previous studies have reported different association results for RA depending on the antiCCP and rheumatoid factor (RF) status, association analysis for the joint dataset was carried out in the different subsets (antiCCP+/- (n=178/138), RF +/- (n=355/111)), showing an association in the antiCCP (p=0.019; OR=1.51 (2.12-1.06) and RF (p=0.010; OR= 1.38 (1.78-1.07)) positive patients, but not in the negative subsets. This work shows that the *LCE3C_LCE3B* deletion is a new genetic risk factor for seropositive RA, and it verifies a pleiotropic effect of a common genetic risk factor for autoimmune diseases, involved in both psoriasis and RA.

## 748/W/Poster Board #406

**Genome-wide analysis of copy number variation implicates *CHL1* in the etiology of age-related macular degeneration.** *K.J. Meyer[1], L.K. Davis[1], E.I. Schindler[1], J. Beck[2], D.S. Rudd[1], J.A. Grundstad[3], T.E. Scheetz[3,4], T.A. Braun[3,4], J.H. Fingert[4], J.C. Folk[4], S.R. Russell[4], T.H. Wassink[1,5], E.M. Stone[1,4,6], V.C. Sheffield[1,2,6].* 1) Interdisciplinary Genetics Program, University of Iowa, Iowa City, IA; 2) Department of Pediatrics, University of Iowa, Iowa City, IA; 3) Center for Bioinformatics and Computational Biology, University of Iowa, Iowa City, IA; 4) Ophthalmology and Visual Sciences, University of Iowa, Iowa City, IA; 5) Department of Psychiatry, University of Iowa, Iowa City, IA; 6) Howard Hughes Medical Institute, University of Iowa, Iowa City, IA.

In recent years genome wide studies of copy number variants (CNVs) have been highly successful in elucidating genetic risk factors for complex disease. Here we report the results of CNV analysis in a cohort of 400 patients with age-related macular degeneration (AMD) and 500 age matched controls. Patient DNA was hybridized to the Affymetrix GeneChip® Human Mapping 500K Array Set or the Affymetrix Genome-Wide Human SNP Array 5.0 and analyzed for CNVs using Copy Number Analyser for GeneChip® (CNAG) 2.0 and PennCNV or dChip and PennCNV, respectively. A list of high interest CNVs was generated using the following criteria: 1) the CNV must be called by two independent tests, 2) the CNV must be present in two or more patients with AMD, and 3) the CNV must not be present in the control set. All high interest CNVs were validated using qPCR. Multiple CNVs in the AMD cohort met criteria for inclusion in the high interest set including a significant finding in the gene *CHL1*. A second set of age-matched controls and a replication cohort is being screened for copy number variation in *CHL1* and other high interest candidate genes using qPCR. In addition to the screen, we used the Panther Classification System to look for a misrepresentation of any protein families, pathways, and/or ontologies in comparison to the human genome and identified an enrichment of CNVs that affect G-protein coupled receptors in the AMD patient sample but not in the control group. This study has identified many interesting CNVs, including a duplication in the gene *CHL1* that may contribute to the etiology of AMD.

## 749/W/Poster Board #407

**Identification of genomic imbalances in cases with cleft lip and palate using array CGH.** *A. Petrin[1], T. Felix[2], S. Daack-Hirsch[3], J. Murray[1].* 1) Department of Pediatrics, University of Iowa, Iowa City, IA, USA; 2) Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, RS, Brazil; 3) College of Nursing, University of Iowa, Iowa City, IA, USA.

The application of high resolution whole genome scanning arrays array CGH (Comparative Genome Hybridization) has helped to identify genomic imbalances including copy number variants (CNV) and isolated insertions/deletions. CNVs are being increasingly well defined for the human genome and contribute substantially to genetic diversity. They result from gains and losses of genomic regions that range from 1000 bp to several megabases and may encompass multiple genes. The use of the array CGH enables the simultaneous testing of multiple loci for insertion/deletion differences at high resolution and allows the identification of genomic imbalances that can be involved on the etiology of common complex diseases. In this study we report the ongoing analysis of a group of cases with craniofacial abnormalities. To date, 40 cases have been studied where 32 have cleft lip and/or palate. We are using the Affymetrix 6.0 SNP array platform that contains 1.8 million genetic markers distributed across the whole genome. We identified 10 different microdeletions/microduplications ranging from 1Mb to 44Mb that include candidate genes for clefting such as FGF12, MEF2, TP63, HHAT, CHURC1, SIX6, MTHFD1, TBX1, DLG1, PAK2, SENP5. Some deletions have involved the binding site for transcription factors (MSX1, MEF2, and BACH2) that play a role in craniofacial development and all the reported genes are involved in pathways that play an important role on craniofacial development and are strong candidates for further studies. All the imbalances were validated by a second method like FISH, sequencing or quantitative PCR. Our study confirms that analysis with array CGH is an important approach for screening and detection of submicroscopic chromosomal abnormalities that are undetectable by current cytogenetic and/or molecular methods and highlight its importance on investigation of genes involved on complex traits such as cleft lip and/or palate.

## 750/W/Poster Board #408

**Copy number variation detection in cleft lip and palate using high resolution array-CGH.** *R.J. PETRIN, A.L. PETRIN, J.C. MURRAY.* PEDIATRICS, UNIVERSITY OF IOWA, IOWA CITY, IA.

Copy number variants (CNVs) have been extensively defined for the human genome contributing to the power of microdeletion/microduplication detection. Over the past few years the use of whole genome scanning arrays has increased and, while generating enormous data, also raises new challenges in detecting, validating and interpreting the CNVs. A number of software packages are available and the combined use of them can increase the analysis confidence and decrease the chance of false positive/negative by the comparison of CNV calls and boundaries. We report the use of 3 software packages: Affymetrix Genotyping Console 3.0.2, Partek® Genomics Suite 6.4TM and Nexus CGH to analyze a group of 40 cases with cleft lip and palate (CL/P) and other craniofacial abnormalities using Affymetrix SNP Array 6.0. All software performs normalization, scaling and signal intensity extraction to enable the detection of CNVs. GTC uses an algorithm called Canary that makes CN state calls that can be exported and treated as SNP genotypes for CNV association studies while Partek uses HMM-based (Hidden Markov Model) based algorithm to detect regions of genomic gains and losses and estimate their boundaries. Nexus CGH uses the built-in Rank Segmentation algorithm for identification of CN change and SNPRank Segmentation that combines B-allele frequency values with log ratios for allelic calls. Both GTC and Partek use Birdseedv2 to perform genotyping calls, perform a multiple analysis to estimate signal intensity for each allele of each SNP and then makes genotype calls using a Gaussian mixture model. Besides the use of software packages it is also very important to use public databases such as UCSC and Database of Genomic Variants that hold a comprehensive summary of structural variation in the human genome and provides a useful catalog of control data for studies that correlate genomic variation with phenotypic data. Among the CNV regions identified by our study we defined as good candidates for causal variants the ones that are not in common region of CNV, that were detected by at least 2 software packages and were validated by a second method (sequencing or qPCR). By using this method we excluded 2 false positive results. Our study shows that the use of high resolution array CGH is a very important tool to detect CNV that can have etiological role in complex diseases such as CL/P and highlights the importance of using different analytical methods to guarantee the data accuracy.

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## 751/W/Poster Board #409

**A CNV in chromosome 5 generating a chimaeric gene is a common protective variant for stroke.** *R. Rabionet[1], J. Aigner[1], S. Villatoro[1], L. Armengol[2], M. García-Aragonés[2], E. Cuadrado-Godia[3], J. Jiménez-Conde[3], A. Ois[3], A. Rodríguez-Campello[3], J. Roquer[3], I. Fernández-Cadenas[4], J. Montaner[4], A. Carracedo[5], E. Martí[1], X. Estivill[1].* 1) Genes & Disease, Center for Genomic Regulation CRG-UPF, Barcelona, Spain; 2) Quantitative Genomics, Barcelona, Spain; 3) Unitat d'Ictus, Servei de Neurologia, Hospital del Mar, Barcelona, Spain; 4) Neurovascular Research Lab, Institut de Recerca, Hospital Vall d'Hebron, Barcelona, Spain; 5) Universidad de Santiago, Santiago de Compostela, Spain.

Stroke is a neurological disease with a high impact in the aging population, being the most important neurological cause of death and disability. Stroke is a heterogeneous disorder with both environmental and genetic risk factors. Up to this date, three genes have been shown to be related to stroke by whole genome analysis, namely, *PDE4D* and *ALOX5AP* in the Icelandic population, and a variant in chromosome 12 (*NINJ2*), recently identified in a GWAS study in four different populations. In this study we have evaluated structural variants as potential risk factors for stroke. We have screened an initial dataset of 169 haemorrhagic stroke, and 729 ischemic stroke cases, and 477 controls, and found an association of an insertion/deletion variant on chromosome 5. The breakpoints of this variant have been identified, and an allele-specific PCR assay has been designed and used for genotyping. Carriers of the intact allele are more frequently affected by stroke, while carrying one or two copies of the deleted allele confers protection against stroke. Association is significant in both the ischemic stroke (p=0.0005; OR= 0.66) and the haemorrhagic stroke subsets (p=0.002; OR=0.58). This result has been replicated in an additional dataset with 475 ischemic stroke cases (p=0.0087; OR=0.71), and further replication in a second haemorrhagic stroke dataset is underway. Surrounding SNPs have been genotyped in order to assess linkage disequilibrium in the region, and identify potential tagging SNPs. The deletion spans 55 kb and affects two genes from the same family, generating a chimaeric gene. Expression in EBV transformed lymphocytes of the two intact genes is correlated with copy number state, while the chimaeric gene is expressed at a lower mRNA level. Downstream expression analysis from expression array data from HapMap samples carriers of zero, one, or two copies of the deleted allele shows differential expression of several genes, including genes involved in blood vessel formation and angiogenesis.

## 752/W/Poster Board #410

**The Crohn's Disease IRGM Copy Number Variant is associated with IgE level in asthma.** *A.J. Rogers[1, 2, 4], J.A. Lasky-Su[1], T. Chilson[1], B. Klanderman[1], K. Darvishi[2, 4], I. Ionita-Laza[3], C. Lee[2, 4], B.A. Raby[1, 2, 4].* 1) Channing Laboratory, Boston, MA; 2) Brigham and Women's Hospital, Boston, MA; 3) Harvard School of Public Health, Boston, MA; 4) Harvard Medical School, Boston MA.

**Rationale**: A common 20kb deletion/insertion copy number variant (CNV) upstream of Immunity-related GTPase Family M (IRGM) is associated with Crohn's disease. This variant is associated with differential IRGM expression in human bronchial smooth muscle cells. We therefore investigated whether the deletion also influences asthma, another common immune-related disease characterized by hyperresponsive airway smooth muscle.

**Methods**: We genotyped 651 asthmatic children and their available parents participating in the Childhood Asthma Management Program, using a TaqMan genotyping assay that interrogates both the deletion and insertion CNV states. We tested for association of the deletion with asthma susceptibility and several intermediate phenotypes, including log-transformed IgE and airway hyper-responsiveness as measured by methacholine bronchoprovocation challenge.

**Results**: Our genotyping success rate was 99.6%. Deletion frequency was 9% among non-Hispanic whites (N=479) and 44% in black subjects (N=67), consistent with HapMap frequency data. The deletion was associated with increased total IgE and increased sensitivity to methacholine in black subjects (p=.02 and .05 in population-based testing). These findings were confirmed by family-based testing for both phenotypes (p=.02 and .03, for IgE and methacholine hyperresponsiveness, respectively). No associations were noted in the non-Hispanic white population. IRGM copy number status was not associated with asthma affection in either subgroup.

**Conclusions**: We provide evidence that a common ancestral CNV upstream of IRGM, known to be associated with Crohn's disease, is also associated with IgE level and methacholine sensitivity in black asthmatic children.

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## 753/W/Poster Board #411

**SPAG16 Gene Variants are Associated with Fasting Glucose and Insulin Sensitivity.** *W.D. Li[1], G.M. Yuan[1], S.F.A. Grant[2], H. Hakonarson[2], R.A. Price[1].* 1) Dept Psychiatry, Univ Pennsylvania Medical Ctr, Philadelphia, PA; 2) Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA.

In our previous genome scan, we found a significant linkage of total cholesterol on human chromosome region 2q34. To fine map the 2q34 region, we selected 65 nuclear families that ranked by family specific LOD scores. We genotyped 125 SNPs within a 6 Mb 2q34 candidate region in those 65 families, FBAT and QTDT analyses showed significant associations on SNPs of the SPAG16-ABCA12 gene region. We further tested SPAG16 gene polymorphisms in a case-control data set. We selected 1,125 unrelated individuals, including 583 cases (BMI>35kg/m2) and 542 controls (BMI<25kg/m2). Body weight (BMI, % fat, waist circumferences), lipids (total cholesterol, triglyceride, LDL and HDL), insulin sensitivity related phenotypes (fasting glucose, insulin, HOMA and QUICKI) were collected in cases/controls. One hundred ninety-six (196) SNPs in the SPAG 16 (Sperm Associated Antigen 16) gene region (213.8-215.0 Mb) were genotyped using ABI and Illumina platforms. Quantitative association studies were performed by PLINK, the most significant result was on SNP rs11677793 for fasting glucose (P = 0.000025). Multiple SNPs were associated with fasting glucose, insulin, HOMA, QUICKI, total cholesterol, triglyceride, %fat and waist circumferences. Haplotype analyses showed multiple significant associations among SPAG12 haplotypes and insulin sensitivity related phenotypes, including a three-SNP haplotype (rs17364234-rs11677793-rs7580474) yielded P = 0.000027 for fasting glucose. Associations among SPAG16 SNPs and glucose and insulin sensitivity phenotypes suggested that the SPAG16 gene could have more functions other than spermatogenesis.

## 754/W/Poster Board #412

**TFAP2A is associated with nonsyndromic Cleft Lip and Palate.** *S. Suzuki[1,6,7], ML. Marazita[2,3], ME. Cooper[3], B. Smith[1], A. Mansilla[1], N. Natsume[6], T. Niimi[6], H. Furukawa[6], K. Minami[6], E. Dragan[1], T. Erkhembaatar[8], JC. Murray[1,4,5].* 1) University of Iowa, Department of Pediatrics, S Grand Avenue, 2182 ML, Iowa City, IA 52242, USA; 2) Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15219, USA; 3) Center for Craniofacial and Dental Genetics, and Department of Oral Biology, and Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA 15219, USA; 4) College of Nursing, The University of Iowa, Iowa City, IA 52242, USA; 5) University of Iowa, Departments of Pediatric Dentistry, Epidemiology and Biology, Iowa City, IA 52242; 6) Maxillofacial Congenital Anomalies, School of Dentistry, Aichi-Gakuin University, 2-11 Suemori-Dori, Chikusa-Ku, Nagoya 464-8651, Japan; 7) Faculty of Psychological and Physical Science, Aichi-Gakuin University, 12 Araike, Iwasaki-cho, Nisshin 470-0195 Japan; 8) Maternal and Children's Health Research Center Hospital, Ulaanbaatar 210624, Mongolia.

Nonsyndromic cleft lip with or without cleft palate (CL/P), a common birth defect, is a complex trait, arising from the influence of genetic variants interacting with environmental factors. A recent study of variants in the IRF6 gene (Rahimov, 2008) showed variants in an AP-2alpha binding site in an IRF6 enhancer is associated with cleft lip. We have also previously reported the linkage of NS CL/P to the 6p23 region that contains TFAP2A gene. TFAP2A is highly expressed in the craniofacial region and knock out mice have multiple facial anomalies. We genotyped 7 SNPs in and near TFAP2A for 175 case-parents trios with nonsyndromic CL/P from Mongolia, large collection of families from Philippines and the US using the TaqMan assay. FBAT was used for transmission disequilibrim test. A statistically significant association between CL/P and TFAP2A (p < 0.001) was shown for rs 1675414. These results support a direct role for variation of TFAP2A in orofacial clefting and suggests that examination of additional members of this pathway and deeper investigation of SNPs at the TFAP2A locus may disclose etiologic variants.

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## 755/W/Poster Board #413

**Expression of a unique neuroendocrine splicing form of TCF7L2 in human and mouse tissues.** *J.L. Hall[1], C. Welch[1], C. Weaver[1], D. Schuweiler[1], N. Adhikari[1], L. Peihl[1], L. Prokunina-Olsson[2].* 1) Medicine, Lillehei Heart Institute, University of Minnesota, Minneapolis, MN; 2) Laboratory of Translational Genomics/DCEG, NCI/NIH, Bethesda, MD.

Background: Genetic variants within the TCF7L2 gene have been identified as the strongest common risk factors for development of type 2 diabetes. Methods and Results: We used expression assays targeting the majority of the TCF7L2 splicing forms observed in human tissues. A unique splicing form of TCF7L2 detected by assay "ex13-13b" was identified in human pancreatic islets, pancreas, gut, and the brain. Further analysis of expression using quantitative reverse-transcriptase PCR (qRT-PCR) showed that compared to the level in the gut/pancreatic islets, expression of this assay was 300 fold higher in the thalamus/hypothalamus area of the brain. Expression of TCF7L2 assay "ex13-13b" was found in 10 human tissues of 25 tissues examined and it was coexpressed with neurohormone Cocaine and Amphetamine Regulated Transcript (CART) in these tissues (n=10, r2=0.88, p= 0.00078) suggesting a neuroendocrine pattern of TCF7L2 expression. Expression of assay "ex13-13b" of TCF7L2 and CART was also detected in murine brain, pancreatic islets, pancreas and gut. Murine expression of "ex13-13b" in the gut was decreased in adults compared to newborn mice by 5 fold (p=0.0021) and 3.6 fold (p=1x10-6) in duodenum and colon, respectfully. Similarly, expression of CART was also decreased 2.7 fold in duodenum (p=0.001) and 2.3 fold in colon (p=0.067) of adult animals compared to newborns. In contrast, expression of 11 other TCF7L2 expression assays was significantly increased up to 10 fold in adult animals compared to newborns. Conclusions: A unique splicing form of TCF7L2 detected by assay "ex13-13b" was identified in human and mouse pancreatic islets, pancreas, gut and brain. Expression of this splicing form correlated with expression of neurohormone CART in human and mouse tissues. Studies are underway in murine tissues to further define the relationship between CART and TCF7L2.

## 756/W/Poster Board #414

**A Non-synonymous *HNF4A* Variant is Associated with Glycemia During Pregnancy and Offspring Head Circumference in Populations of European Ancestry in the HAPO Study.** *M.G. Hayes[1], H. Lee[1], R.M. Freathy[1,2], M. Urbanek[1], L.P. Lowe[1], C. Ackerman[1], N.J. Cox[3], D.B. Dunger[4], A.R. Dyer[1], A.T. Hattersley[2], B.E. Metzger[1], W.L. Lowe[1], The HAPO Study Cooperative Research Group.* 1) Northwestern Univ, Chicago, IL; 2) Univ Exeter, UK; 3) Univ Chicago, Chicago, IL; 4) Univ Cambridge, UK.

The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study is a multicenter, international study, which examined the association of maternal glucose levels with fetal growth and outcome in 25,000 pregnant women from multiple ethnic groups to demonstrate a continuous relationship between maternal glucose measures and birth size throughout the range of glucose concentrations. We hypothesize genetic factors contribute to these phenotypes, and examined 1536 fetal and maternal SNPs in 79 candidate loci previously implicated in insulin secretion or sensitivity to determine associations with maternal glycemia and insulin secretion (fasting glucose and C-peptide and 1-hr glucose from the OGTT) at ~28 weeks gestation and/or offspring size at birth (birth weight, length, head circumference, and sum of skinfolds) for HAPO mothers of European (Belfast and Manchester, UK, and Brisbane and Newcastle, Australia; N=3828) and Asian (Bangkok, Thailand; N=1813) ancestry and their offspring. Associations were assessed through linear regressions with the single trait/outcome under an additive genetic model adjusting for known confounders. Among our strongest signals was rs1800961G>A, which encodes a Thr>Ile amino acid change in exon 4 of *HNF4A*, recently identified in a GWAS meta-analysis as a variant associated with decreased HDL levels. In the HAPO study, this SNP was strongly associated with increased fetal head circumference (0.5cm [95%CI: 0.3-0.7] per maternal minor allele; $P=1.2x10^{-7}$) in those of European descent. The maternal minor allele was also weakly associated with 1-hour glucose (4.3mg/dL [95%CI: 0.5-7.9]; P=0.03), birth length (0.7cm [95%CI: 0.2-1.1]; P=0.003), birth weight (52.6g [95%CI: -8.0-113.3]; P=0.09), and sum of skinfolds (0.3cm [95%CI: -0.1-0.6]; P=0.13). This same minor allele in the fetal genome was weakly associated with cord C-peptide (0.1ug/dL [95%CI: 0.01-0.22]; P=0.03), and head circumference (0.2cm [95%CI: -0.1-0.4]; P= 0.08). The same trends were observed among the Thai, although not significantly probably due to a reduction in power from the low risk allele frequency (<2%). These results suggest that *HNF4A*, a locus previously implicated in insulin secretion and sensitivity and dyslipidemia, has a significant impact on birth size, particularly head circumference at birth.

## 757/W/Poster Board #415

**Leveraging Gene Ontology Information to Identify Gene-gene Interactions Underlying Type 2 Diabetes-related Traits.** *R.M. Watanabe[1,2], D.V. Conti[1], N. Cremer[3], H. Allayee[1,4], J.M. Lawreance[5], A.H. Xiang[1], T.A. Buchanan[6,2].* 1) Dept. of Preventive Medicine, USC Keck School of Medicine, Los Angeles, CA; 2) Dept. of Physiology & Biophysics, USC Keck School of Medicine, Los Angeles, CA; 3) Dept. of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany; 4) Institute for Genetic Medicine, USC Keck School of Medicine, Los Angeles, CA; 5) Research and Evaluation, Kaiser Permanente of Southern California, Pasadena, CA; 6) Dept of Medicine, Division of Endocrinology, USC Keck School of Medicine, Los Angeles, CA.

Complex traits are thought to be partly determined by gene-gene (GxG) interactions. Genome-wide association (GWA) studies have identified loci underlying complex diseases like type 2 diabetes (T2D), but provide no context for how these loci may be interacting. Recent development of gene ontology (GO) databases provides a rich resource regarding known biology of specific loci. We sought to leverage this information to identify potential GxG interactions underlying T2D-related traits. We downloaded GO terms for a list of 81 candidate loci underlying T2D risk or variability in T2D-related traits identified by GWA. We generated pair-wise correlations among loci based upon the congruence of GO terms. The analysis dataset comes from the BetaGene study, consisting of Mexican American families of a proband with or without previous gestational diabetes. DNA and fasting blood (glucose, insulin, and lipids) were collected on all subjects (n=1,650), and the proband generation (n=976) were additionally phenotyped by oral and intravenous glucose tolerance test to obtain measures of glucose effectiveness, insulin sensitivity, insulin secretion, and beta-cell function. The GO-based correlations can be utilized in two ways. First, pairs of loci with the strongest correlations can be directly tested for interactions with T2D-related traits. Second, the correlation matrix can be used to guide a Bayes model selection approach to detect interacting loci. The GO correlation analysis revealed two loci, *PPARG* and *HNF4A*, that were correlated (r=0.35), which we previously showed interacted to alter insulin sensitivity, thus acting as a positive control. Preliminary pair-wise interaction analysis of loci using traditional linear modeling methods revealed the interaction between *KCNJ11* and *G6PC2* to be associated with glucose effectiveness (interaction p=0.018) and *FTO* and *IGF2BP2* to be associated with 2-hour insulin levels (interaction p=0.028). Both of these interactions tend to cluster together in a hierarchical clustering of GO terms. These initial analyses suggest the biologic information in GO can be used to inform GxG analyses. Additional analyses based on linear modeling and on a Bayes model selection approach are on-going.

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## 758/W/Poster Board #416

**Biological and statistical interaction between type 1 and type 2 diabetes genes.** *J.J. Zhang[1,2,3], K. Pearce[1,2,3], S.H. Li[4], M. Serafin[1,2,3], J. Basken[1,2,3], R. Hoffmann[4], P. Simpson[4], C. Sarti[5], S. Ghosh[1,2,3].* 1) 1Max McGee National Research Center for Juvenile Diabetes, Department of Pediatrics, Medical College of Wisconsin, Milwaukee, Wisconsin; 2) Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, Wisconsin; 3) Children's Research Institute, Children's Hospital of Wisconsin, Milwaukee, Wisconsin; 4) Quantitative Health Sciences, Department of Pediatrics and Population Health, Medical College of Wisconsin, Milwaukee, Wisconsin; 5) National Public Health Institute, Helsinki Finland.

Type I diabetes (T1D) is a polygenic, T-cell-dependent autoimmune childhood disease, characterized by the selective destruction of the $\beta$ cells of the islets of Langerhans. In contrast, Type 2 diabetes (T2D) is the end point of a progressive insulin secretory defect on a background of insulin resistance and typically occurs later in life. Both diseases are of multifactorial etiology, in which genetic predisposition plays a critical role and underpins each complex trait. In T1D, even though about 40 loci have been discovered, the class II HLA region (HLA-DRB, HLA-DQA and HLA-DQB) and insulin genes (INS) have the largest effect. Insulin is thought to be a major type 1 diabetes autoantigen presented to the immune system by HLA molecules. For T2D, there are at least 20 gene loci with the largest effect due to TCF7L2. To date, no genetic overlap exists between T1D and T2D loci. INS functions at the level of the thymus in T1D to reduce the impact of clonal deletion of insulin-specific, autoreactive T cells. In contrast, some T2D susceptibility genes could reduce insulin production at the level of the beta cell and potentially decrease insulin antigen load, giving rise to delayed autoimmune, T-cell dependent diabetes that would manifest later and only in young adults. To test this genetic hypothesis, we studied a sample of 748 T1D cases (age of onset 15-39) and 1549 control subjects from Finland, which has the highest incidence of T1D in the world. Our results showed that INS (p<0.0001) and HLA (p<0.0001) are strongly associated with disease, but none of the T2D genes tested were (TCF7L2, PPAR$\gamma$, SLC30A8, KCNJ11, MTNR1B). In contrast, using logistic regression, there was an interaction seen between INS and TCF7L2 under a risk model which assumed INS was recessive whereas TCF7L2 was acting in a dominant manner (p=0.02). Results were verified using a non-parametric, classification tree approach. The finding will be confirmed in a second, independent sample. In summary, our data suggest that the delay in T1D in a young adult sample could be due to an interaction between a T1D and a T2D gene. The biological significance of the finding is that diminished insulin secretion due to a T2D gene could reduce the extent of autoimmunity by decreasing antigen load and hence delaying onset of disease. Thus, even though there is no overlap between T1D and T2D genes, they may interact to produce delayed diabetes in young adult life.

## 759/W/Poster Board #417

**Evidence for two susceptibility loci for epilepsy on chromosomes 9q33-q34 and 20p12-q13 in a Finnish family.** *A. Polvi[1], A. Siren[1,2], H. Rantala[3], A.-E. Lehesjoki[1].* 1) Dept Medical Genetics, Biomedicum Helsinki, Helsinki, Finland; 2) Department of Pediatrics, University Hospital of Tampere, Finland; 3) Department of Pediatrics, Oulu University Hospital, Finland.

Epilepsy is a brain disorder characterized by recurrent epileptic seizures and divided to many syndromes with distinct seizure types. In many epilepsy families, the patients do not share the same seizure phenotype. Idiopathic epilepsies occur in the absence of detectable brain abnormalities and are assumed to be mainly polygenic or multifactorial in origin. Mutations in single genes, mostly encoding ion-channels, have been reported in rare families with apparently dominant inheritance. In the majority of idiopathic epilepsy patients the underlying genetic defect remain unknown. A multiplex family including four patients with febrile seizures, 11 with focal seizures and two with generalized and febrile seizures was recognised and clinically characterized. Blood samples from 59 family members were collected after obtaining informed consent. A genome-wide microsatellite marker scan (GWS) was performed. Multipoint location scores were calculated with the SIMWALK2 2.91 program using the nonparametric linkage (NPL) analysis option. Regions with a multipoint location score > 1.5 were fine mapped and further analysed by NPL. Functional candidate genes were sequenced from genomic DNA. In the GWS two loci (9q33-34 and 20p12-q13) gave evidence for linkage by showing multipoint location score values of 2.11 and 2.85. After fine mapping, maximum multipoint NPL scores of 2.16 (p= 0.0069) at 9q33-34 and 2.314 (p=0.0049) at 20q11-12 were detected. The susceptibility regions on 9q33-34 and 20p12-q13 were 20.5 and 21.0 Mb long and contained 284 and 290 genes, respectively. Sequencing of one functional candidate gene, STXBP1, did not reveal a pathogenic alteration. Clinical variability within the family suggests the contribution of several genes. Our data support the existence of at least two epilepsy susceptibility loci in the family.We are in the process of recruiting more family members with epilepsy to increase the statistical power with the ultimate aim of narrowing the regions of interest to allow identification of the underlying genes.

## 760/W/Poster Board #418

**Genome-wide study of families with absolute pitch reveals linkage to 8q24.21 and locus heterogeneity.** *E. Theusch, A. Basu, J. Gitschier.* Univ California, San Francisco, San Francisco, CA.

Absolute pitch (AP) is the rare ability to instantaneously recognize and label tones with their musical note names, without using a reference pitch for comparison. The etiology of absolute pitch is complex, with prior studies implicating both genetic and environmental factors in its genesis, yet the molecular basis for absolute pitch remains unknown. To locate regions of the human genome that may harbor AP-predisposing genetic variants, we performed a genome-wide linkage study on 73 multiplex absolute pitch families by genotyping them with 6,090 single nucleotide polymorphism (SNP) markers. Non-parametric multipoint linkage analyses were conducted, and the strongest evidence for linkage was observed on chromosome 8q24.21 in the subset of 45 families with European ancestry (exponential logarithm of the odds (LOD) score = 3.464, empirical genome-wide P = 0.03). Other regions with suggestive LOD scores included chromosomes 7q22.3, 8q21.11, and 9p21.3. Of these four regions, only the 7q22.3 linkage peak was also evident when 19 families with East Asian ancestry were analyzed separately. Though only one of these regions has yet reached statistical significance individually, we detected a larger number of independent linkage peaks than expected by chance overall, indicating that absolute pitch is genetically heterogeneous.

## 761/W/Poster Board #419

**ADIPOQ gene variation is associated with plasma adiponectin and HDL cholesterol in Yup'ik Eskimos: The CANHR study.** *B. Boyer[1], R. Pasker[1], M. Irvin[2], H. Weiner[2], D. O'Brien[1], M. Jeannet[1], K. Stanhope[3], P. Havel[3], G. Mohatt[1], H. Tiwari[2].* 1) Institute of Arctic Biology, University of Alaska-Fairbanks, Fairbanks, AK; 2) Department of Biostatistics, University of Alabama at Birmingham, Birmingham, AL; 3) Department of Nutrition, University of California Davis, Davis, CA.

Background: Adiponectin is an insulin-sensitizing hormone, and several linkage and association studies implicate the adiponectin locus in the development of obesity and diabetes. Dietary factors including marine derived polyunsaturated fatty acids (EPA/DHA) may decrease the risk of obesity and diabetes by modifying the association of genetic variants with obesity phenotypes. Objectives: The objective of this study was to determine if EPA/DHA modify the association between obesity phenotypes and four candidate genes in the pathway leading to adiponectin synthesis (PPARG, PPARA, PPARGC1A and ADIPOQ). Design: 981 non-pregnant Yup'ik Eskimos $\geq$14 years old were selected from the CANHR study - a cross sectional study investigating genetic, nutritional, and behavioral risk factors for obesity. A total of thirteen single nucleotide polymorphisms (SNPs) were genotyped. Mixed models were used to assess whether EPA/DHA influence the relationship between candidate genes and obesity phenotypes, using a biomarker of red blood cell EPA/DHA that was recently validated for Yup'ik Eskimos (the nitrogen isotope ratio, $\delta$15N). Results: Two SNPs in ADIPOQ, rs1784686 and SNP 2 (identified through re-sequencing), were associated with plasma adiponectin levels, while rs17300539 was associated with HDL cholesterol. EPA/DHA also modifies the association between PPARG rs3856806 (H447H) with plasma adiponectin. Conclusions: Polymorphisms in ADIPOQ are associated with plasma adiponectin and HDL levels. Associations between rs3856808 in PPARG and plasma adiponectin levels is modified by EPA/DHA intake.

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## 762/W/Poster Board #420

**Polymorphisms in *IFNG* show sex-specific associations with IFN-γ response and asthma in early childhood.** *D.A. Loisel[1], Z. Tan[1], G. Du[1], C. Tisler[2], K.A. Roberg[2], R.E. Gangnon[3], M.D. Evans[3], J.E. Gern[2], R.F. Lemanske[2,4], C. Ober[1,5].* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Pediatrics, University of Wisconsin-Madison, Madison, WI; 3) Department of Biostatistics and Medical Informatics, University of Wisconsin-Madison, Madison, WI; 4) Department of Medicine, University of Wisconsin-Madison, Madison, WI; 5) Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL.

Sex differences in the prevalence and severity of asthma begin in childhood and are maintained through adulthood. The activation and expression of the Th1 cytokine, IFN-γ, may play a central role in the development of sex-specific differences in immune response and asthma risk. Previously, we reported sex differences in the IFN-γ response to PHA in peripheral blood mononuclear cells from children in the Childhood Origins of ASThma (COAST) birth cohort study; boys had significantly higher IFN-γ responses at 1 and 3 years of age (Uekert et al. *JACI* 2006; 118:1375). Here we examined sex-specific effects of 5 polymorphisms in the *IFNG* gene on two phenotypes, IFN-γ response (at ages 3 and 5 years) and asthma (assessed at age 8), in 134 boys and 100 girls. The median IFN-γ response was significantly higher in boys compared to girls at age 5 years ($P$=0.0004), similar to ages 1 and 3 years. Only the -1616 SNP (C/T), located in the *IFNG* promoter region, showed main effects on IFN-γ response ($P$=0.024 for age 3; $P$=0.047 for age 5). The effect of the -1616 SNP on IFN-γ response differed by sexthe association was significant in girls ($P$=0.0038 and 0.045, respectively) but not in boys ($P$=0.47 and 0.78, respectively). Moreover, the -1616 SNP was associated with asthma in girls ($P$=0.032) but not in boys ($P$=0.38) or in the full cohort ($P$=0.36) (genotype-by-sex interaction $P$=0.059). Girls homozygous for the minor allele at -1616 had the lowest IFN-γ responses and highest prevalence of asthma. Two other polymorphisms, a 3'UTR SNP (A/G) and a microsatellite in intron 1, were also associated with asthma in girls ($P$=0.048 and 0.00013, respectively) but not in boys or in the full cohort. These two loci, which were in strong LD with each other but not with the -1616 SNP, showed significant genotype-by-sex interactions on asthma status ($P$=0.0043 and 0.0010, respectively). Overall, we found that IFN-γ response was sexually dimorphic throughout early childhood (ages 1 through 5) in COAST children. We identified an *IFNG* promoter SNP that was associated with sex-specific variation in IFN-γ response, and an intronic and/or 3'UTR SNP showing significant interaction effects with sex on risk for developing asthma by age 8. The observed sex-specific associations and genotype-by-sex interactions on asthma suggest that variation in the IFN-γ pathway may contribute to asthma risk during childhood in a sex-specific manner. Supported by NIH grant P01 HL070831.

## 763/W/Poster Board #421

**The cellular role of DYX1C1 - estrogen receptor pathways and cell migration.** *K. Tammimies[1], I. Tapia-Páez[1], S. Massinen[2], S. Le Guyader[1], I. Fransson[1], H. Matsson[1], M-E. Hokkanen[3], E. Castrén[3], S. Strömblad[1], J-A. Gustafsson[1,4], E. Treuter[1], J. Kere[1,2,3].* 1) Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden; 2) Department of Medical Genetics, University of Helsinki, and Folkhälsan Institute of Genetics, Helsinki, Finland; 3) Neuroscience Centre, University of Helsinki, Finland; 4) Center for Nuclear Receptors and Cell Signaling, Department of Biology and Biochemistry, University of Houston, Houston, Texas, USA.

The focus of research in molecular biology of dyslexia has shifted towards explaining the function of genes associated with increased risk for dyslexia. So far six dyslexia candidate genes have been identified: DYX1C1, ROBO1, DCDC2, KIAA0319 MRPL19 and C2ORF3. Four of these genes, including DYX1C1, have been implicated in neuronal migration and/or axon guidance suggesting a role for specific neurobiological pathway/s in dyslexia. Although some common biological mechanisms have been identified, relatively little is known about the interactions and interplay of the known dyslexia candidate genes. We have focused on identifying the cellular role and regulation of DYX1C1. The DYX1C1 gene encodes a 420-aa protein with a p23 domain in the N-terminus and three tetratricopeptide repeat domains (TPRs) towards the C-terminus. We have previously shown that allelic differences in the promoter or 5' UTR of DYX1C1 affect transcription factor binding and also regulation of the gene. Recently, we have also identified that DYX1C1 interacts with the estrogen receptors alpha and beta (encoded by the ESR1 and ESR2 genes), and overexpression of DYX1C1 downregulates their protein levels and activity on estrogen responsive elements (EREs). We are now studying the consequences of the DYX1C1-ER protein interactions by testing rapid signaling pathways such as mitogenin-activated protein kinases ERK1/2. To investigate other effects of overexpression of DYX1C1 in a cell line, in particular cell migration and its molecular mechanisms, we are using transient transfections of the neuroblastoma SH-SY5Y cell line, live cell imaging and transcriptome analyzes. Our preliminary results using single cell tracking show that overexpression of DYX1C1 increases random migration of SH-SY5Y cells. The total and final distance of the DYX1C1 cells (n= 73) were approximately 50% more than GFP transfected control cells (n= 79). Taken together, the dyslexia candidate gene DYX1C1 can modulate estrogen signaling by direct effects on estrogen receptors, and current work aims at elucidating the molecular mechanisms of DYX1C1 effects on cell migration.

## 764/W/Poster Board #422

**Comprehensive evaluation of FTO genetic variation shows associations with obesity in two populations of African origin.** *A. Adeyemo[1], G. Chen[1], J. Zhou[1], D. Shriner[1], Y. Chen[2], A. Doumatey[1], H. Huang[1], C. Rotimi[1].* 1) CRGGH/IDRB, National Human Genome Research Institute, Bethesda, MD; 2) National Human Genome Center, Howard University, Washington, DC.

The FTO gene has been one of the most consistently replicated loci for obesity. However, data from populations of African origin are scanty. We have undertaken a comprehensive evaluation of variation in the FTO gene by genotyping a set of 262 tag SNPs across the entire span of the gene in two populations of African origin. The study samples comprised 968 African Americans (59% female, mean age 49 years, mean BMI 30.8 kg/m$^2$) and 517 West Africans (58% female, mean age 54 years, mean BMI 25.5 kg/m$^2$). Both samples showed weaker linkage disequilibrium (LD) patterns across the gene when compared with other continental (e.g. European and Asian) populations. Multiple SNPs in intron 8 and the downstream region showed significant associations with body mass index, waist circumference and percent fat mass in both study samples. Two reported associations with intron 1 SNPs (rs1121980 and rs7204609) were replicated at a p level of 0.05 among the West Africans. In summary, the FTO gene shows significantly differences in allele frequency and LD patterns between populations of African ancestry compared to other continental populations. Despite these differences, we observed significant associations with obesity in African Americans and West Africans. This comprehensive approach of characterizing genetic variation within candidate gene improved our study design and chances of identifying novel variants and successful replication of reported variants.

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## 765/W/Poster Board #423

**Positional cloning of a quantitative trait locus in KCNQ5 gene influencing lung function.** *E. Bouzigon[1], H. Aschard[1], H. Tharrault[1], M-H. Dizier[1], R. Matran[2], M. Lathrop[3], F. Kauffmann[4], F. Demenais[1], EGEA cooperative group.* 1) U946, INSERM, Paris, France; 2) Univ Lille Nord de France, CHU, Lille, France; 3) Commissariat à l'Energie Atomique, Institut de Génomique, Centre National de Génotypage, Evry, France; 4) U780, INSERM, Villejuif, France.

A previous genome-wide linkage scan conducted in French EGEA families (Epidemiological study on the Genetics and Environment of Asthma) identified linkage of 6q14 to $FEV_1$ (forced expiratory volume in one second expressed as a percentage of predicted values based on age, height and gender), with a higher signal detected in adult offspring (16 years of age or older). We investigated further this region by genotyping a panel of 399 SNPs (spanning 30Mb) in 203 EGEA families (337 adult offspring). To reduce the problem of multiple testing, we used a two-stage approach based on two multi-marker methods: the Local Score and multi-marker FBAT (FBAT-M). This strategy allows detection of sets of adjacent markers showing aggregation of high statistical scores while taking into account linkage disequilibrium between markers. We identified five marker sets associated with $FEV_1$ (P-values ranging from 0.005 to 0.0008). The most significant marker set was located within *KCNQ5* gene and included 11 SNPs of which four were strongly associated with $FEV_1$ (P-values ranging from 0.0007 to 0.0001). We checked that these polymorphisms explained the original linkage signal. These associations were replicated in two independent samples: 1) 476 parents of EGEA families (P-values ranging from 0.05 to 0.01 for five SNPs) and 2) a set of 267 adult French controls (P-values ranging from 0.03 to 0.002 for four SNPs). The combination of P-values from our three samples, using Fisher's combined probability test, enhanced the evidence for association of $FEV_1$ with three SNPs (combined P-values ranging from $2x10^{-4}$ to $2x10^{-5}$). KCNQ5 belongs to a family of genes which encode voltage-gated potassium channels which are key regulators of cellular activity. KCNQ5, which is expressed in bronchial epithelium and is a determinant of airway-surface liquid, is therefore a strong candidate for lung function regulation. Funded by: French Ministry of Higher Education and Research, Afsset-APR-SE-2004, ANR 06-CEBS, GABRIEL.

## 766/W/Poster Board #424

**Missense mutations in the MEFV gene are associated with fibromyalgia syndrome and correlate with elevated IL-1β plasma levels.** *J. Feng[1,2], Z. Zhang#[2], W. Li[1], X. Shen[1], W. Song[1], C. Yang[1], F. Chang[2], J. Longmate[3], C. Marek[4], R.Paul St.Amand[4], T. Krontiris[5], J. Shively*[2], S. Sommer*[1].* 1) Department of Molecular Genetics; 2) Department of Immunology; 3) Department of Biostatistics, City of Hope Natl Medical Ctr, Duarte, CA; 4) The Fibromyalgia Treatment Center, Los Angeles, CA; 5) Department of Molecular Medicine, City of Hope Natl Medical Ctr, Duarte, CA.

Fibromyalgia syndrome (FMS), a common, chronic, widespread musculoskeletal pain disorder with a preponderance of 85% in females has both a genetic and environmental factor basis. Patients and their parents have high plasma levels of the chemokines MCP-1 and eotaxin, providing evidence for both a genetic and an immunological origin for the syndrome (Zhang et al., 2008, Exp. Biol. Med. 233: 1171-1180). Since FMS symptoms are similar to, but milder than, Familial Mediterranean Fever (FMF), a disease linked to compound heterozygous mutations in the FMF gene, MEFV, we have analyzed the exons in this gene in 100 probands with FMS and their parents. A total of 2.63 megabases of genomic sequence of the MEFV gene were scanned by direct sequencing. One common and 10 rare missense mutations were identified, all of which are found in the FMF database. The rare missense mutations had a significantly elevated frequency of transmission to affecteds (p= 0.017). Our data provide evidence for an association between rare missense variants of the MEFV gene and a subset of 15% of FMS patients. When this subset was reanalyzed for plasma cytokine and chemokine levels, a correlation (p= 0.019) was found with high levels of IL-1β, a cytokine associated with fever and muscle aches. Since mis-regulation of IL-1β expression has been predicted for patients with mutations in the MEFV gene, we conclude that heterozygotes for rare variants of this gene may be predisposed to FMS, possibly a milder form of FMF, while FMF requires compound heterozygotes. # J.Feng and Z.Zhang contributed equally to the results.*Corresponding authors.

## 767/W/Poster Board #425

**The functional variant (gly307ser) in CD226 is associated with susceptibility to multiple autoimmune diseases.** *A. Maiti[1], X. Kim-Howard[1], P. Viswanathan[1], L. Guillén[2], X. Qian[3], A. Rojas-Villarraga[4], C. Sun[1], C. Cañas[5], G. Tobón[5], K. Matsuda[6], N. Shen[3], A. Cherñavsky[2], J. Anaya[4], S. Nath[1].* 1) Genetic epidemiology Unit, Oklahoma medical Research Foundation, Oklahoma, OK; 2) Immunogenetic Laboratory, Universidad de Buenos Aires, Buenos Aires, Argentina; 3) JiaoTong University School of Medicine, China; 4) Center for Autoimmune Diseases Research (CREA), Universidad del Rosario, Bogota, Colombia; 5) Rheumatology Unit, Fundación Valle del Lili, Cali, Colombia; 6) Human Genome Center, the University of Tokyo, Tokyo, Japan.

Objectives: Recently, A non-synonymous (Gly307Ser) variant, rs763361, in the CD226 gene is shown to be associated with multiple autoimmune diseases (ADs) in European populations. However, shared autoimmunity with CD226 has not been evaluated in non-European populations. The aim of this study is to assess the association of this SNP with ADs in Hispanic and Asian populations Methods: To replicate its association with other ADs, we evaluated case-control association between rs763361 and celiac disease (CED) samples from Argentina; systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Type-1 diabetes (T1D) and primary Sjögren's syndrome (pSS) from Colombia; and SLE samples from China and Japan. We genotyped rs763361 for evaluation of association and 3 other unlinked SNPs for assessing population substructure. Genetic associations were tested with the chi-square test. Results: All our populations are homogenous as both cases and controls are collected from endogenous local populations and association results are not affected by population substructures. In population substructure analysis, the subdivision coefficient among the Argentinean and Colombian cases and controls, was not significantly different from zero (Fst = 0.01). We show that rs763361 is significantly associated with CED (p=0.0009, OR=1.60), RA (p=0.047, OR=1.25), and SLE (p=0.01, OR= 1.19) from China. We also observed a trend of possible association with SLE (p=0.0899, OR=1.24) and pSS (p=0.09, OR=1.33) in Colombians. A meta-analysis across all ADs reinforces the association with this coding variant (Pmeta=2.9x10-6, OR (95% CI)=1.23 (1.13-1.33). Conclusions: Our results demonstrate that this coding variant rs763361in cd226 gene is associated with multiple ADs in non-European populations.

## 768/W/Poster Board #426

**A *RET* founder mutation in Chinese Hirschsprung's patients.** *B.K. Cornes[1], C.S. Tang[2], T.Y.Y. Leon[1], M.T. So[1], P.C. Sham[2], P.K.H. Tam[1], M.M. Garcia-Barcelo[1].* 1) Paediatric Surgery Division, Department of Surgery, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong SAR, China; 2) Department of Psychiatry, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China.

Hirschsprung's disease (HSCR) is a congenital disorder associated with the lack of intramural ganglion cells in the myenteric and sub-mucosal plexuses along varying segments of the gastrointestinal tract. Rearranged during transfection (*RET*) gene is implicated in HSCR and is the major gene of this gastrointestinal disease. To date, over 200 low frequency recurrent RET coding sequence (CDS) mutations have been identified in HSCR patients. However, a highly recurrent $RET^{R114H}$ mutation has been identified in 10% of the Chinese HSCR patients which has never been found in Caucasians patients or controls nor in 400 Chinese controls. The high frequency of $RET^{R114H}$ in our population together with the fact that it is not a *"de novo"* mutation in the context of the most HSCR-associated *RET*-haplotype, suggests that it may be a founder HSCR mutation in the Chinese population. Initial investigation involved applying a Bayesian method to 21 single nucleotide polymorphisms (SNPs; across a ~62kb region of *RET*) genotyped in 421 Chinese HSCR patients (of which 24 individuals had the mutation) to predict the approximate age of $RET^{R114H}$. The approach allowed the inference of the mutation age based on the observed linkage disequilibrium (LD) at multiple SNPs which predicted the mutation to be between 12 and 13 generations old. Including SNPs from a recently obtained genome-wide 500K dataset for 181 of the above mentioned patients (which now only included 14 patients who had the $RET^{R114H}$ mutation), we applied haplotype estimation methods to determine whether there were any segments shared between patients with the $RET^{R114H}$ compared to those without the mutation and controls. Data consisted a total of 92 SNPs spanning a 510kb region over the RET gene. Analysis yielded a 256kb (76 SNP) shared segment over the *RET* gene (and downstream) in only those patients with the mutation with no similar segments found among other patients or controls. This suggests that $RET^{R114H}$ is a possible founder effect for Hirschsprung's disease in the Chinese population.

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## 769/W/Poster Board #427

**Association analysis of exonic polymorphisms in TNIP1 and TNFAIP3 with psoriasis.** *R.P. Nair[1], P.E. Stuart[1], T. Tejasvi[1], H.W. Lim[2], J.J. Voorhees[1], J.T. Elder[1].* 1) Dept Dermatology, Univ Michigan, Ann Arbor, MI; 2) Henry Ford Hospital, Detroit, MI.

Psoriasis is a complex disease with genetic, environmental and imunological etiology. Our recent genome-wide association study (GWAS, Nair et al, Nature Genetics, 2009) identified seven replicated associations, including three previously known ones, and implicated IL-23 and NF-κB mediated pathways in psoriasis pathogenesis. Here we examine the association of exonic polymorphims in two of the genes identified by the GWAS - TNIP1 and TNFAIP3 - that act downstream of TNF in NF-κB regulation. The best associated GWAS markers near these genes are rs17728338, 18 kb upstream of TNIP1 on chromosome 5q33.1, and rs610604 in intron 3 of TNFAIP3 on chromosome 6q23.3. By data mining of public databases we identified 13 potential non-synonymous variations in TNIP1 and 6 in TNFAIP3. Testing of these variations in a subset of DNA samples showed that only 4 SNPs in TNIP1 and one in TNFAIP3 are present in our samples with a minor allele frequency > 1%. These SNPs and the associated GWAS SNPs were genotyped in our entire sample of 2,302 psoriasis cases and 2,132 controls of European ancestry. The GWAS marker for TNIP1 (rs17728338) showed allelic association with genome-wide significance ($p = 1.1 \times 10^{-9}$, OR=1.67) and the TNFAIP3 marker (rs610604) approached genome-wide significance ($p = 4.5 \times 10^{-6}$, OR = 1.23). None of the exonic SNPs tested showed significant association. When multi-marker haplotypes for each gene were tested for association, only haplotypes containing the risk allele for the GWAS markers showed significance (TNIP1: $p = 2.2 \times 10^{-6}$, OR = 1.6; TNFAIP3: $p = 5.2 \times 10^{-7}$, OR = 1.26). These results show that the known non-synonymous SNPs in TNIP1 and TNFAIP3 are unlikely to be causal in psoriasis. A detailed characterization of these loci by re-sequencing and testing of the full spectrum of allelic variations in our large case-control collection is in progress.

## 770/W/Poster Board #428

**Evaluation of Pooled Sequence Variation in SERPINE2 Using the Illumina Genome Analyzer.** *M.H. Cho, G.M. Oliveria, A.L. Donohue, B.J. Klanderman, B.A. Raby, E.K. Silverman.* Channing Lab, Brigham & Women's Hosp, Boston, MA.

Background: Genetic association and expression studies have implicated SERPINE2 in the pathogenesis of COPD; however the SNPs responsible for these genetic associations are not known. We sought to sequence the exons and conserved regions of this gene in a pilot project using subject and amplicon pools in the Illumina Genome Analyzer. Methods: We created 7 equimolar pools of genomic DNA each from 20 subjects from 80 cases from the National Emphysema Treatment Trial and 60 controls from the Normative Aging Study. We performed standard PCR amplification, and ligated amplicons to create large concatamers for Illumina sequencing. Sanger sequencing was performed on 20 individuals from one of the pools as a reference. Sequences were aligned with maq, and variants were called and compared based on allele frequency. Results: In our reference pool, 29/30 Sanger sequenced SNPs were identified; with the missing SNP present at a frequency of < 1% in the pool. Of the total discovered SERPINE2 variants, there were no significant differences in allele frequency between the pooled cases and controls. Conclusions: Pooling DNA is a feasible option for comprehensive variant discovery in case-control studies, though variability in pooling must be considered. In this limited sample, we found no obvious conserved region or exonic variation accounting for the role of SERPINE2 in COPD.

## 771/W/Poster Board #429

**Development of methods for resequencing of genes using indexed DNA samples and the SureSelect target enrichment system.** *E.M. Kenny, A.S. Gates, L.E. Cochrane, M. Gill, A.P. Corvin, D.W. Morris.* Neuropsychiatric Genetics Research Group, Dept. of Psychiatry, Trinity College Dublin, Dublin, Ireland.

Next-generation sequencing technology has allowed sequencing of whole genomes to be carried out in standard molecular genetics laboratories. However, an important application of this technology is sequencing of specific genomic regions, for example disease genes in patient samples. In order to sequence parts of the genome of interest, a number of methods have been developed including long range (LR) PCR and microarray capture. Both methods have been shown to work but are costly in terms of time for LR PCR and DNA for microarray capture (20μg input DNA required). Agilent Technologies have developed the SureSelect Target Enrichment System. This method allows targeting of 3.3Mb of the genome by using cRNA baits. Indexing methods have been developed for next generation sequencing that allow multiplexing of samples in one sequencing library. We have combined the SureSelect Target Enrichment System with an indexing protocol to develop a cost-efficient method for targeting smaller regions of the genome (e.g. 200Kb) in multiple DNA samples. We evaluated this method by comparing sequence data produced to data generated by a LR PCR enrichment of the same target sequence. For the LR PCR method, primers were designed to generate amplicons that tile across the genomic region of interest. All amplicons for each individual sample were pooled such that an equimolar amount of PCR-amplified DNA was added for each fragment and the total amount of DNA in the pool was 1-5ug. For the SureSelect method, unique baits were designed to capture genomic fragments in the target region and 3μg of genomic DNA was used as input for the Illumina genomic DNA library prep method. For both enrichment methods, the Illumina genomic DNA library prep method was modified to allow indexing of more than one sample in each sequencing library according Craig et al (2008). We will present data on the performance of the SureSelect method in comparison to the LR PCR method in HapMap test samples and present a protocol that will be extremely useful in the rapid sequencing of target genomic regions.

## 772/W/Poster Board #430

**Replication of genome-wide association signal on 8q associated with cleft lip and palate.** *T. Goto[1,5], M.A. Mansilla[1], A.L. Petrin[1], S.A. Bullard[2], J. Dierdorff[2], A.L. Lidral[2], K. Christensen[3], K. Durda[1], M. Marazita[4], M. Cooper[4], K. Arakaki[5], H. Sunakawa[5], L.M. Moreno-Uribe[2], J.C. Murray[1].* 1) Pediatrics, University of Iowa, Iowa City, IA; 2) University of Iowa, Department of Orthodontics, Iowa City, Iowa 52242; 3) University of Southern Denmark, Odense, Denmark; 4) University of Pittsburgh, Department of Oral Biology, Pittsburgh, Pennsylvania 15219; 5) University of the Ryukyus, Department of Medicine,Okinawa, Japan.

Cleft lip and/or palate is a common birth defect of complex etiology with modest success in finding genetic contributors. The first genome-wide association published on cleft lip and palate (Birnbaum et al.,2009) has reported a highly significant association with markers in a gene poor region at 8q24 in a German population. We have replicated the associated SNP (rs987525) with the lowest p value in multiple world-wide populations (US (684 cases), Denmark (571 cases), Colombia (744 cases), Brazil (284 cases), Philippines(1470 cases)) using case/parent trios and the TDT test. In European derived populations, there is very strong association (excepting Brazil) with the rs987525 (US p=0.0000024; Denmark 0.0000015; Colombia 0.0029; Brazil 0.101995). We also stratified the results by cleft phenotype and found stronger association with cleft lip with cleft palate than for cleft lip alone. However, no association with this marker was noted in a large Filipino sample set (1470 case-parent trios, 5850 persons); nor is there an association identified with any of an additional 20 SNPs tested in Filipinos in the 640 kb critical region identified in the published work. We have also sequenced 180 Filipino cleft cases and controls, as well as 90 US cases and controls in two highly conserved regions in the 8q24 critical region but failed to identify any obvious etiologic mutations. These results confirm the previous report of genome-wide association in cleft lip and palate for this novel locus but fail to identify any specific causal variations. This suggests that the effect may be European-population specific; at present, there is no evidence for association in South Asian populations.

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## 773/W/Poster Board #431

**Genomewide association scan of neurophysiological endophenotypes reveals transregulational effects on *SLC2A3* in dyslexic children.** *P. Hoffmann[1,2], D. Roeske[3], K.U. Ludwig[1,2], N. Neuhoff[4], J. el Sharkawy[4], J. Becker[1,2], J. Schumacher[1], B. Müller-Myhsok[3], G. Schulte-Körne[4], M.M. Nöthen[1,2].* 1) Intitute of Human Genetics, University of Bonn, Bonn, Germany; 2) Department of Genomics, Life and Brain Center, University of Bonn, Bonn, Germany; 3) Max-Planck Institute of Psychiatry, Munich, Germany; 4) Department of Child and Adolescent Psychiatry and Psychotherapy, University Hospital Munich, Munich, Germany.

In the present study we tested for association with mismatch-negativity (MMN), an endophenotype measuring the children's reaction to a change in speech stimuli, in a whole-genome association data set of 200 German dyslexic children. We selected 19 SNPs showing significant p-values and being located in chromosomal regions with previous linkage evidence for independent replication in a sample of 186 German dyslexics. We found that SNP rs4234898 on chr 4 was significantly associated with the subphenotype MMN3 in both samples. The association result in the combined sample withstood genome-wide correction for multiple testing (p=6.09e-08 carrier T model). rs4234898 lies within a gene desert, with the nearest genes being more than 150kb away. None of the neighboring genes shows evidence for brain-specific expression or function or is known to be related to neurophysiological phenotypes. Analysis using publicly available GWA expression data yielded evidence for possible trans-regulation effects of rs4234898 on *SLC2A3* (also called *GLUT3*), the predominant facilitative glucose transporter in neurons. We could confirm the trans-regulation of *SCL2A3* by own experiments in EBV-transformed cell lines from 17 dyslexic children, with 10 individuals carrying at least one T-allele showing lower expression of SLC2A3 (p=0.028 one-sided Wilcoxon-test). When performing further analysis we found that rs11100040 on chr 4 was also significantly associated the differential expression of SLC2A3 in the public expression databases. Interestingly, rs4234898 and rs11100040 form a haplotype which is associated with MMN3 (p=6.71e-08 in combined sample). In conclusion, we performed the first genome-wide association study focussing on a neurophysiological endophenotype of dyslexia. Our results suggest a genomic region on chromosome as a factor contributing to variable expression of this trait, possibly conferring its functional effect through trans-regulation of *SLC2A3*.

## 774/W/Poster Board #432

**Variants in *GIPR* are associated with insulin response to oral glucose load: results from the first genome-wide meta-analysis of 2h glucose levels.** *V. Lyssenko[1], R. Saxena[2,3], M-F. Hivert[3], C. Langenberg[4], T. Tanaka[5], J.S. Pankow[6], P. Vollenweider[7], N. Boutia-Naji[8], L. Groop[1], R.M. Watanabe[9].* 1) Clinical Sciences, Lund University, Malmö, Sweden; 2) Massachusetts General Hospital, Boston, MA, USA; 3) Broad Institute of Harvard and MIT, Cambridge, MA, USA; 4) MRC Epidemiology Unit, Cambridge, UK; 5) Medstar Research Institute, Baltimore, MD , USA; 6) University of Minnesota, Minneapolis, MN, USA; 7) Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; 8) Pasteur Institute, Lille, France; 9) University of Southern California, Los Angeles, CA, USA.

Genome-wide association studies (GWAS) have largely contributed to our understanding of the pathogenesis of T2D in the past. The collaborative effort called MAGIC (Meta-Analyses of Glucose and Insulin-related traits Consortium) has now extended the search for genetic variants influencing diabetes-related traits to postprandial insulin and glucose levels during OGTT. A meta-analyses of 2h glucose levels using 24 studies totaling up to 40,442 non-diabetic individuals identified a SNP in the *GIPR* (glucose-dependent insulinotropic peptide receptor) gene achieving genome-wide significance level (meta $P=2.05x10^{-9}$). GIP (Glucose-dependent Insulinotrophic Peptide or Gastric Inhibitory Polypeptide) is released after food ingestion from intestinal K cells to stimulate insulin secretion in a glucose-dependent manner (insulinotropic effect). We investigated whether the SNP in *GIPR* would influence insulin response to oral glucose in more than 22,000 non-diabetic subjects from 17 studies, insulin response to intravenous glucose in 562 subjects as well as incretin effect in 351 Botnia Study participants. The 2hr glucose raising allele of the *GIPR* variant was associated with lower 2h insulin levels (adjusted for 2h glucose $P=4.40x10^{-15}$; BMI-adjusted $P=8.18x10^{-11}$, n= 28,965), lower insulinogenic index ($P=2.44x10^{-20}$; BMI-adjusted $P=1.00x10^{-17}$, n=22,529) and lower ratio insulin-to-glucose area under the curve (AUC) during the OGTT ($P=3.39x10^{-20}$; BMI-adjusted $P=9.50x10^{-17}$, n=22,251). There was no association of *GIPR* with acute insulin response during the intravenous glucose tolerance test (IVGTT; P= 0.28, n=562), consistent with its presumed role in the incretin effect. Finally, *GIPR* did influence the incretin effect estimated as 100% x (AUCins OGTT-AUCins IVGTT)/AUCins OGTT (P=0.003). In conclusion, genetic variation in GIPR is associated with elevated postprandial glucose levels and impaired insulin secretion during OGTT, consistent with a receptor defect in mediating the incretin effect of GIP.

## 775/W/Poster Board #433

**Investigating the complex associations of MHC with RA susceptibility.** *G. Orozco, W. Thomson, A. Barton, S. Eyre, X. Ke.* arc Epidemiology Unit. University of Manchester, Manchester, United Kingdom.

The major rheumatoid arthritis (RA) susceptibility locus is *HLA-DRB1*, which lies within the major histocompatibility complex (MHC) at 6p21. There is evidence of additional RA loci in this region, although interpretation of these associations is made difficult by the presence of strong linkage disequilibrium. Our aim was to investigate the complex association pattern of the MHC region with RA susceptibility to identify effects independent of *HLA-DRB1*. A total of 1,804 RA cases and 1,474 1958 Birth Cohort controls were included in the study. High resolution HLA-DRB1 typing was performed using Dynal RELITM SSO kits. Subjects were genotyped for 1,546 single nucleotide polymorphisms (SNPs) using Affymetrix GeneChip 500K, as part of the Wellcome Trust Case Control Consortium (WTCCC) study. Statistical analysis was carried out using PLINK and Stata. Shared epitope (SE) alleles were strongly associated with RA (OR 4.25 95% CI 3.52-5.12 and OR 17.44 95% CI 13.18-23.07) for the carriage of one and two copies of the SE, respectively). In particular, the *0101, *0404 and *0408 alleles showed the strongest association with increased RA risk. Therefore, our RA cohort showed a similar pattern of HLA associations to that previously shown for other European populations. We found 745 SNPs significantly associated with RA (*P* trend<0.05). Of these, 398 SNPs showed association at a significance level of P trend<0.001. Many of the strongest associations were observed in the vicinity of the *HLA-DRB1* locus. To avoid confounding by RA-associated DRB1 alleles, we analyzed MHC SNPs using a data set with pairwise matching of cases and controls on DRB1 genotypes. A total of 594 case-control pairs with identical DRB1 genotypes were identified and used in a subsequent meta-analysis and conditional logistic regression. After this adjustment, 104 SNPs remained significantly associated with RA (*P*<0.05), suggesting that additional effects can be found in the HLA region. Of these, 3 loci showed the strongest associations with RA (*P*<0.003): Locus 1: close to *ZNF391* in extended class I Locus 2: close to *OR2H1* in extended class I Locus 3: close to *HLA-DPB1/DPB2* class II In conclusion, this analysis has revealed that multiple independent effects contribute to RA susceptibility in the MHC region. Validation is now being carried out using independent external datasets.

## 776/W/Poster Board #434

**Characterization of Candidate Loci for Autism Spectrum Disorders.** *M. Rossi[1, 2], K. Rehnström[1, 2, 3], E. Kempas[1, 2], L. von Wendt[4], L. Peltonen[1, 2, 3, 5, 6].* 1) Institute for Molecular Medicine Finland, Helsinki, Finland; 2) National Institute for Health and Welfare, Public Health Genomics Unit, Helsinki, Finland; 3) University of Helsinki, Department of Medical Genetics, Helsinki, Finland; 4) Hospital for Children and Adolescents, Unit of Child Neurology, Helsinki, Finland; 5) Program in Medical and Population Genetics, The Broad Institute, MIT and Harvard University, Cambridge, MA, USA; 6) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK.

Autism and Asperger syndrome (AS) are the most common of autism spectrum disorders (ASDs). These disorders are characterized by impairments in social interaction and communication, and restricted and repetitive interests and activities. Despite several cytogenetic, candidate gene, linkage and association studies conducted in ASDs only a few susceptibility loci have been successfully replicated. Here we have genotyped the best SNPs (n=65) from a genome-wide association (GWA) study performed in autism and AS families originating from an internal isolate of Finland, in our nationwide autism and AS samples as well as in the Autism Genetic Research Exchange (AGRE) families. The Finnish autism and AS families were genotyped with iPLEX and the analysis were performed using PLINK, Pseudomarker and HBAT software. Publicly available AGRE data, genotype using Illumina 550 BeadChip was obtained from the AGRE website. This replication study revealed several susceptibility loci, the most promising being ALS2CR11. This locus on chromosome 2 showed evidence for contributing to ASDs in the AGRE sample, both in single SNP association (rs10931963 and rs12464623, both p<0.018) and haplotype analysis (haplotype consisting of rs4675170, rs10931963, rs12464623, rs17384203, p= 0.02). The Finnish autism and AS families instead showed the most significant associations to several loci (2q32.3, 4p16.2, 6q14.3 and PSD3 at 8p22), many of which have previously been reported in association and linkage studies. Of these the most significant was PSD3 at 8p22 (Pseudomarker analysis, rs7009615 p<0.013). PSD3 codes for Pleckstrin and Sec7 domain containing protein 3 that acts as a guanine nucleotide exchange factor for ADP-ribosylation factor (ARF6) and is located in cell junction, synapse, postsynaptic cell membrane and postsynaptic density. These results agree well with previous reports of synaptic dysfunction in ASDs. The most significant results of this replication do not overlap with previously reported ASD linkage studies and the association is less significant in this replication study compared to the original autism GWA study. The results point towards several genomic regions in different chromosomes, and the predisposing variants differ between populations. Differences in the results between autism and AS families suggest that the predisposing variants are phenotype specific.

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## 777/W/Poster Board #435

**Germline variation at the micro-seminoprotein beta (*MSMB*) locus results in physiological differences in serum and seminal levels of MSP/PSP94 and other major prostate secretory products.** *X. Xu[1], C. Valtonen-André[2], C. Sävblom[2], C. Halldén[2], A. Giwercman[3], H.G. Lilja[2,4], R.J. Klein[1].* 1) Program in Cancer Biology & Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA; 2) Department of Laboratory Medicine, Division of Clinical Chemistry, Lund University, Malmö University Hospital, 205 02 Malmö, Sweden; 3) Reproductive Medicine Centre, Lund University, Malmö University Hospital, 205 02 Malmö, Sweden; 4) Department of Clinical Laboratories, Surgery (Urology) and Medicine (GU-Oncology), Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA.

**Objectives**: Prostate cancer is a very common and highly heritable disease. Recent association screens have identified a single nucleotide polymorphism (SNP) consistently associated with prostate cancer risk, rs10993994. rs10993994 is located 57bp upstream of the transcription start site of a gene, micro-seminoprotein beta (*MSMB*). *MSMB* codes for one of the three major secretory products of the prostate, MSP/PSP94. Though its physiological functions are unknown, MSP has been implicated in prostate cancer tumorigenesis. Meanwhile, rs10993994 genotype has also been associated with blood levels of prostate specific antigen (PSA), a major prostate-specific secretory product and widely used diagnostic biomarker for prostate cancer. Thus, we hypothesize that germline variation at the *MSMB* locus results in physiologically detectable differences in MSP and PSA levels.

**Methods**: Serum and seminal fluid collected from 304 healthy young Swedish men (aged 18-21) were assayed for levels of MSP, PSA and other prostate biomarkers. Matching genomic DNA was extracted from blood and genotyped for several SNPs around the *MSMB* locus. SNPs passing quality control filters were analyzed for association with blood or semen biomarker levels. Empirical p-values were obtained through label-swapping permutation testing, with independence of each SNP's effect determined through conditional haplotyping.

**Results**: In healthy young Swedish men, rs10993994 was significantly associated with serum and semen levels of MSP and PSA. Additional copies of the risk allele for prostate cancer resulted in lower levels of MSP in semen and serum, but higher levels of PSA in semen and serum. Additionally, novel SNPs further upstream of the *MSMB* locus existing as part of a single large linkage disequilibrium block were - independent of rs10993994 - significantly associated with MSP levels in semen and serum. Finally, a novel SNP was found to be associated with semen levels of PSA, but not MSP itself.

**Conclusions**: rs10993994 genotype is significantly associated with physiologically detectable variation in MSP and PSA levels in seminal fluid and serum of healthy young men. Additionally, novel SNPs near the *MSMB* locus are independently associated with MSP and PSA levels.

## 778/W/Poster Board #436

**HLA-DRB1 and Pediatric Multiple Sclerosis.** *A.D. Sadovnick[1,2], G. Disanto[3,4], A. Handel[3,4], K.M. Morrison[3,4], G. Ebers[3,4], B. Banwell[5], D. Arnold[6], A. Bar-Or[6], Canadian Pediatric Demyelinating Disease Study Group.* 1) Department of Medical Genetics, University of British Columbia, Vancouver, Canada; 2) Faculty of Medicine, Division of Neurology, University of British Columbia, Vancouver, Canada; 3) Wellcome Trust Centre for Human Genetics, Oxford UK; 4) Department of Clinical Neurology, Oxford University, Oxford UK; 5) Department of Pediatrics, Division of Neurology, The Hospital for Sick Children, University of Toronto, Toronto, Ontario; 6) Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Quebec.

Susceptibility to adult onset multiple sclerosis (AOMS) in Northern European populations has been fine mapped to the extended HLA class II haplotype. HLA-DRB1*15 allele frequency is 0.33 in AOMS and 0.13 in controls. We studied 21 children with pediatric onset MS (POMS) [16/21 Caucasian], 148 children with Acquired Demyelinating Syndrome (ADS) [117 Caucasian] and 196 Caucasian controls. HLA-DRB1 alleles were typed by an allele-specific PCR amplification method. Allele frequencies were calculated using the Unphased-2.404-w32 program. Chi-square distribution or Fisher's exact test was used to assess significance. To avoid any population stratification, the analysis was first restricted to Caucasian individuals. Allelic frequencies of HLA-DRB1 alleles for Caucasians were 0.31 (POMS), 0.19 (ADS) and 0.14 (controls). The frequency of HLA-DRB1*15 in POMS was significantly higher than healthy controls (p=0.011) with an odds ratio of 2.73. When non-Caucasian POMS and ADS were included, the HLA-DRB1*15 allele frequencies were 0.36 and 0.21 respectively (OR=2.05, $\chi 2=4.31$, p=0.03). The association of HLA-DRB1*15 with POMS very closely resembles that seen in AOMS, thus unifying these phenotypes. Genotyping data distinguished ADS from adult clinically isolated syndrome (CIS). Thus studies of the interactions of this allele with known environmental factors associated to MS (e.g. vitamin D; Epstein Barr virus) may be key in elucidating the causal cascade of POMS.

## 779/W/Poster Board #437

**In search for biological basis of music listening: Association analysis of five candidate genes in Finnish families with musical aptitude.** *L. Ukkola[1], P. Onkamo[2], P. Raijas[3], K. Karma[4], I. Järvelä[1,5].* 1) Dep Med Gen, Univ Helsinki, Helsinki, Finland. M.Sc; 2) Dep Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland, Ass. Prof; 3) Sibelius Academy, DocMus Department, Helsinki, Finland, D.Mus., M.Soc.Sc; 4) Sibelius Academy, Department of Music Education, Helsinki, Finland, Adj. Prof; 5) Laboratory of Molecular Genetics, Helsinki University Central Hospital, Helsinki, Finland, Adj. Prof.

Listening to music is able to evoke a wide spectrum of emotions that are unique in its scale. Listening to music has been seen as rewarding, and pleasurable human experience. The reasons why music is commonly practiced in all societies as well as why music has been preserved in human evolution remain largely unknown. During human history, music has been used as therapy for neuropsychiatric disorders, to ameliorate anxiety and pain. Still, the biological mechanisms underlying the therapeutic effects of music are largely unknown. In order to understand the neurobiological pathways of listening to music we analyzed polymorphisms of the arginine vasopressin receptor 1A (AVPR1A), serotonin transporter (SLC6A4), catecol-O-methyltranferase (COMT), dopamine receptor D2 (DRD2) and tyrosine hydroxylase 1 (TPH1), genes associated with cognitive functions and emotions in 19 Finnish multigenerational families (n=343 members) with professional musicians and/or active amateurs. All family members were tested for musical aptitude using the auditory structuring ability test (Karma Music test; KMT) and Carl Seashores tests for pitch (SP) and for time (ST) and their education in music, active and passive listening to music and creative functions in music (composing, improvising and/or arranging) were surveyed using a web-based questionnaire. The motivations to listen to music were asked using four questions, whether music was listened due to: 1) relaxation, 2) different emotional states, 3) learning and/or 4) concentration. We found that active listening to music before 20 years of age was associated with higher music test scores. Active music listening at the ages of 12-20 years had a significant effect on KMT (Kruskall Wallis Test (K-W) p=0.005) and suggestive effect on SP (K-W p=0.025). Here, average 7+ weekly hours of active music listening resulted in the highest music test scores. We found statistically significant correlation between active listening to music during childhood and creative activity in music (p<0.0001;K-W). Our preliminary results suggest an association with the AVPR1A gene (marker RS1;allele 3) and active listening to music between age 12-20 years (corrected p=0.007) using FBAT. Listening to music due to different emotional states was associated with 5-HTTLPR+VNTR haplotype of the SLC6A4 gene (S+LG/12 repeats) (p=0.006;corrected 0.005) and the AVPR1A gene AVR+RS1 haplotype 5 4 (corrected p=0.005).

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## 780/W/Poster Board #438

**Comprehensive analysis of genes in the type I interferon pathway and their association with SLE.** *J.K. Sandling[1], S. Garnier[1], S. Sigurdsson[1,2], C. Wang[1], M-L. Eloranta[3], G. Nordmark[3], I. Gunnarsson[4], E. Svenungsson[4], G. Sturfelt[5], A. Jönsen[5], A. Bengtsson[5], L. Truedsson[6], C. Eriksson[7], S. Rantapää-Dahlqvist[8], R.R. Graham[9], T.W. Behrens[9], G. Alm[10], L. Rönnblom[3], A-C. Syvänen[1].* 1) Molecular Medicine, Department of Medical Sciences, Uppsala University, Uppsala, Sweden; 2) Current address: Functional Genomics, Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden; 3) Section of Rheumatology, Department of Medical Sciences, Uppsala University, Uppsala, Sweden; 4) Rheumatology Unit, Department of Medicine, Karolinska Institutet/Karolinska University Hospital, Stockholm, Sweden; 5) Department of Clinical Sciences, Section of Rheumatology, Lund University, Lund; 6) Institute of Laboratory Medicine Section of MIG, Lund University, Lund, Sweden; 7) Department of Clinical Immunology, Umeå University Hospital, Umeå, Sweden; 8) Department of Rheumatology, Umeå University Hospital, Umeå, Sweden; 9) Genentech Inc., South San Francisco, CA, USA; 10) Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Systemic Lupus Erythematosus (SLE) is a complex systemic autoimmune disorder in which the interferon system plays a crucial role. Increased levels of interferon-alpha (IFN-$\alpha$) in patient serum and increased expression of type I IFN-inducible genes in cells playing key roles in the development of SLE have been observed. The serum IFN-$\alpha$ levels correlate both with disease activity and severity, and individuals treated with IFN-$\alpha$ have been known to develop autoimmune diseases such as SLE as a treatment side-effect. We have previously found that three genes in the type I interferon pathway, the interferon regulatory factor 5 *(IRF5)*, the tyrosine kinase 2 *(TYK2)* and the signal transducer and activator of transcription 4 *(STAT4)*, are strongly associated with risk for SLE. Here we investigate the association of 87 genes involved in this pathway with the aim of identifying additional risk genes for SLE. In the first stage 1536 single nucleotide polymorphisms (SNPs) from these 87 genes were genotyped in 485 Swedish SLE patients and 563 controls. Ten genes showing p-values < 0.01 in the initial screen were followed up in an additional 376 Swedish patients and 679 controls. In order to find further support for the importance of the most promising genes we also present data from a genome-wide association study on SLE performed in American Caucasian cases and controls. Analysis of gene expression in lymphoblastoid cell lines reveals that the SLE associated alleles are expressed in an allele-specific manner, indicating the mechanism by which these polymorphisms could influence SLE susceptibility. Our results further highlight the importance of type I IFN system genes in SLE.

## 781/W/Poster Board #439

**Association of HTR3a with Autism.** *O.J. Veatch[1], N. Schnetz-Boutaud[1], A.M. Wotawa[1], B.M. Anderson[1], A.M. Halstead[1], K. Brown[1], H.H. Wright[3], R.K. Abramson[3], M.L. Cuccaro[2], J.R. Gilbert[2], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Center for Human Genetics and Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN; 2) Miami Institute for Human Genomics, University of Miami, Miller School of Medicine, Miami, FL; 3) W.S. Hall Psychiatric Institute, University of South Carolina, Columbia, SC.

Autism is the most severe form of a broad spectrum of complex neurodevelopmental disorders known as Autistic Spectrum Disorders (ASDs). It has a high degree of heritability but due to the complexity and heterogeneity of the disorder, genome-wide screens have been unsuccessful in identifying a single risk locus for autism. However, multiple studies have proposed association of the disease to variations in genes in the dopaminergic and serotonergic pathways. Individuals with autism are also known to have increased levels of serotonin in their blood when compared with unaffected individuals. In this study, a total of 75 single nucleotide polymorphisms (SNPs) were genotyped in 13 genes related to the serotonin pathway in an independent dataset of 400 Caucasian American families (1656 individuals). We used the Pedigree Disequilibrium Test (PDT) to assess family-based allelic association and the Genotype-PDT (GenoPDT) to look for genotypic association with autism risk. The most significant result from the GenoPDT, with a p-value of 0.0002, was found for SNP rs1150220 located in the HTR3a gene. Multifactorial Dimensionality Reduction (MDR) analysis was also used to detect multilocus interactions. We tested for all two-way and three-way interactions in a total of 400 cases and 400 “pseudo” controls, using the non-transmitted alleles of the parents. A significant three-way interaction (p-value < 0.01) was found among rs1150222 in the HTR3a gene, rs4947644 in the dopa decarboxylase gene, and rs37020 in the SLC6A3 dopamine transporter gene showing interaction across the dopaminergic and serotonergic pathways. SNP rs1150222 is not only located in the HTR3a gene, it is also in linkage disequilibrium (LD) with the most significantly associated SNP determined via GenoPDT, rs1150220 (D’ = 0.93), indicating strong non-random association between the two loci. These results suggest association of risk for autism with variations in the HTR3a gene which is a member of the serotonin receptor family and is unique from other family members because it contains an integral agonist ion channel and is not a G protein-coupled receptor. Also, these data point to the possibility of interaction across two neurotransmitter pathways.

## 782/W/Poster Board #440

**Different IRF5 Alleles Associate with Distinct Serologic Subphenotypes in Lupus, Revealing Ancestral Differences in Association.** *T.B. Niewold[1], J.A. Kelly[2], S.N. Kariuki[1], K. Thomas[2], D. Walker[2], S. Kamp[2], J.M. Frost[3], A.K. Wong[4], J.T. Merrill[2,5], M.E. Alarcón-Riquelme[2,6], M. Tikly[3], P.ROFILE Study Group[7], D.L. Kamen[8], G.S. Gilkeson[8], T.J. Vyse[9], J.A. James[2,5], P.M. Gaffney[2], K.L. Moser[2], M.K. Crow[10], J.B. Harley[2,5,11].* 1) Section Rheumatology, University of Chicago, Chicago, IL; 2) Oklahoma Medical Research Foundation, Oklahoma City, OK; 3) University of the Witwatersrand, Johannesburg, South Africa; 4) MRC Unit for Longlife, Health & Ageing, London, UK; 5) Department of Medicine, University of Oklahoma, Oklahoma City, OK; 6) University of Uppsala, Uppsala, Sweden; 7) Northwestern University Feinberg School of Medicine, University of Texas-Houston Health Science Center, Johns Hopkins University, and University of Alabama at Birmingham; 8) Medical University of South Carolina, Charleston, SC; 9) Imperial College, London, UK; 10) Mary Kirkland Center for Lupus Research, Hospital for Special Surgery, New York, NY; 11) US Department of Veterans Affairs Medical Center, Oklahoma City, OK.

Interferon-alpha (IFN-$\alpha$), interferon regulatory factor 5 (IRF5) haplotypes, and various specific autoantibodies all are strongly associated with systemic lupus erythematosus (SLE). Logistic regression of phenotypic variation in European-derived subjects resolved the IRF5 SLE-risk haplotype into allelic associations with particular autoantibodies [rs2004640T with anti-dsDNA (OR=2.41, p=3.3x10-14), rs10488631C with anti-Ro (OR=2.46, p=2.8x10-13), and a minor haplotype with anti-La (OR=1.96, p=0.035)]. Anti-nRNP and anti-Sm autoantibodies were not associated with IRF5 haplotypes. Meanwhile, in African-Americans SLE risk is confined to the same IRF5 haplotype, which in African-Americans is of European genomic origin and similarly associated with anti-dsDNA and anti-Ro. IFN-$\alpha$ levels are elevated in SLE cases with IRF5 risk haplotypes only in the setting of anti-dsDNA and anti-Ro autoantibodies. This suggests that specific SLE-associated autoantibodies cooperate with IRF5 variants to dysregulate IFN-$\alpha$ production and consequently increase risk of SLE. These results demonstrate the power of phenotypic variability when informed by genomic origin to disentangle complex genetic relationships.

## 783/W/Poster Board #441

**Association study of citokines genes with juvenile rheumatoid arthritis in Mexican population.** *J. Ramirez[1,4], V. Baca[2], S. Jimenez[1], G. Escamilla[3], L. Orozco[1,4].* 1) Instituto Nacional de Medicina Genómica, Ssa. Mexico City, Mexico; 2) Centro Médico Nacional Siglo XXI, IMSS, Mexico City, México; 3) Instituto Nacional de Pediatría, Ssa. Mexico City, Mexico; 4) Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México. Mexico City, México.

Introduction. Juvenile rheumatoid arthritis (JRA) represents the most common chronic autoimmune disease in childhood. Like in others autoimmune disorders, there are strong evidences suggesting that multiple genes are involved in the JRA etiopathogenesis. Genes encoding to central mediators of inflammation, such as IL2, IL8, IL17 and IL18 cytokines are important candidate genes for susceptibility to immune diseases. Actually, single nucleotide polymorphisms (SNPs) located in these cytokines have been identified as genetic risk factors to rheumatoid artrhitis susceptibility, systemic lupus erhytematosus, multiple esclerosis, etc. Objective. The present study was undertaken in order to investigate whether there is association between polymorphisms in IL2, IL8, IL17 and IL18 and JRA in Mexican patients. Samples and methods. This study included 170 pediatric Mexican patients that fulfilled the American College of Rheumatology (ACR) criteria for JRA, and 370 healthy controls without antecedents of JRA or other autoimmune diseases; all them were recruited from Mexico City. The genomic DNA was extracted from peripheral blood leukocytes according to standard protocols (Genomic DNA Kit; Qiagen). This study included five SNPs (IL2: -91A/C, IL8: -251T/A, IL17: -692T/C and IL18-667A/C and -618G/T). Genotyping analysis was performed using TaqMan assay. The association test and Hardy-Weinberg equilibrium (H-WE) were determined using EPI-DAT and FINETTI softwares, respectively. Results. Both cases and controls were in H-WE. Non differences between cases and controls were observed when we compared the genotype and allele distributions (-91A/C IL2: genotype A/A vs A/C, OR 1.16, p=0.45, C allele OR 1.13, p=0.38. -251T/A IL8: genotype T/T vs TA, OR 0.92, p=0.67, A allele OR 0.93, p=0.59. -692T/C IL17: genotype T/T vs T/C, OR 1.33, p=0.17, C allele, OR 1.14, p=0.41. -667A/C IL18: genotype A/A vs A/C, OR 1.16, p=0.51, C allele, OR 1.10, p=0.45. -618G/T IL18: genotype G/G vs G/T, OR 1.15, p=0.53, T allele, OR 1.12, p=0.38). Gender stratification showed association of -91A/C IL2 with JRA only in the male group (genotipe A/A vs A/C, OR 2.08, p=0.015, C allele, OR 1.57, p=0.03). Conclusion. SNP analyzed in the cytokine genes did not show association with susceptibility to JRA in Mexican population. Our results suggest that -91A/C IL2 SNP could be a risk genetic factor to JRA in a gender-dependent manner.

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## 784/W/Poster Board #442

**Loss of *TLR1* function is under selection and protects against leprosy.**
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Natural selection has driven population differentiation in humans and shaped genomic regions affecting complex phenotypes. Several genome-wide studies have shown that the Toll-like receptor (*TLR*) locus at chromosome 4p14 is highly differentiated between populations. We genotyped polymorphisms in this locus and show that the *TLR1* I602S variant is extremely differentiated between populations ($F_{ST}$>99$^{th}$ percentile). This non-synonymous variant prevents receptor translocation to cell surface and abrogates TLR1 signalling. Mapping the allele frequency in 15 human populations indicated that this variant has expanded out of Africa consistent with strong positive selection. We further investigated this locus in infectious diseases by genotyping 76 SNPs in the 80kb flanking region, and observed strongly significant association of the variant with protection against leprosy in a New Delhi case-control cohort of 558 cases and controls ($P$=1.3x10$^{-6}$, OR=0.27, 95% CI=0.15-0.47). The association was replicated in an independent cohort of 393 cases and controls from Kolkata of India ($P$=0.022, OR= 0.48, 95% CI=0.26-0.91). Our results identify *TLR1* as a major susceptibility locus for leprosy and strongly support the hypothesis that *TLR1* has evolved through positive selection.

## 785/W/Poster Board #443

**Association between HLA-A, -B and -DRB1 alleles and pulmonary tuberculosis in Western Javanese Indonesia.**
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Pulmonary tuberculosis (PTB) is caused by the interplay between environmental and genetic factors and still one of the deadliest diseases in the world. The genetic studies of PTB have been reported in several populations, including human leukocyte antigen (HLA) gene. However the results are still conflicting. The stronger role of the gene in more severe form of tuberculosis also has been reported. In the present study, HLA class I and class II alleles and haplotypes were investigated to ascertain the role of those alleles in the susceptibility/resistance to new PTB and recurrent PTB in Western Javanese Indonesia. HLA typing was performed for HLA-A, -B and -DRB1 loci on 237 ethnically matched healthy control individuals and 257 PTB patients (216 new and 41 recurrent PTB patients) by the high resolution PCR-Luminex method using WAKFLOW typing kit. The statistical analyses were performed using the Arlequin software based on the expectation maximization (EM) algorithm to determine the haplotypes and using Chi-square and Fisher's exact tests to calculate the frequency difference of the alleles between case and control groups. A total of 18 A, 40 B and 20 DRB1 alleles were identified. The most frequent HLA-A, -B and -DRB1 alleles in control group were HLA-A*2407 (21.6%), HLA-B*1502 (11.7%), HLA-B*1513 (11.2%) and DRB1*1202 (37.5%), respectively. The frequently observed 2-locus and 3-locus haplotypes were HLA-A*2407-B*3505 (7%), HLA-B*1513-DRB1*1202 (9.2%), HLA-A*3401-B*1521-DRB1*150201 (4.6%), HLA-A*2407-B*3505-DRB1*1202 (4.3%) and HLA-A*330301-B*440302-DRB1*070101 (4.2%), respectively. None of the indentified alleles in 3 HLA loci was found to be significantly associated with new PTB. Interestingly, among recurrent PTB, the allele frequency of HLA-B*1802 was significantly higher than that in control sample (p=0.0026). However, when the correction for multiple comparisons was applied, the result became insignificant. The possible role of the allele was supported by the fact that in two-factor analysis, the presence of both HLA-B*1802 and HLA-DRB1*1202 among recurrent PTB was more frequent than that among both new PTB and healthy control with odd ratio 4.15 and 3.98, respectively. This study suggested that HLA-B*1802 had a tendency to be associated with recurrent PTB rather than new PTB. The result of this study need to be further confirmed using more recurrent PTB samples.

## 786/W/Poster Board #444

**Muscle contracture genes play a role in isolated clubfoot.**
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Clubfoot is a common birth defect that affects 4000 newborns in the US each year. Isolated clubfoot (no other malformations) is characterized by an inward turning of the foot towards the midline of the body and is held in a rigid, downward position. Calf muscles in the affected leg(s) are underdeveloped and remain small even after corrective treatment. Clubfoot is a complex multifactorial disorder caused by both genes and environmental factors. Syndromes with a complex phenotype have been used to identify candidate genes for isolated conditions. Distal Arthrogryposis (DA) is a rare genetic disorder that is characterized by congenital contractures with clubfoot associated with some DA subtypes. Currently, there are nine different types of DA classified. Mutations in five different genes that encode for proteins that play a key role in muscle contracture are the cause for four types of DA. This study was undertaken to investigate whether variation in muscle contracture genes (n= 15), including those that cause DA, contribute to clubfoot. Seventy-four single nucleotide polymorphisms (SNPs) spanning these genes were genotyped in 160 nonHispanic whites (NHW) and 213 Hispanic simplex trios and 121 NHW and 93 multiplex families. The analysis was stratified by population, because the allele frequencies differed between populations. In the NHW group, one SNP in TPM2 and MYH3 showed strong association (p<0.01). Pairwise haplotype analysis detected strong altered transmission for 5 haplotypes with the strongest haplotype association identified for rs3744550 and rs2240579 in MYH13 (p=0.000038). In the Hispanic group, one SNP in TNNT3 and MYH13 were significantly altered transmitted (p=0.006 and p=0.003, respectively). All the genes interrogated play vital roles in muscle contracture, thus gene-gene interaction analysis was performed. Multiple significant interactions (p<0.01) were identified in both the nonHispanic White (19 interactions) and Hispanic groups (26 interactions). Identification of an association with MYH13 and clubfoot is a novel finding as this gene has not been implicated in any type of DA. These results suggest that genetic variation in genes involved in muscle contracture could play a role in clubfoot. These findings further support our working clubfoot model in which variation in multiple genes play a key role in the onset of clubfoot and its severity.

## 787/W/Poster Board #445

**Integration of human and mouse genome-wide association studies (GWAS) to identify genetic determinants for lung development and asthma.**
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Objectives: Chord length, the distance between alveolar walls, can assess lung development. Reduced chord length may result in low lung reserve and predispose to lung diseases such as asthma. A critical phenotype in asthma is airway hyper-responsiveness (AHR). We hypothesize that integration of GWAS for chord length and AHR may identify disease-causing alleles that determine lung development and asthma. Methods: We measured chord length in 6-10 female mice in 30 inbred strains and used strain means for haplotype association mapping. This method detects associations between haplotype blocks and strain phenotypes with a Hidden Markov Model to identify SNPs. Using the gene lists from Biomart, we searched for the genomic location of such SNPs and identified the mouse genes that included or were in close proximity to a detected SNP. Because disease-causing genes are often the same in mouse and human, we integrated these mouse data with human asthma GWAS data. SNP genotyping was performed in 422 families (parent-child trios) from the Childhood Asthma Management Program (CAMP) cohort using the Infinium II HumanHap550v3 Genotyping BeadChip (Illumina, San Diego, CA). We searched these data for SNPs that were in, or close to, the orthologous genes previously identified in the mouse. Results: We identified 23 genes in the mouse by a total of 71 SNPs ($P$<10-5) within or very close to the genes. We then asked whether any of these mouse genes were also detected as significant for asthma in the human GWAS. To do this, we used the mouse genes to find the orthologous region in humans, and examined each region to determine whether it contained any of the SNPs that were significantly different between case and control in the CAMP GWAS at $P$<0.05. Of the 23 genes identified in the mouse, 9 had significant SNPs in the orthologous human gene and the remaining 14 had a significant SNP in very close proximity (<700 kb) to the human gene. Interestingly, one gene, *Irx1*, is expressed during early lung development. Conclusion: Integrating murine and human GWAS data and application of bioinformatics tools may speed the identification of disease causing genes that are important in lung development and asthma.

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## 788/W/Poster Board #446

**Skin pigmentation and the *MC1R* gene are associated with central corneal thickness.**

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The cornea is the transparent cover at the front of the eye. It plays a critical role in maintaining normal vision through both refraction of light and maintaining a transparent visual axis. The thickness of the cornea, termed central corneal thickness (CCT), is a normally distributed quantitative trait. Thin CCT is a known risk factor for the sight-threatening condition open-angle glaucoma and extreme CCT measurements have been reported in a variety of Mendelian disorders. CCT is highly heritable ($h^2$=0.7-0.95) but as yet no genes have been identified for normal CCT variation. CCT varies significantly between different ethnic groups. We have previously observed an association with degree of skin pigmentation. To further assess the relationship between CCT and skin pigmentation we measured CCT in 13 different inbred strains of mice. There were significant differences in CCT between strains ($p < 0.001$). A general trend was noted, with pigmented strains trending to the lower end of the range and albino strains to the upper end. CCT was significantly associated with pigmentation when the 13 strains of mice were stratified into pigmented and albino strains: Albino = 83.5 ± 11.1 μm (n = 79), Pigmented = 78.3 ± 8.8 μm (n = 53) ($p = 0.008$). To explore this association further in humans, we genotyped SNPs in several genes known to influence pigmentation in humans including *TYR, OCA2, ASIP, SLC45A2* and *MC1R*. All genes were screened in a population based cohort of 953 Caucasian subjects from Australia. The strongest association was found with the MC1R gene. Two tag SNPs in MC1R were significantly correlated with CCT under a dominant model: rs2270459 ($p = 0.0008$) and rs3212346 ($p = 0.0062$). This gene is known to control eye and hair colour in Caucasians. The finding of an association between *MC1R* and CCT in Caucasians provides support for the hypothesis of pigmentation playing a role in the development of the clear cornea suggesting that these genes may have a broader function than what is currently recognized. Through discovery of the genes involved in the determination of CCT, the association of this trait with open-angle glaucoma will become better understood which may lead to improved methods of diagnosis and treatment of this important disease.

## 789/W/Poster Board #447

**Variation in *FGFR2* Expression Correlates with Genotype at SNPs Associated with Nonsyndromic Cleft Lip/Palate and Breast Cancer.**

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**Background:** Epidemiologic data has shown an increased incidence of breast cancer in women born with nonsyndromic cleft with or without cleft palate (CL/P). GWAS studies of both breast cancer and nonsyndromic CL/P have found association within intron 2 the *FGFR2* locus. We have confirmed that single nucleotide polymorphisms (SNPs) within intron 2, rs2981582 and rs3750817 previously associated with breast cancer, are significantly associated with CL/P in three populations, Denmark, Iowa and Filipino ($p < 0.01$). We have also identified a single SNP downstream of *FGFR2* (rs10466215) that is significantly associated with CL/P in these populations ($p < 0.01$) which is near two SOX9 binding sites. **Objectives:** Continue fine mapping sequencing techniques to identify rare SNPs associated with CL/P that may have functional importance and correlate significant SNPs associated with CL/P to functional changes at the *FGFR2* locus. **Methods:** Sequencing areas of high conservation near associated SNPs to identify rare alleles, not initially screened, associated with CL/P. Analysis of *FGFR2* expression in mRNA isolated from foreskin of healthy term males (n = 150) by qRT-PCR. DNA from the same foreskin samples was genotyped at SNPs in intron 2. Analysis of variance (ANOVA) of expression levels was performed on the three genotype groups. **Results:** Preliminary sequencing results identified one rare variant near a SOX9 binding site near rs10466215 in the Filipino population. ANOVA results at 3 SNPs show significant genotypic differences in FGFR2 expression normalized to ACTB expression with the most significant SNP being rs2912787 (ANOVA p = 0.02). **Conclusions:** Two areas of *FGFR2* are focal points of significant association with CL/P. One of those areas, intron 2, is also significantly associated with breast cancer. Current sequencing analysis of samples from Iowa will expand the number of cases in the study and add information from another population. In addition, work is currently ongoing to expand the number of intron 2 SNPs genotyped and determine *FGFR2* isoform specific expression based on SNP genotype. This work continues to narrow in on the region of *FGFR2* association with CL/P as well as provide functional clues of the role *FGFR2* plays in CL/P and breast cancer risk.

## 790/W/Poster Board #448

**Linkage analysis in a large Amish pedigree with von Willebrand Disease identifies regions suggestive of linkage and candidate modifier genes.**

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Von Willebrand Disease (VWD), the most common genetic bleeding disorder, is characterized by incomplete penetrance and variable expressivity. The ABO blood group is the only well-characterized modifier of VWF levels. We identified and characterized an Amish pedigree composed of more than 3000 members of whom 828 have been enrolled to identify genetic modifiers of VWD. To date we have evaluated 395 individuals. Sequence of VWF demonstrated heterozygosity for a missense mutation at position 4120 (C>T) in 71 individuals, that predicts an arginine to cysteine change at position 1374 (R1374C) in the A1 domain of the mature VWF molecule. Variability of both von Willebrand factor levels and bleeding phenotype (determined by a bleeding survey) are present among family members with and without the mutation. Significant heritability was found for different measures of the VWF molecule: VWF:Ag (h2=0.49, p< 0.00001), VWF:RCo (h2=0.54, p< 0.00001), and FVIII:C (h2=0.51, p< 0.00005). A primary genetic screen with 400 microsatellites was performed to construct a 10 cM-resolution genetic map of the first 395 pedigree members. Dichotomous trait two-point linkage analysis was performed using MLINK for five traits: bleeding score ≥3, bleeding score ≥4, VWF:RCo ≤50 IU/dL, VWF:RCo ≤25 IU/dL, and mutation status. All individuals with VWF:RCo ≤25 IU/dL are heterozygous for the C>T mutation. Modeling VWF:RCo ≤25 IU/dL as an autosomal dominant trait (allele frequency=0.1, penetrance=0.6), the highest LOD score (19.27) corresponded to marker GATA49D12 on chromosome 12p13.3 (to where VWF maps). Other regions suggestive of linkage to VWF levels include 6q25.3 (LOD = 2.18), 9q22.3 (LOD =1.03), 9q34 (LOD = 1.32), 14q12, (LOD =1.21), 16q12.2 (LOD = 1.20), 18q12.1 (LOD = 1.16) and 21q22 (LOD= 2.46). Three regions suggested linkage with both bleeding score phenotypes: 7q32 (LOD=2.52 and 1.52), 12q12 (LOD=1.13 and 1.26) and 16q12 (LOD=1.23 and 1.48). Regions suggestive of linkage were identified for both VWF:RCo ≤25 IU/dL and bleeding score phenotypes: 12p13.3, 16p12.2 and 21q22. Similar results were obtained by MCMC multipoint linkage using MORGAN. Candidate modifier genes include ABO (9q34), ADAMTS13 (9q34), ADAMTSL2 (9q34), CD9 (12p13), MMP2 (16q13), and COL6A1 and COL6A2 (21q22). This analysis in a very large pedigree with a homogeneous primary cause for VWD has identified several regions that will be investigated at higher resolution for the presence of modifier genes and alleles.

## 791/W/Poster Board #449

**Two novel associations between the fractalkine receptor (CX3CR1) common polymorphisms and overweight.**

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Overweight is a sub-phenotype of obesity characterized by an inflammatory component. The CX3CR1 gene, which encodes the fractalkine (CX3CL1) receptor, has two coding single nucleotide polymorphisms (SNPs), V249I and T280M, which have been linked to a decrease in the incidence of other inflammatory diseases, such as coronary artery disease and asthma. To determine whether CX3CR1 is involved in overweight expression, we genotyped the V249I and T280M polymorphisms of the CX3CR1 gene using High Resolution Melt (HRM) technique in 267 subjects with a body mass index (BMI) ≥ 30 kg/m2 and 453 control subjects with a BMI < 27 kg/m2. Binary logistic regression models revealed that 280MM genotype is associated with an increased risk of presenting a BMI ≥ 30 kg/m2 (P corrected = 0.044). A gender-specific one-way analysis of variance was also conducted to investigate mean BMI and waist circumference differences between genotypes of each polymorphism. Significant mean BMI differences were observed for the two polymorphisms between heterozygotes and wild homozygotes in men (TM > TT, P = 0.032 for the T280M polymorphism; VI > VV, P = 0.021 for the V249I polymorphism). The results showed that for each polymorphism, mutant homozygotes had significant increased waist circumference compared to wild homozygotes in women (MM > TT, P = 0.016 for the T280M polymorphism; II > VV, P = 0.008 for the V249I polymorphism). Significant increase of waist circumference was also observed for the mutant homozygotes compared to heterozygotes for the V249I polymorphism (II > VI, P = 0.025). Men mean waist circumference was also increased in heterozygotes when compared to wild homozygotes for the T280M polymorphism (TM > TT, P = 0.027). In conclusion, this study demonstrates that the T280M and V249I polymorphisms of the CX3CR1 gene are associated with overweight. Further genetic and functional studies are required to better define the role of the CX3CR1 in overweight.

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## 792/W/Poster Board #450

**Association studies of continuously distributed traits: dissection of human sweet taste perception.** *A. Fushan[1], C. Simons[2], J. Slack[2], D. Drayna[1].* 1) NIDCD/National Institutes of Health, Rockville, MD; 2) Givaudan Flavors Corp., Cincinnati, OH.

Substantial heritable variation for the perception of sweet taste compounds exists in humans. However, sweet taste sensitivity phenotypes display a unimodal continuous distribution in the population, and Mendelian transmission has not been reported for variation in sweet sensitivity. We evaluated candidate genes encoding the components of the sweet taste transduction pathway by DNA sequencing, and used R-index analysis to generate area under the curve (AUC) scores for threshold and suprathreshold perception of sucrose in a group of 166 normal, unrelated subjects. Using ANOVA analysis, we observed strong association of AUC scores with two C/T SNPs, residing 1266 bp and 1572 bp upstream of the coding sequence of the TAS1R3 gene, which encodes a component of the G protein coupled sweet taste receptor. Together these SNPs explain 16% of the variance in sucrose AUC scores, with the T alleles associated with reduced sucrose sensitivity in vivo. Luciferase reporter constructs assayed in HuCCT1 and NCI-H716 cells demonstrate that the T alleles of these SNPs significantly reduce TAS1R3 promoter activity, suggesting lower protein expression in vivo. Worldwide population surveys demonstrate highest frequency of the T alleles in sub-Saharan African populations, and a gradient of frequency extending across Eurasia, with lowest frequencies of the T alleles in western European populations. Our results suggest that population-specific genetic differences in sweet taste perception may have significant effects on differences in food choices and consumption.

## 793/W/Poster Board #451

**Genetic polymorphism of the type II deiodinase (DIO2) gene is associated with bipolar disorder.** *N. Zhong[1, 2], B. He[2], E.C. Jenkins[1], G. Wang[2], W.T. Brown[1].* 1) Department of Human Genetics, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY; 2) Peking University Center of Medical Genetics, Beijing, China.

Genetic factors play a critical role in the etiology of bipolar disorder. Previous studies suggested an association between thyroid dysfunction and bipolar disorder. We hypothesized that genetic variations in the type II deiodinase (DIO2) gene that possibly alter the bioactivity of thyroid hormones are associated with bipolar disorder. A case-control association study was conducted in a subset of Chinese Han population. Two single nucleotide polymorphisms (SNP), open reading frame a (ORFa)-Gly3Asp (rs12885300) and Thr92Ala (rs225014) with potential functions on the activity of DIO2, were selected. The frequencies of allele, genotype and haplotype of the two SNPs were compared between the bipolar disorder patients and the control group. Statistical significance between the bipolar disorder patients and the control group was observed for the allele ($\chi2$=7.746, P=0.005, df=1) and genotype frequencies ($\chi2$=8.158, P=0.017, df=2) at the locus of ORFa-Gly3Asp, and for the allele ($\chi2$=15.838, P=7.00e-005, df=1) and genotype frequencies ($\chi2$=17.236, P=0.0002, df=2) at Thr92Ala. Distribution of allele 3Gly and 92Ala were significantly higher in the bipolar disorder patients, with odds ratios of 1.489 and 1.616, respectively. Individuals with two copies of the variant 3Gly or 92Ala were at greater risk of bipolar disorder than individuals with one copy (dose-response manner). Haplotypes ORFa-3Asp-92Ala and ORFa-3Gly-92Ala indicated higher susceptibility for bipolar disorder with odds ratios of 3.759 and 1.292, respectively, while ORFa-3Asp-92Thr probably played a protective role with an odds ratio of 0.395. Data generated from this study supported our hypothesis that genetic variations of the DIO2 gene were associated with bipolar disorder and suggested further consideration on the possible involvement of these functionally active variants in the pathophysiology of bipolar disorder.

## 794/W/Poster Board #452

**Variants of gene for microsomal prostaglandin E synthase 1 show association with disease and severe inflammation in rheumatoid arthritis.** *M. Korotkova[1], M. Seddighzadeh[1], B. Ding[2], N.A. Daha[3], S. Lindblad[1], T. Huizinga[3], R.E.M. Toes[3], L. Alfredsson[2], L. Klareskog[1], P-J. Jakobsson[1], L. Padyukov[1].* 1) Department of Medicine, Rheumatology Unit, Karolinska Institutet/Karolinska University Hospital, Stockholm, Sweden; 2) Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 3) Leiden University Medical Center, Leiden, The Netherlands.

mPGES1 is a terminal enzyme in induced state of PGE2 synthesis and constitutes a novel therapeutic target for rheumatoid arthritis (RA) treatment. SNPs in the mPGES1/PTGES gene could modify enzyme expression levels or activity, alter induced PGE2 biosynthesis and lead to the inter-individual differences in susceptibility to or severity of RA. Aim: to study the association of the PTGES gene polymorphism with susceptibility to and severity of RA and to relate variations in the gene to its expression and function. Methods: The PTGES gene polymorphism (SNPs and haplotypes) was analyzed in 2157 incident RA cases and 1068 age and sex matched controls from EIRA, a population based case-control study in Sweden, using TaqMan allelic discrimination. We generated the dense map of the locus including neighboring PRPX2 and TOR1 genes. Baseline disease activity score (DAS28) was employed as disease severity measure. mPGES1 mRNA and protein expression in LPS-stimulated PBMC was analyzed by qPCR and flow cytometry respectively in 25 healthy individuals with known genotypes. PGE2 was measured in cell supernatants by EIA. Results: Among women, a significant association with RA risk was observed for three SNPs from the same recombination block (rs10988484, rs11999368 and rs16931419, p=0.0014, 0.0028, 0.0011, significant after permutation test). In addition, haplotype frequency analysis based on 3 SNPs in a recombination block downstream to PTGES demonstrated a significant association between haplotype TTC and susceptibility to RA in women (p=0.0054) that was substantiated in an independent study from the Dutch population with overall OR 1.28 (95%CI 1.09-1.51). No significant association with RA either for a single SNP or in the haplotype analysis was found in men. One of the SNPs (rs4837404) was associated with lower DAS28 in women with RA (p=0.0028). Rare genotypes of another two SNPs, rs2241270 and rs230282 were consistent with 3 and 10 years earlier onset of RA (p<0.05). There were no significant differences in the expression of mPGES1 protein or PGE2 production by LPS-stimulated PBMC from healthy individuals in relation to genotypes. mRNA expression tended to be lower in PBMC from individuals with protective haplotype. Conclusions: Our data reveals an association between polymorphisms at the PTGES gene locus and susceptibility to and severity of RA in women, which may contribute to the differences in the expression of PTGES at the site of inflammation.

## 795/W/Poster Board #453

**Functional Validation of Variants in Interleukin-23 Receptor (IL23R) Associated with Crohn's Disease.** *S. Pidasheva, N. Ghilardi, HF. Clark.* Bioinformatics & Immunology, Genentech, S San Francisco, CA.

Crohn's disease (CD) is an inflammatory bowel disease (IBD), which occurs when the immune system attacks the gastrointestinal tract. Genome-wide association (GWA) studies for CD in several populations demonstrated that one of the strongest association signals mapped to the IL23R gene. IL23 promote, the expansion and development of pro-inflammatory Th17 cells. Thus, perturbation of IL23 signaling in Th17 cells could play a pivotal role in the pathogenesis of CD. In this study, we investigated the effect of coding single nucleotide polymorphisms (cSNP) in IL23R for potential functional roles in susceptibility to CD. To address this issue, we have analyzed wild type (WT) and ten IL23R cSNPs. The main focus of this study was on R381Q a highly associated protective variant. To investigate the effects of cSNPs we used retrovirally transduced BaF3 cells as well as polyclonal T cell lines. We analyzed cell surface expression, proliferation in response to IL23R agonist and STAT3 phosphorylation. CD4+ T cell lines were generated from healthy donors with WT and R381Q haplotypes. Donors with protective haplotype had significantly reduced STAT3 phosphorylation compared to the WT counterparts. Reduced STAT3 signaling may lead to decreased production of pro-inflamatory cytokines like, IL17, explaining the protective effect of this variant. Our genetic research provides better understanding of the pathogenic mechanisms of IL23R's contribution to CD and may contribute to the development of targeted drug therapy.

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## 796/W/Poster Board #454

**A comprehensive strategy for uncovering functional elements underlying non-genic disease associations.** *N.F. Wasserman, M.A. Nobrega.* Department of Human Genetics, The University of Chicago, Chicago, IL.

In the classical model of human disease genetics, mutations in coding regions of the genome are assumed to underlie disease phenotypes. It is only recently that functional non-coding regions - such as enhancers, silencers, and insulators - have been implicated in disease states. Numerous genome-wide association studies (GWAS) have identified risk variants in non-genic regions for common diseases, including various cancers and obesity. We propose a novel framework within which to view these GWAS results, hypothesizing that the genetic reasons for the non-genic associations stem from mutations in long-range *cis*-regulatory elements controlling the temporal- and tissue-specific expression of nearby genes. Our platform builds upon recent association studies and illustrates an efficient strategy to identify, map, and functionally characterize long-range regulatory elements implicated in complex human diseases. We use this approach to analyze two associations; between multiple cancers and a gene desert on 8q24, and between an intronic region of *FTO* and obesity. GWAS investigations have identified independent risk alleles within a 620kb non-genic region on 8q24 for prostate, colorectal, breast, ovarian, and urinary bladder cancer. Although there are no well-characterized genes in the interval, the proto-oncogene *MYC* lies just downstream, suggesting that the associated region may contain functional elements implicated in *MYC* regulation. Using a combination of bioinformatics, comparative genomics, and mouse transgenic *in vivo* assays, we have successfully identified a prostate-specific enhancer within the associated interval; other tissue-specific enhancers implicated in *MYC* mis-regulation are currently being mapped. A tissue-specific brain enhancer has also been identified in the associated region of *FTO*, partially recapitulating the endogenous gene expression pattern. *In vivo* and *in vitro* studies are being performed to characterize the functional implications of variation within these enhancers, as polymorphisms within the elements are theorized to predispose to and/or cause the tissue-specific mis-regulation of *MYC/FTO*, leading to the initiation and/or progression of cancer/obesity. These findings strongly support the hypothesis that long-range *cis*-regulatory elements are localized within non-genic regions associated with human diseases, and emphasize that investigations into regulatory potential should be a priority in the follow-up to GWAS inquiries.

## 797/W/Poster Board #455

**The single nucleotide polymorphisms in the VEGF, MMP-2 and MMP-9 are associated with risk of endometriosis.** *M. SAARE[1,5], M. Lamp[1], T. Kaart[3], H. Karro[1,4], Y. Kadastik[4], A. Metspalu[2,5], M. Peters[1,5], A. Salumets[1,2].* 1) Department of Obstetrics and Gynaecology, University of Tartu, Estonia; 2) Department of Biotechnology, Institute of Molecular and Cell Biology, University of Tartu, Estonia; 3) Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, Estonia; 4) Tartu University Hospital's Women's Clinic, Estonia; 5) Estonian Biocentre, Estonia.

Background: The extracellular matrix remodeling and excessive endometrial angiogenesis are proposed to be two important mechanisms in pathogenesis of endometriosis. It has been proposed that vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMP) play an active role in the establishment and progression of endometriosis since altered VEGF, MMP-2 and MMP-9 gene expression profiles have been reported in eutopic and ectopic endometrial tissues obtained from women with endometriosis. The aim of this study was to investigate the associations between the promoter region polymorphisms of VEGF, MMP-2 and MMP-9 and risk of endometriosis. Methods: 150 Estonian patients with endometriosis and 199 healthy women were studied. PCR based restriction fragment length polymorphism analysis was used to detect SNPs in VEGF (-2578 C/A, -1154 G/A; -634 C/G, -936 C/T), MMP-2 (-735 C/T, -790 T/G, -1575 G/A) and MMP-9 (-1562 C/T). Results: Women with VEGF-2578 (rs699947) AA/AC genotype had more than twofold higher risk (p=0.008) of endometriosis than women with CC genotype and women with MMP-2 -735 (rs2285053) TC and TT genotype had a lower risk (p=0.016) of developing endometriosis compared to the CC genotype carriers. MMP-9 -1562 (rs3918242) TT genotype or TT/TC genotype carriers had higher risk to develop moderate-severe endometriosis than CC genotype carriers (p=0.013 and p=0.027, respectively). There were no significant differences in the distribution of the MMP-2 -790 T/G,-1575 G/A and VEGF-1154 G/A; -634 C/G, -936 C/T SNPs between studied groups. Conclusions: Current study showed that polymorphisms in VEGF, MMP-2 and MMP-9 promoter regions may influence the development of endometriosis.

## 798/W/Poster Board #456

**Detection of Excess Rare Damaging Variants in Surfactant Associated Genes among Infants with Respiratory Distress Syndrome Using Pooled Next Generation Sequencing.** *D. Wegner[1], S. Robison[1], K. DePass[1], T. Druley[1,2], F. Vallania[2], R. Mitra[2], A. Hamvas[1], F.S. Cole[1].* 1) Pediatrics, Washington Univ, St. Louis, MO; 2) Genetics, Washington Univ, St. Louis, MO.

**Background:** Heritability of neonatal respiratory distress syndrome (RDS), a complex disease, is likely associated with rare, damaging variants in many genes related to the pulmonary surfactant metabolic pathway.

**Objective:** To determine the contribution of rare damaging variants associated with neonatal RDS in surfactant associated pathway genes.

**Methods:** We evaluated race-specific, collapsed frequencies of disruptive, rare variants (MAF <0.02) in 5 genes in the pulmonary surfactant metabolic pathway (*SFTPC, ABCA3, CHPT1, LPCAT1,* and *PCYT1B*) in 346 African-American infants (160 cases and 186 controls) and 331 European-American infants (200 cases and 131 controls). We used an Illumina next generation sequencing platform and normalized pooled samples. Identification of rare variants was performed with our in-house software program (SPLINTER). Statistical approaches included Fisher's exact tests on collapsed rare variant frequencies and in silico evaluation of genetic disruption with SIFT, PolyPhen, and Pmut.

**Results:** Among African-American infants, we found no disruptive, rare variants in *SFTPC, ABCA3, CHPT1,* and *PCYT1B*. We found 17 disruptive, rare alleles in *LPCAT1* among 346 infants (collapsed frequency 0.049). The difference in frequency between cases and controls was not significant (P= 0.46). Among European-American infants, we found no disruptive, rare alleles in *SFTPC, LPCAT1,* and *PCYT1B*. We found 11 disruptive, rare *ABCA3* alleles among 331 infants (collapsed frequency 0.033), and the difference in frequency between cases and controls was significant (P= 0.004). We also found 4 disruptive, rare variants in *CHPT1* (collapsed frequency 0.012), and the difference in frequency between cases and controls was significant (P=0.02). However, the disruptive variants were overrepresented in the control infants.

**Conclusion:** Rare disruptive variants in pulmonary surfactant metabolic pathway genes contribute to the relative risk of neonatal RDS. Collapsing rare variants at individual gene loci permits sufficient statistical power to identify genes and gene pathways associated with complex diseases despite modest case-control cohort sizes.

## 799/W/Poster Board #457

**Genome-wide association study for osteoarthritis- Novel polymorphisms in TIMP-2 are associated with susceptibility to osteoarthritis.** *B. Keam, J.Y. Heo, M.S. Park, J.Y. Lee, J.Y. Lee, B.G. Han, J. Lee.* Center for Genome Science, National Institute of Health, Korea Centers for Di, Seoul, Seoul, Korea.

Objective: Osteoarthritis (OA) can cause serious medical, social and economic problems affecting the elderly population worldwide. Not only clinical factors such as age, sex, and obesity but also genetic factors contribute to the risk of OA. The aim of this study was to identify the associations between single nucleotide polymorphisms (SNPs) and OA using a genome-wide approach and to find novel candidate genes for OA. Methods: We conducted a genome-wide association study using 3,793 subjects recruited from a community-based epidemiological study. A total 476 OA cases and 3,317 controls were genotyped on a single platform using the Affymetrix Genome-Wide Human SNP array 500K chip. Of the SNPs assayed on the chip, 179,626 SNPs were excluded because they showed: 1) a call rate lower than 96.0% in cases or controls; or 2) a minor allele frequency (MAF) <1% in the population; or 3) a significant distortion from Hardy-Weinberg equilibrium in the controls (p<0.05). The population stratification was negligible ($\lambda$= 1.008772). Results: A total of 320,942 SNPs passed all quality control filters. We identified 42 SNPs that were significantly associated with OA. Two SNPs (rs4789934 and rs9914634) in the TIMP-2 (tissue inhibitor of metalloproteinase-2) gene exhibited the most significant association with OA (age, sex adjusted odd ratio, 2.064 and 2.174, adjusted p-values, 4.01×10-6 and 6.69×10-6, respectively). Six SNPs in NKAIN2 (Na+/K+ transporting ATPase interacting 2) and one SNP in SPOCK1 (sparc/osteonectin) also showed significant associations with OA. SNPs in several other genes, FLJ44379, HDAC9, SH3GL2, RASEF, SH3RF1, PALLD, LHFPL4, VSTM2L, ZFHX3, and C10orf72, exhibited a significant association with OA. Additionally, 18 significantly associated SNPs were located in intergenic regions. To consider the joint effects and SNP-SNP interactions among the identified 42 SNPs, we conducted multiple stepwise logistic regression analyses, and SNPs in TIMP-2 and NKAIN2 remained significantly associated with OA. Conclusion: We identified novel genetic polymorphisms in TIMP-2 and NKAIN2 that are associated with OA. Further investigations are warranted to fully characterize these associations.

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## 800/W/Poster Board #458

**Are there common genes affecting both hip osteoarthritis and lumbar disc degeneration?** *A. Näkki[1,2,3,4], T. Videman[4,5], U.M. Kujala[6,7], M. Suhonen[8,9], L. Peltonen[1,2,10], M.C. Battie[4,5], J. Kaprio[1,4,11], J. Saarela[1,2].* 1) Institute for Molecular Medicine Finland FIMM, Helsinki, Finland; 2) Unit of Public Health Genomics, National Institute for Health and Welfare, Helsinki, Finland; 3) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 4) Department of Public Health, University of Helsinki, Helsinki, Finland; 5) Faculty of Rehabilitation Medicine, University of Alberta, Edmonton, Canada; 6) ORTON Orthopedic Hospital, Invalid Foundation, Helsinki, Finland; 7) Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland; 8) Suomen Terveystalo, Helsinki, Finland; 9) Department of Clinical radiology, Kuopio University Hospital, Kuopio, Finland; 10) Wellcome Trust Sanger Institute, Cambridge, United Kingdom; 11) Department of Mental Health, National Institute for Health and Welfare, Helsinki, Finland.

Objective. Our aim was to study if the gene variants, previously identified to associate lumbar disc degeneration phenotypes (Videman et al. 2009; Arthritis and Rheumatism), were also associated with another degenerative connective tissue disease, hip osteoarthritis (OA). Methods. We used a population based twin cohort, consisting of 588 Finnish monozygotic or dizygotic male twins, aged 35-70 years. Osteoarthritis-related changes that extend to bone were graded using magnetic resonance imaging (MRI) and used as the hip OA phenotype. A total of 99 single nucleotide polymorphisms (SNPs) in 25 structural, degenerative and inflammatory candidate genes were genotyped and the association was monitored using the Pseudomarker analysis package, which can utilize combined data of individuals and sib pairs. Results. Of the 18 SNPs previously shown to associate with disc degeneration, only one SNP in the COL9A2 gene (rs7533552) was also significantly associated with the hip OA phenotype ($p = 9*10^{-5}$) in this twin cohort. The G allele of this non-synonymous SNP, changing a codon of a glutamine to either arginine or to tryptophan, was observed to predispose both to hip OA and lumbar disc degeneration. Conclusion. A common non-synonymous SNP (rs7533552, other alias rs2228564) in the COL9A2 gene was identified to associate with hip OA and disc degeneration in a Finnish population based twin cohort. It was located next to the rare variant (rs12077871) coding for the degenerative connective tissue disease associated Trp2 allele together with the G allele in rs7533552. Our finding identifies the COL9A2 gene as an interesting target for future studies in common degenerative connective tissue diseases.

## 801/W/Poster Board #459

**Analysis of Multiple Sclerosis Candidate Genes Stratified by HLA Genotype Reveals Novel Associations.** *J. Haines[1], R. Zuvich[1], J. McCauley[2], Y. Bradford[1], N. Schnetz-Boutaud[1], A. Compston[3], P. De Jager[4], D. Hafler[4], S. Hauser[5], J. Oksenberg[5], S. Sawcer[3], M. Pericak-Vance[2].* 1) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN 37232; 2) Miami Institute for Human Genomics, Miller School of Medicine, University of Miami, Miami, FL 33136; 3) Department of Clinical Neurosciences, Addenbrooke's Hospital, University of Cambridge, Box 165, Hills Road, Cambridge CB2 2QQ, United Kingdom; 4) Division of Molecular Immunology, Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; 5) Department of Neurology, School of Medicine, University of California, San Francisco, CA 94143.

Multiple sclerosis (MS) is etiologically a complex disease that is primarily characterized as a neurodegenerative, autoimmune disorder. The autoimmune nature of MS led to the early discovery of the Major Histocompatibility Complex (MHC) involvement in the pathogenesis of MS. The strongest association has been subsequently localized to the HLA-DRB1*1501 allele. Since this discovery, numerous genome-wide linkage screens have been performed to identify disease loci, but have had little success. Through these and more recent linkage studies, it became clear that the only reliably strong linkage signal exists in the MHC. Because of the strong signal (odds ratio ~2) and the difficulties in identifying other loci harboring disease alleles, it is plausible that common alleles with much smaller effects are being masked by the strong MHC signal. Given this hypothesis, we stratified the results of a SNP analysis on a set of candidate genes derived from a candidate pathway analysis of MS by the presence of an HLA risk allele. In a case-control dataset of 2,722 samples genotyped for 7,865 single-nucleotide polymorphisms (SNPs), we found several genes that had suggestive association only in the stratum of individuals without HLA-DRB1*1501 (HLA-), which contained 1,668 individuals. Before stratification, the genes IRS1, BCL2, IL12A, and SOCS1 had p-values of 1.66x10-3, 0.04, 1.43x10-3, and 2.53x10-3, respectively. After stratification, the results were much stronger, with max p-values of 5.90x10-5, 1.90x10-4, 5.47x10-4, and 5.55x10-4, respectively in the HLA- subset. In an independent balanced case-control dataset of ~4,000 samples, two of these gene regions (IL12A and SOCS1) replicated in the HLA- stratum. The SNP near SOCS1 has a p-value of 5.55x10-4 with an estimated genotype relative risk (GRR) of 1.47; whereas, in the overall dataset, the SNP has a p-value of 2.5x10-3 with an estimated GRR of 1.28, and in the stratum of individuals with HLA-DR*1501 (HLA+), the SNP has a p-value of 0.24. The SNP in IL12A has a p-value of 6.67x10-4 with an estimated GRR of 1.28; whereas, in the overall dataset, the SNP has a p-value of 1.9x10-3 with an estimated GRR of 1.18, and in the HLA+ stratum, the SNP has a p-value of 0.25. These results suggest effects that are independent of HLA.

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## 802/W/Poster Board #460

**A Pathway Approach to Decoding Multiple Sclerosis reveals several new associated loci.** *R. Zuvich[1], J. McCauley[2], Y. Bradford[1], N. Schnetz-Boutaud[1], A. Compston[3], P. De Jager[4], D. Hafler[4], S. Hauser[5], J. Oksenberg[5], S. Sawcer[3], M. Pericak-Vance[2], J. Haines[1].* 1) Human Gen,519 Light Hall, Vanderbilt Univ, Nashville, TN 37232; 2) Miami Institute for Human Genomics, Miller School of Medicine, University of Miami, Miami, FL 33136; 3) Department of Clinical Neurosciences, Addenbrooke's Hospital, University of Cambridge, Box 165, Hills Road, Cambridge CB2 2QQ, United Kingdom; 4) Division of Molecular Immunology, Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; 5) Department of Neurology, School of Medicine, University of California, San Francisco, CA 94143.

Multiple sclerosis (MS) is characterized as an autoimmune, demyelinating disease. Through various family studies, there is strong evidence of a genetic component underlying its etiology. After several decades of frustration in identifying genetic risk factors beyond the MHC, technological advances have enabled the identification of new genes associated with MS. In this study, we used the paradigm of examining genes within the extended pathway of previously identified genetic loci to find novel associations. Using available pathway information, we identified 73 genes associated with the IL7RA pathway, a gene recently identified and subsequently confirmed as a risk locus for MS. These genes are involved in the signaling cascade downstream of IL7RA or affected by varying IL7 concentrations. Genes were represented by single-nucleotide polymorphisms (SNPs) within the gene or in the 50kb region upstream and downstream of each gene. We genotyped the resulting 7,865 SNPs on 2,722 case-control samples using the Illumina iSelect Custom BeadChip assay. Our results suggest that five gene regions are associated with MS risk: IL7 with max p-value 2.46x10-5, the region flanking SOCS1 with max p-value 4.33x10-4, PRKCE with max p-value 3.47x10-4, Tyk2 with max p-value 3.97x10-4 and BCL2 with max p-value 4.32x10-4. Two of the gene regions, IL7 and SOCS1, had SNPs that replicated in an independent case-control dataset of ~2,000 cases and 2,000 controls. The SNPs in IL7 had a combined max p-value of 8.29x10-6 with an estimated genotype relative risk (GRR) of 1.14. The SNPs around SOCS1 had a combined max p-value of 3.48x10-7 with an estimated GRR of 1.25. Both of these genes have biological relevance: IL7 is the ligand of a known susceptibility gene (IL7RA) and vital for the development, maturation, and survival of T cells and SOCS1 is involved in suppression of cytokine signaling. Thus, our data indicates that using the pathway approach is an effective way to identify novel associations in a complex disease. This experiment lends further support that multiple common alleles with modest effects contribute significantly to MS susceptibility.

## 803/W/Poster Board #461

**Modeling Complex Genetic and Environmental Influences on Comorbid Bipolar Disorder with Tobacco Use Disorder.** *R. McEachin[1,4], N. Saccone[2], S. Saccone[3], L. Bierut[3], J. Cavalcoli[4], M. McInnis[1,4].* 1) Psychiatry, Univ Michigan, Ann Arbor, MI; 2) Genetics, Washington Univ, St. Louis, MO; 3) Psychiatry, Washington Univ, St. Louis, MO; 4) National Center for Integrative Biomedical Informatics, Univ Michigan, Ann Arbor, MI.

Comorbidity of psychiatric and substance use disorders represents a significant complication in the clinical course of both disorders. Bipolar Disorder (BD) is a psychiatric disorder that has significant negative effects on the lives of those affected, as well as a high rate of comorbid Tobacco Use Disorder (TUD). For patients with BD, the risk for TUD is almost 3 times that for the general population, based on our meta-analysis of the seven epidemiological studies that have been published to date. Notably, the observed bi-directional increased relative risk for both disorders is consistent with some common underlying etiology for BD and TUD. Given this potential for common etiology, as well as evidence of genetic influences on both BD and TUD, we hypothesized a common underlying genetic etiology, interacting with environmental nicotine exposure, influencing susceptibility to both BD and TUD. We used multiple bioinformatics resources to establish validated candidate genes for comorbid BD with TUD and explore the likely roles of these genes in the comorbidity. We then hypothesized networks of candidate genes for the comorbidity, based on their interactions with the validated candidate genes, and tested these hypothesized networks for association with BD and TUD. Consistent with our hypothesis, the selected genetic network shows statistically significant over-representation of genes associated with both BD and TUD, as well as significant over-representation of genes differentially expressed with exposure to tobacco smoke. This network reveals novel inference on the multiple genetic influences on the comorbidity of BD and TUD, establishes novel candidate genes for follow-on testing, and models the environmental effect of nicotine on the genetic network. We provide two sets of SNPs that have been prioritized for follow-on testing. The first set is prioritized by functional and conservation data and includes variants in our validated candidates (COMT, SLC6A3, and SLC6A4), as well as their interactors. The second set, a subset the first, also incorporates evidence of association with nicotine and BD.

## 804/W/Poster Board #462

**Peptide YY (PYY): Discrete polymorphisms in the 3'-UTR and proximal promoter regulate gene expression in cella as well as PYY secretion and metabolic syndrome traits in vivo.** *P. Shih, L. Wang, G. Wen, C. Nievergelt, M. Mahata, F. Rao, D. O'Connor.* Sch Med, Univ California, San Diego, San Diego, CA.

Rationale: Obesity is a heritable trait that contributes to hypertension and subsequent cardio-renal disease risk; thus the investigation of genetic variation that predisposes individuals to obesity is an important goal. Circulating Peptide YY (PYY) is known for its appetite and energy expenditure-regulating properties; linkage and association studies have suggested that PYY genetic variation contributes to susceptibility for obesity, rendering PYY an attractive candidate for study of disease risk. Study Design: To explore whether common genetic variation at the human PYY locus influences plasma PYY or metabolic traits, we systematically resequenced the gene for polymorphism discovery, then genotyped common SNPs across the locus in an extensively phenotyped twin sample to determine associations. Finally, we experimentally validated the marker-on-trait associations using in cella PYY 3'-UTR/reporter analysis in neuroendocrine cells. Results: 4 common genetic variants were discovered across the locus, and 3 were typed in phenotyped twins. Plasma PYY was highly heritable ($p<0.0001$), and pleiotropy was noted for plasma PYY and body mass index (BMI) ($p=0.03$). A PYY haplotype extending from the proximal promoter (A-23G) to the 3'-UTR (G1134A) predicted not only plasma PYY ($p=0.009$) but also other metabolic syndrome traits. 3'-UTR variant G1134A disrupted a microRNA recognition motif, while proximal promoter variant A-23G extended the TATA box by one base. Functional studies with transfected luciferase reporters confirmed regulatory roles in altering gene expression for both 3'-UTR G1134A ($p<0.001$) and promoter A-23G ($p=0.0016$). Conclusions: Functional genetic variation at the PYY locus influences multiple heritable metabolic syndrome traits, likely conferring susceptibility to obesity and subsequent cardio-renal disease.

## 805/W/Poster Board #463

**The asthma associated G-protein coupled receptor NPSR1 affect downstream signalling in a splice variant specific manner.** *C. Orsmark Pietras[1], J. Vendelin[2], S. Bruce[1], F. Anedda[1], M. D'Amato[1], C. Söderhäll[1], J. Kere[1,2,3].* 1) Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden; 2) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 3) Clinical Research Centre, Karolinska University Hospital, Stockholm, Sweden.

Neuropeptide receptor 1 (NPSR1, GPRA) was first identified as an asthma candidate gene through positional cloning. Since then many studies have shown that NPSR1 not only is an asthma susceptibility gene, but also involved in settings such as inflammatory bowel disease, sleep and circadian phenotypes and anxiety. NPSR1 encodes two functional membrane receptors, NPSR1-A and NPSR1-B, generated by alternative splicing of the last exon resulting in unique intracellular C-termini. The isoforms show distinct expression patterns in tissues and cells, but previous studies have failed to show any functional difference between NPSR1-A and -B. To investigate differences in NPSR1 isoform specific signalling we looked at regulation of genes downstream the receptors. Human epithelial kidney cells were transiently transfected with NPSR1-A or -B. The transfected cells were stimulated with NPS, the ligand for NPSR1, and RNA was collected. We performed an isoform specific expression array experiment, looking at 30 000 potential target genes. The results showed that NPSR1-A and -B regulated the same genes but with a clear difference between the two isoforms, NPSR1-A constantly yielded a stronger induction of genes than NPSR1-B. These results were verified by both NPS dose-response and time series analyses that showed NPSR1-A responses consistently above those of NPSR1-B. We verified the results on selected genes also in human lung epithelial cells and neuroblastoma cells relevant for NPSR1 signalling. Using luciferase-based reporter assays we could confirm that NPSR1-A is the stronger activator of transcription factors in three different pathways, cAMP/PKA, MAPK/ERK and MAPK/JNK, providing a mechanistic explanation for the difference between the two isoforms. In conclusion, we show that NPSR1-A and -B regulate the same set of genes but that -A is a more potent activator, possibly based on differences in phosphorylation between the two isoforms.

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## 806/W/Poster Board #464

**Role of Genetic Polymorphisms in the Chemokine Pathway in Asthma and Atopy: Molecular and Clinical Genetic Study.** *S.A. AL-ABDULHADI[1], M.W AL-RABIA[2].* 1) MOLECULAR PATHOLOGY, SAAD SPECIALIST HOSPITAL, KHUBAR, Saudi Arabia; 2) IMMUNOLOGY DEARTMENT, UM AL-QURA UNIVERSITY, MAKKAH, Saudi Arabia.

Chemokines and their receptors contribute to the asthmatic airway inflammation by acting on mononuclear cells. Their expression has been reviewed for a number of human diseases with an inflammatory component. Possible associations between CCR5Δ32, CCR2V64I, CCR3Y17Y, and the RANTES-G403A promoter and asthma and related phenotypes in high-risk families. 154 families (453 individuals), with at least two affected children with physician-diagnosed asthma (PDA) and atopy were studied. Both TDT and PDT demonstrated similar non-significant associations between CCR5Δ32 with asthma or atopy. CCR2V64I was significantly associated with absence of asthma FEV1% but not with s-IgE. CCR3Y17Y was preferentially transmitted with asthma and BHR. The -403 G/A RANTES polymorphism was transmitted with atopy and atopic asthma although its contribution appeared to relate more to atopy than asthma and BHR. Both linkage and haplotype analyses confirmed the association between chromosome 3 and asthma status. Haplotype analyses suggested significant linkage disequilibrium between these three polymorphisms and asthma status. Log linear analyses revealed that the CCR2 mutant allele was most strongly associated with protection against atopic asthma whereas the CCR3 mutant allele was associated with increased the risk of disease. Imprinting analyses failed to demonstrate an increased risk of disease when effector alleles were transmitted from the mother. A significant interaction was found between maternal smoking, and carriage of the -17YCCR3 for an increased the risk of atopic asthma in child probands. Domestic animal exposure (cat or dog) showed no significant interaction with the polymorphisms of interest. The bioinformatic databases BLAST, and MatInspector analyses suggested the SNP-64I within the CCR2 gene and the SNPs -17Y, -21G, and 5UTR within CCR3 were functional. The -403A and -28C SNP's in the RANTES promoter were identified as belonging to common functional binding sites. These analyses suggested that environmental exposures could interact with genetic constitution in influencing disease expression. Overall this study suggested new mechanisms relevant to asthma that might effect into the future therapy and genetic counseling education session and clinical pathway. However, this suggested mechanisms and the possible functional effects of the studied polymorphisms clearly need verification in model biological systems and possible multi-ethnic large populations.

## 807/W/Poster Board #465

**Genome wide meta-analysis for Crohn's disease and celiac disease identifies new shared risk loci.** *E. Festen[1,2], T. Green[3], P. Goyette[4], G. Trynka[2], P. Dubois[5], R. Scott[6], S. Brant[7,8], J. Cho[9], M. Silverberg[10], K. Taylor[11], P. Stokkers[12], D. McGovern[11], J. Achkar[13], R. Duerr[14,15], R. Weersma[1], D. Van Heel[5], J. Rioux[4], C. Wijmenga[2], M. Daly[3,16].* 1) Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands; 2) Department of Genetics, University Medical Center Groningen and University of Groningen, Groningen, the Netherlands; 3) Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, 185 Cambridge Street, Boston, Massachusetts 02114, USA; 4) Laboratory in Genetics and Genomic Medicine of Inflammation, Montreal Heart Institute Université de Montréal, Montreal, Canada; 5) Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, London, E1 2AT, UK; 6) Division of Gastroenterology, Hepato logy and Nutrition, Department of Medicine, University of Pittsburgh School of Medicine, UPMC-PUH, Mezzanine Level, C-Wing, 200 Lothrop Street, Pittsburgh, PA 15261, USA; 7) Harvey M. and Lyn P. Meyerhoff Inflammatory Bowel Disease Center, Department of Medicine, Johns Hopkins University, 1503 East Jefferson Street, Baltimore, Maryland 21231, USA; 8) Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, 615 E. Wolfe Street, Baltimore, Maryland 21205, USA; 9) Departments of Medicine and Genetics, Division of Gastroenterology, Inflammatory Bowel Disease (IBD) Center, Yale University, 300 Cedar Street, New Haven, Connecticut 06519, USA; 10) Mount Sinai Hospital IBD Centre, University of Toronto, 441-600 University Avenue, Toronto, Ontario M5G 1X5, Canada; 11) Medical Genetics Institute and Inflammatory Bowel Disease (IBD) Center, Cedars-Sinai Medical Center, 8700 W. Beverly Blvd., Los Angeles, California 90048, USA; 12) Department of Gastroenterology and Hepatology, Academic Medical Center, Amsterdam, the Netherlands; 13) Center for Inflammatory Bowel Disease, Department of Gastroenterology & Hepatology, Cleveland Clinic, Cleveland, Ohio, USA; 14) Division of Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Pittsburgh School of Medicine, UPMC-PUH, Mezzanine Level, C-Wing, 200 Lothrop Street, Pittsburgh, PA 15261, USA; 15) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, 130 Desoto Street, Pittsburgh, Pennsylvania 15261, USA; 16) Molecular Biology Department, Massachusetts General Hospital, Harvard Medical School, 185 Cambridge Street, Boston, Massachusetts 02114, USA.

Celiac disease and Crohn's disease are chronic inflammatory diseases, most commonly affecting the small intestine. Several studies have reported the co-occurrence of celiac disease and Crohn's disease within families and individual patients. GWAS studies have identified some 40 Crohn's disease loci and more than 15 celiac disease loci. Interestingly, two risk loci are shared between the diseases: IL2/IL21 (interleukin 2 and 21) and IL18RAP (interleukin 18 receptor accessory protein). This suggests that these diseases have a shared genetic background. We hypothesize that the two identified shared genes represent the tip of the iceberg, leaving more shared risk loci to be discovered. The aim of our study is to perform a meta-analysis on GWAS data from both diseases in order to identify new shared genes for celiac disease and Crohn's disease. For Crohn's disease, data was used from a meta-analysis on three GWAS containing 3230 Crohn's disease cases and 4829 controls (Barrett e.a. Nat Gen 2008). Data for the replication in Crohn's disease was obtained by sequenom genotyping of 973 Crohn's disease cases and 1000 controls. Celiac disease data was taken from a GWAS on 768 cases and 1422 controls (Hunt e.a. Nat Gen 2008). Data for the replication was obtained from a recently completed GWAS on celiac disease containing 3149 cases and 6325 controls. The meta-analysis was performed using an unweighed Z-score method, to control for the differences in study sizes. We took all loci associated to Crohn's disease with P<1x10-3, associated to celiac disease with P<1x10-2 and an overall P value in the meta-analysis of <1x10-5 for replication. The most associated markers in loci meeting the replication criteria were analyzed in the replication cohorts. Sixteen loci met these criteria and eleven of these loci have not yet been implicated in either Crohn's disease or celiac disease. Replication is currently ongoing. Our meta-analysis has identified 11 potentially new loci for Crohn's disease and celiac disease. Most of these risk loci contain genes involved in T cell development and function, similar to those already proven to be shared between celiac disease and Crohn's disease. This finding is in agreement with the known pathology of both diseases in which the initial inflammatory response follows a T helper 1 pattern and a subsequent T helper 17 response in longstanding inflammation.

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## 808/W/Poster Board #466

**TNFR2 associated with hand OA and evidence of quantitative trait loci (QTL) on chromosome 16: the CARRIAGE family study.** *HC. Chen[1], D. Thompson[1], SH. Shah[2,3], YJ. Li[2], S. Nelson[2], C. Haynes[2], J. Johnson[2], T. Stabler[1], ER. Hauser[2], SG. Gregory[2], WE. Kraus[3], VB. Kraus[1].* 1) Division of Rheumatology; 2) Center for Human Genetics; 3) Division of Cardiology, Department of Medicine, Duke University Medical Center.

Background: Osteoarthritis (OA) is a multifactorial disorder with genetic predisposition. Tumor necrosis factor alpha (TNF) has been identified as an important role in cartilage degeneration which initiates a cascade of inflammatory reactions via the TNFR1 and TNFR2 receptors. The aim of this study was to evaluate the soluble tumor necrosis factor (TNF) receptor levels in individuals with hand osteoarthritis (OA) and their genetic contribution in an extended family. Methods: The extended CARRIAGE (CARolinas Region Interaction of Aging Genes and Environment) family consists of 3357 pedigreed members dating back ten generations in the USA. Hand OA status (by American College of Rheumatology Criteria), and serum levels of TNF alpha and TNF receptors were evaluated in 271 family members. The levels of TNF alpha and TNF receptors were measured by ELISA (Biosource, Camarillo, California, USA). Statistical significance between groups (hand OA and non-hand OA) was analyzed using unpaired t test. The genotyping was performed using Illumina BeadChip with 6,090 SNP markers with average spacing of 0.58 cM covering the whole genome. The SOLAR (Sequential Oligogenic Linkage Analysis Routines) package was used to calculate two-point and multi-point probabilities of identical by descent (IBD) using a polygenic model. Results: Mean ln TNFR2 was significantly higher in the hand OA group, compared with the non-hand OA group (7.96+0.65 vs 7.61+0.66, p<0.01), but was not significant after age-adjustment (p=0.16). However, the difference was significant with age-adjustment comparing hand OA and non-hand OA in the > 60 year old individuals (8.08+0.70 vs 7.75+0.68, p=0.039, age-adjusted). There was no difference in TNF alpha and ln TNFR1 between the two groups (2.95+0.50 vs 3.05+0.49, p=0.35 and 8.59+0.49 vs 8.68+0.48, p=0.40, respectively). The maximum multipoint LOD score of 2.0 was achieved for TNFR2 on chromosome 16q12.1 between 51-71 cM. For TNFR1, the maximum QTL were also identified on chromosome 16q (LOD=1.2, 65-87 cM). TNF alpha was also observed to have a peak (LOD=1.2, 49-67 cM) overlapping the regions of TNFR1 and TNFR2. Conclusion: We demonstrated that TNFR2 levels are significantly increased in hand OA individuals in this extended family. This finding has been shown for the disease of rheumatoid arthritis (RA) but not previously for OA phenotypes. We hypothesize that genetic variations linked to TNFR2 may influence the risk for the hand disease of OA.

## 809/W/Poster Board #467

**Linkage and association studies of joint morbidity from rheumatoid arthritis.** *K. Min[1], J. Min[2], S. Cho[3], J. Sung[3].* 1) Ajou University School of Medicine, San 5, Wonchon-dong, Yeontong-gu, Suwon, 443-721, Republic of Korea; 2) Institute of Health & Environment, Seoul National University, 28 Yeongun-dong, Jonno-gu, Seoul, 110-460, Republic of Korea; 3) Department of Epidemiology, School of Public Health, Seoul National University, 28 Yeongun-dong, Jonno-gu, Seoul, 110-460, Republic of Korea.

Purpose.Rheumatoid arthritis (RA) is a chronic and progressive inflammatory disease occurring in the synovial membrane that can lead to joint deformity and cartilage destruction. We investigated the relationship between genetic susceptibility and rheumatoid arthritis (RA) severity in terms of joint morbidity, using a linkage study and family-based association tests. Methods. The dataset included 723 families with 990 affected sibling pairs with RA. The Illumina linkage panel IV included 5,858 SNPs, with 5,744 SNPs passing quality control filters. The phenotypic variables analyzed were the level of rheumatoid factor (RF) and score on the Joint Alignment and Motion (JAM) scale. In affected siblings, the mean values were 261.6 IU/ml (range = 8-6920 IU/ml) for RF levels and 30.8 (range = 0-118) on the JAM scale. We modified the scale dividing by RF values relevant to disease severity (JAM-RF ratio = JAM scale score/RF). Patients with lower ratios presented more severe symptoms in terms of joint mobility and/or higher RF levels. Linkage analysis for affected sibling pairs was done using the MERLIN program, and family-based association tests were carried out by using PLINK and FBAT software. Results. For linkage analysis, we found a high peak (log of the odds [LOD] = 2.66; nonparametric linkage [NPL] Z = 3.23) near the HLA-DRB1 region on chromosome 6; HLA-DRB1 may be not only the major region of genetic susceptibility to RA but may be also a potential region for the joint deformity and loss of joint motion that occurs with RA. The linkage at 6p24 at rs1410766 (LOD = 3.29; NPL Z = 4.07) was statistically significant. The linked region on chromosome 6p24 is located less than 1 cM from the bone morphogenetic protein 6 (BMP6) gene. Two other regions also showed possible linkage peaks: chromosome 7q30 at rs322812 (LOD = 2.47; NPL Z = 3.39) and chromosome 15p34 at rs347117 (LOD = 1.95; NPL Z = 2.80). For the family-based association study, there were seven SNPs, for which only three genes could be found: triadin (TRDN), Src homology 2 domain containing family member 4 (SHC4), and hypothetical protein FLJ23834 (FLJ23834). Conclusion. We found two significant linkage at 6p21-24 and suggestive linkage peaks at 7q30 and 15p34. Seven SNPs were detected from family-based association tests. Our results imply that genetic variations may lead to an enhanced risk of joint damage and increased levels of RF.

## 810/W/Poster Board #468

**Percent African Ancestry is Significantly Related to Hip Structural Geometry.** *L. Qi[1], Z. Chen[2], T. Beck[3], J. Robbins[4], G. Wu[2], B. Lewis[5], J. Cauley[6], N. Wright[2], M. Seldin[1].* 1) Rowe Program in Human Gen, Univ California, Davis, Davis, CA; 2) Division Epi/Biostat, U Arizona, Tucson, AZ; 3) Quantum Medical Metrics, LLC, Baltimore, MD; 4) Dept. General Med., Univ California, Davis, Davis, CA; 5) Univ of Alabama at Birmingham, Birmingham, AL; 6) Univ of Pittsburgh, Pittsburgh, PA.

African Americans have a significant lower fracture risk in comparison to non-Hispanic whites in the United States. Higher bone density and favorable femur geometry have been found in African Americans, but the relative contributions of genetic vs. environmental components to apparently stronger bones in African Americans remains an interesting research area. In this study we investigated the association between percentage of African ancestry and femur BMD and geometry in a subgroup of participants from the Women's Health Initiative. All participants underwent hip dual-energy x-ray absorptiometry (DXA) and femoral narrow neck structural geometry was analyzed using Beck's method on hip DXA scans. Based on >1000 well distributed ancestry informative markers that distinguish between sub-Saharan African and European ancestry, the percentage of African ancestry for each African American individual was determined. Of the 739 African American Women, 19 women had <50% African ancestry, 162 women had 50-74% and 558 women had 75-100% African ancestry. A total of 8,206 self-identified non-Hispanic white were included as the comparison group. BMD, cross-sectional area and section modulus (an index of bone bending strength) significantly increased with increased % African ancestry. Buckling ratio (an index of susceptibility to cortical failure under compression) reduced significantly with increasing % African ancestry. Outer diameter at the femoral narrow neck region was slightly smaller in women with high % African ancestry. While women with higher percent African ancestry were younger and heavier, the racial differences in femoral neck geometry still existed after adjusting for age, weight, total calcium intake and hormone use. In conclusion, indices of bone strength increased in women with higher percentage of African heritage, suggesting a significant genetic contribution to reduced fracture risk observed in the African Americans. Larger cross-sectional area plays a major role in the increased bone strength in older African American women.

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## 811/W/Poster Board #469

**Failure to confirm association of three polymorphisms of rheumatoid arthritis susceptibility identified by the Wellcome Trust Case Control Consortium in a Japanese population.** 
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Rheumatoid arthritis (RA) is believed to be a complex multifarious disease that is influenced by both genetic and environmental factors. There is increasing evidence that genome-wide association studies (GWAS) represent a powerful approach to the identification of genetic markers. The Wellcome Trust Case Control Consortium (WTCCC) published GWAS using 1860 RA patients and 2938 controls in a British population. The WTCCC study confirmed the association of SNPs with previously identified loci within the HLA region and PTPN22 gene, and nine more loci were found to be associated in the next level (our replication study on these SNPs have been presented at the ASHG meeting 2008). Subsequently, they reported 49 SNPs were that associated with RA in the third level ($P=1 \times 10^{-4}$ - $1 \times 10^{-5}$). Three of these could be replicated in a validation cohort of 4106 patients and 11238 controls; PRKCQ (rs4750316), KIF5A (rs1678542) and IL2RB (rs3218253). The aim of our study is to replicate the association of these SNPs with susceptibility to RA in a Japanese RA cohort. The present study is part of a RA cohort project with an enrollment of nearly 5000 patients established by the Institute of Rheumatology, Tokyo Women's Medical University. DNA samples from 1504 patients and 752 controls were used for the study. Genotyping was performed using the TaqMan assay according to the manufacture's instructions (Applied Biosystems, Japan). Chi-squared test was performed for statistical analysis, using the R software package. No significant differences were observed in allele frequencies of the three SNPs between cases and controls (rs4750316 [P=0.51, OR=1.07, 95%CI= 0.87-1.32], rs1678542 [P=0.15, OR=1.12, 95%CI=0.95-1.31], rs3218253 [P=0.88, OR=1.02, 95%CI=0.78-1.34]). Our data could not support the evidence of association between these SNPs and RA. Although strong association was found in European descents, these reported susceptibility genes analyzed here are not associated with RA susceptibility in a Japanese population. The result suggests that the SNPs may be associated with RA susceptibility in a specific ethnic group.

## 812/W/Poster Board #470

**The histidine-triad nucleotide binding protein is associated with nicotine dependence and is increased in the nucleus accumbens after chronic nicotine exposure.** 
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The histidine triad nucleotide binding protein (HINT1) is widely expressed in mesocortical and mesostriatal brain regions; however, little is known about the physiological function of this protein. Recent expression and association studies indicate that the HINT1 gene is associated with schizophrenia. Additionally, HINT1 knockout mice display altered responses to morphine and amphetamine. Nicotine dependence is highly comorbid with other substance abuses and schizophrenia. Although studies implicate a role for HINT1 in schizophrenia and drug dependence, there are no available studies assessing the role of this protein in nicotine dependence. Thus, using association and expression studies, this study aimed to examine the involvement of the HINT1 gene in nicotine dependence. Association analyses revealed that variants in the HINT1 gene previously found to be associated with schizophrenia, are also associated with nicotine dependence. Further, human mRNA expression data revealed that smoking significantly influences HINT1 expression in the brain. Additionally, western blot analysis of HINT1 protein level in the mouse PFc, hippocampus, nucleus accumbens (NAc), and ventral tegmental area (VTA) were assessed following acute or chronic nicotine, and after nicotine withdrawal. Results show no change in protein level after acute nicotine; however, there was a significant increase in HINT1 protein level in the NAc after chronic nicotine exposure, which was reduced after precipitated and spontaneous nicotine withdrawal. These results demonstrate a genetic association between variants in HINT1 and nicotine dependence, and indicate that nicotine-induced increases in HINT1 level may compensate for the HINT1 deficiency observed in the PFc of schizophrenia patients. Future studies will further examine the role of this gene in nicotine dependence by testing HINT1 knockout mice in established nicotine reward and withdrawal behavioral models. Overall, these studies will aid in elucidation of genetic and molecular mechanisms that contribute to excess smoking in schizophrenia patients.

## 813/W/Poster Board #471

**Familial aggregation of melanoma and co-aggregation with other cancers.** 
*S.V. Ward[1], R.J. Webster[1], J.G. Dowty[2], J.L. Hopper[2], L.J. Palmer[1].* 1) Centre for Genetic Epidemiology and Biostatistics, The University of Western Australia, Nedlands, Western Australia, Australia; 2) Centre for Molecular, Environmental, Genetic and Analytic (MEGA) Epidemiology, The University of Melbourne, Melbourne, Victoria, Australia.

Melanoma is the most aggressive form of skin cancer and Australia has one of the highest rates in the world. Epidemiological research has identified some of the environmental and genetic risk factors of the disease, including family history of melanoma. However, little is known about the relationship between family history of melanoma and an individual's risk of developing other forms of cancer. Some evidence exists for familial aggregation of melanoma with pancreatic cancer and glioma but little research has been undertaken in the Australian population. The purpose of this study was to investigate the familial aggregation of melanoma and its co-aggregation with other types of cancer in the Western Australian (WA) general population. WA has the second highest rate of melanoma in Australia. All data were obtained from the WA Data Linkage System, a population based database linking WA's core health datasets. Linked data from the Family Connections database, which maps family history, and the WA Cancer Registry were used for this study. Cancers considered were invasive breast, lung, prostate, cervical, colorectal, thyroid, brain, mouth, testicular, and kidney cancers, non-melanoma skin cancers, lymphoma and melanoma, and were all diagnosed between 1981 and 2006. The total sample size was 1,530,147 individuals, which included 5,478 individuals with invasive melanoma. The number and type of cancers diagnosed during the time period were calculated for each individual. Risk models were then developed using Cox regression modeling and survival analyses. Analyses showed that individuals who had one or more first degree relatives (FDRs) with melanoma had an increased risk of developing melanoma themselves ($p<0.001$). The risk increased by a factor of 4.15 (95% confidence interval (CI) 3.25-5.3) for each FDR with melanoma. This result is consistent with findings in other populations that family history of melanoma is a risk factor for the disease. Individuals who had one or more FDR with melanoma were also at an increased risk of developing non-melanoma cancer (p=0.047). For each FDR with melanoma, the risk of developing non-melanoma cancer increased by a factor of 1.32 (95% CI 1-1.73). Further, the risk of melanoma increased for individuals by a factor of 1.43 (95% CI 1.09-1.87) for each FDR with a non-melanoma cancer (p=0.0094). These findings provide additional support for familial aggregation between melanoma and other forms of cancer.

## 814/W/Poster Board #472

**Familial aggregation of breast cancer with cancers at other sites: Survival analysis of data from the Western Australian Data Linkage System.** 
*R.J. Webster[1,3], J.G. Dowty[2,3], J.L. Hopper[2], L.J. Palmer[1].* 1) Centre for Genetic Epidemiology and Biostatistics, The University of Western Australia, WA, Australia; 2) Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne, VIC, Australia; 3) Authors contributed equally.

Family history is one of the biggest known risk factors for many common cancers, indicating that factors pertaining to the shared environment and/or genetic makeup of family members predispose to these diseases. Numerous epidemiological studies have also shown familial aggregation of different types of cancer, suggesting that those cancers may have similar such risk factors in common. Breast cancer risk has a strong familial component, and among the many susceptibility alleles now identified are the BRCA1 and BRCA2 mutations, which predispose to both breast and ovarian cancer. The purpose of this study was to investigate the co-aggregation of breast cancer with cancers at other sites within families in a Western Australian population. The study group was derived from the Western Australian Data Linkage System (WADLS), a dataset that comprises over 30 years of linked pedigree and health data on the entire population of the state of Western Australia, and is one of the largest population-based datasets in the world. The study group includes 1,530,147 individuals with a median age of 31 (95% confidence interval (CI) 3-60) years. The median family size is 4 (95% CI 3-10). Analyses aimed to determine whether the relatives of women with breast cancer have a heightened risk of other cancers. Survival analyses using the Cox proportional hazards model were performed with the time to development of different types of cancer as the end-points. Risk ratios corresponding to covariates derived from the individual's family history of breast cancer were estimated. The risks of invasive melanoma and colorectal cancer were found to be greater in the first degree relatives of patients with invasive breast cancer (p=0.04 and p=0.04, respectively), with the risks multiplicatively increased by a factor of 1.76 (95% CI 1.02-3.04) and 3.20 (95% CI 1.03-9.97), respectively, for each first degree relative with invasive breast cancer. These results suggest that familial risk factors for breast cancer may also increase susceptibility to melanoma and/or colorectal cancer.

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## 815/W/Poster Board #473

**Association of Sirtuin 1 (SIRT1) gene variation and transcription levels with severe obesity.** *J. Andersson[1], M. Falchi[1], S. Clark[1], B. Olsson[2], D. Meyre[3], C. Lecouer[3], P. Jacobson[2], L. Sjöström[2], L. Carlsson[2], P. Froguel[1,3], A. Walley[1].* 1) Section Genomic Medicine, Imperial College London, London, United Kingdom; 2) Department of Molecular and Clinical Medicine and Center for Cardiovascular and Metabolic Research, The Sahlgrenska Academy, Gothenburg, Sweden; 3) CNRS 8090-Institute of Biology, Pasteur Institute, Lille, France.

**Background:** The SIRT1 gene is important in the regulation of lipid and glucose metabolism. Variants in the gene have been associated with levels of energy expenditure and activators of the SIRT1 protein protect against obesity making it a clear candidate gene for common polygenic obesity. This study was designed to investigate a possible association between SIRT1 variants and obesity in both severely obese adults and children and to examine whether expression levels of the SIRT1 gene affect susceptibility to the disease. **Subjects and Methods:** Three tag-SNPs together with twenty-four additional SNPs chosen for even coverage of the 34kb SIRT1 gene were selected for genotyping purposes. The genotyping was carried out using Sequenom iPLEX assays. Case-control analyses were performed using 1533 obese subjects (896 adults with BMI over 40kg/m$^2$ and 637 children with a BMI>97$^{th}$ percentile for age and sex) and 1237 non-obese controls, all French Caucasians. Expression of SIRT1 was measured in the siblings from the SibPair cohort, which comprises 154 nuclear families (732 subjects) from Sweden, each containing an obesity-discordant sib pair (at least 10 kg/m$^2$ difference in body mass index). Subcutaneous adipose tissue was obtained via punch biopsy. Analysis was only carried out using the same sex BMI-discordant sibpairs to avoid the effect of gender-discordance. **Results:** Two SNPs, rs33957861 and rs11599176 were found to be associated with adult obesity (p-values: 0.005 and 0.003 respectively) and a third SNP, rs2234975 was associated with childhood obesity (p=0.006). These p-values remained significant after correction for multiple testing using the method of Nyholt. However, no significant association was observed with BMI adjusted for age and sex in either adults or children. SIRT1 transcript expression was found to be negatively correlated to BMI and lower SIRT1 expression was associated with obesity (p-values: 3.7x10$^{-19}$ in 76 female sibpairs and 0.0025 in 12 male sibpairs). Genotyping in these families also revealed an association between four SIRT1 SNPs (rs11599176, rs12413112, rs33957861 and rs35689145) and BMI (p values= 0.0004, 0.0006, 0.0004 and 0.002 respectively). **Discussion:** We have demonstrated that variants in the SIRT1 gene are associated with severe obesity in both adults and children and that SIRT1 expression is associated with the disease.

## 816/W/Poster Board #474

***NRF2* polymorphisms are not associated with the childhood-onset asthma susceptibility in Mexican population.** *E. Cordova[1], F. Centeno[1], S. Jiménez[1], NE. Martínez-Aguilar[2], BE. Del Rio-Navarro[3], J. Gómez-Vera[4], E. Navarro-Olivos[5], L. Orozco[1,6].* 1) Investigacion, Inst Natl de Medicina Genomica, Mexico D.F., Mexico; 2) Servicio de Alergia, Hospital Regional 1 de Octubre, ISSSTE; 3) Departamento de Alergia, Hospital Infantil de México; 4) Servicio de Alergia, Hospital Regional Adolfo López-Mateos; 5) Programa de Maestría en Ciencia Médicas, Instituto Politécnico Nacional; 6) Posgrado Ciencias Genómicas, Universidad Autónoma de la Ciudad de México.

Oxidative stress has been recognized as an important factor in asthma pathogenesis. Recent reports have shown that the activation of the Nuclear erythroid 2-related factor 2 (*NRF2*) gene confers cellular protection against different asthma-related factors such as cigarette smoke, DEPs and atmospheric particulate matter. Nrf2 is a basic leucine zipper transcription factor that regulates the expression of antioxidant and detoxificant genes. Germinal deletion of *nfr2* in mice increases cell susceptibility to several environmental insults and pathogenic states. In addition, SNPs located in the promoter region of *NRF2* (-653G/A, and -617C/A), which decrease NRF2 gene expression, have been associated to different diseases. In this same context, *nrf2* -/- mice showed a higher susceptibility to asthma development, after a challenge with ovoalbumine. These data strongly suggest a possible role of Nrf2 in asthma susceptibility. Thus, the aim of this study was to determine whether the -653G/A, and -617C/A *NRF2* polymorphisms are associated to susceptibility to childhood-onset asthma in a Mexican-Mestizo population. We studied 242 unrelated patients with childhood-onset asthma and 358 unrelated sex-matched healthy individuals. All the participants were recruited from four tertiary level Institutions from Mexico City and have Mexican-mestizo origin. Genotyping of -653G/A and -617C/A SNPs was performed by TaqMan assay. The association test, Hardy-Weinberg equilibrium (H-WE) and haplotypes were determined using EPIDAT, FINETTI and Haploview softwares, respectively. The two Nrf2 SNPs were found in Hardy-Weinberg equilibrium, both in cases and controls. The *NRF2* polymorphisms, showed an strong linkage disequilibrium (LD) (D'=0.97) and a high frequency of the risk alleles -653A and -617A was observed (0.40; an 0.24;, respectively). No significant differences were observed in the genotype and allelic distribution between healthy and asthma individuals, even after gender stratification. Similarly, haplotypes analysis failed to detect any association with asthma. Despite we were unable to observe an association between *NRF2* gene variants and asthma manifestation, we cannot discard that this gene could be associated with severity of asthma. Further studies with careful and deep emphasis in clinical manifestation of asthma are necessary to define the role of *NRF2* in this disease.

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## 817/W/Poster Board #475

**Functional Variant of the Bitter Taste Receptor Gene Associated with Oral Microbial Profiles in the COHRA Study.** *K.T. Cuenco[1,2,3], S.K. Wendell[1,2], J.G. Thomas[4], D.M. Gray[4], X. Wang[1,2], R.J. Weyant[5], R.M. Crout[6], D.W. McNeil[7], M. Marazita[1,2,3,8].* 1) Center for Craniofacial and Dental Genetics, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; 2) Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; 3) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 4) Department of Pathology, School of Medicine, West Virginia University, Morgantown, WV; 5) Department of Dental Public Health/Information Management, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; 6) Department of Periodontics, School of Dentistry, West Virginia University, Morgantown, WV; 7) Dental Practice and Rural Health, West Virginia University, Morgantown, WV; 8) Clinical and Translational Sciences Institute and Department of Psychiatry, School of Medicine, University of Pittsburgh, Pittsburgh, PA.

Our understanding of the role of host genetics and oral microbes on caries has been evolving. Estimates of caries heritability range from 0.39 to 0.66 (see abstract submission #21309). Host susceptibility to pathogen colonization in the oral cavity may be dictated by innate taste preference, establish preferential microbial profiles, and explain the shared genetic component underlying caries. A major pathogen related to caries is *Streptococcus mutans*, but recent evidence suggests that other flora in addition to *S mutans* affect caries development. Microbial testing for *S mutans*, periodontal-related flora, and common nosocomial pathogens (including *Staphylococcus aureus*) found in the oral cavity and proximal throat sites have been assessed in COHRA families from the Appalachian region. We evaluated 8 SNPs in taste genes (TAS2R38, TAS1R1, TAS1R2, and GNAT3) with selected microbial joint outcomes in 705 families. Single outcome association between *S mutans* and caries was mild. However, joint assessment of multiple microbial organisms suggested an association with increased odds of severe caries. Based on these epidemiologic evaluations of microbes, the joint microbial profile of nosocomial and oral disease pathogens were implemented in family based SNP association analyses. A mis-sense mutation (rs10246939) in TAS2R38 was associated with a specific microbial profile (125 informative families, $p = 0.0146$) and persists after adjustments for potential confounding by oral disease. This finding suggests that a taste receptor variation can impact host microbial communities in the oral cavity and may have a fundamental role in microbial colonization that dictates subsequent oral health outcomes. We are currently more closely assessing the effects of oral disease, evaluating other potential health outcomes (e.g. body mass index, caries endophenotypes), and their relationship with this taste receptor variant and corresponding microbial profiles. These findings additionally highlight the importance of assessing joint microbial profiles in genetic epidemiologic studies. R01 DE 014899.

## 818/W/Poster Board #476

**ITGAM and Systemic Lupus Erythematosus (SLE) Susceptibility: A Meta-analysis.** *X. Kim-Howard, S. Nath.* Oklahoma Medical Research Foundation, Oklahoma City, OK.

Introduction: We identified ITGAM as a strong systemic lupus erythematosus (SLE) susceptibility gene which was also replicated in 3 genome-wide association studies (GWAS). We narrowed down the association interval and pinpoint the causal association with a coding variant, rs1143679 (A/G) at exon-3. Using imputation-based association analysis followed by conditional analysis we showed that rs1143679 was the only causal SNP within ITGAM. We also replicated this association in 5 ethnically diverse populations. Recently 2 other reports also replicated this association. Objectives: The objectives of this study are to 1) perform a meta-analysis on 23 independent data sets and 2) perform geographic region-specific meta-analyses and assess the heterogeneity of the genetic effect between these regions. Methods: For our meta-analysis we used 23 independent data sets from 17 countries which represented 5 geographic regions. To test for geographic region-specific effect associated with rs1143679, we performed broad region-specific meta-analyses including European (10 data sets, N=4324), European-American (2 data sets, N=5602), African-derived (2 data sets, N=1560), Latin-Americans (4 data sets, N=2832), and Asians (5 data sets, N=3849). Results: The overall meta-analysis combining European-derived, African-derived, Latin-American, and some Asian independent data sets greatly reinforces the genetic association (Pmeta=2.15x10-67, OR=1.79, 95% CI=1.68-1.92). The overall heterogeneity of the odds ratio (OR) was not significantly different across populations (p=0.50). Robustness of rs1143679 association with SLE was confirmed in 5 ethnically diverse groups, including European (Pmeta=1.13x10-25), European-American (Pmeta=1.20x10-24), African-derived (Pmeta=1.00x10-5), Latin-Americans (Pmeta=9.55x10-13), and some Asian populations (Pmeta=4.68x10-6; 2 data set) but not in other Asian populations (3 data sets), where rs1143679 was monomorphic for the non-risk allele (G). The heterogeneity of the ORs were not significant across any of the regional associations (lowest p=0.26) Conclusions: The SNP rs1143679 has shown robust association across geographically diverse populations. From the allele frequency spectrum it is shown that the risk allele (A) is most prevalent in European populations (12%-31%) and least prevalent in Asian populations (0%-6%). Although significant differences exist in allele frequency heterogeneity, ORs were homogeneous across populations.

## 819/W/Poster Board #477

**Association and interaction analyses of GABBR1 and GABBR2 with nicotine dependence.** *M. Li[1], J.E. Mangold[1], G.-B. Chen[1], C. Seneviratne[1], J.Z. Ma[2], X.-Y. Lou[1], T.J. Payne[3].* 1) Department of Psychiatry and Neurobehavioral Sciences, University of Virginia, Charlottesville, VA; 2) Department of Public Health Sciences, University of Virginia, Charlottesville, VA; 3) ACT Center for Tobacco Treatment, Education and Research, Department of Otolaryngology and Communicative Sciences, University of Mississippi Medical Center, Jackson, MS.

Previous studies demonstrate that the $\gamma$-aminobutyric acid type B (GABAB) receptor plays an essential role in modulating neurotransmitter release and regulating the activity of ion channels and adenyl cyclase. However, whether GABAB receptor subunit genes are associated with nicotine dependence (ND) remains largely unknown. In this study, we genotyped 6 and 33 single nucleotide polymorphisms (SNPs) for GABAB receptor subunit 1 and 2 genes (GABBR1, GABBR2), respectively, in a sample of 2037 individuals from 602 nuclear families of African American (AA) or European American (EA) origin. Association analyses were conducted for each gene with ND at both the individual SNP and haplotype levels. We also conducted interactive analysis of the two genes in affecting ND by using a pedigree-based generalized multifactor dimensionality reduction method. We found that several SNPs and haplotypes in GABBR2 were significantly associated with ND in both ethnic samples. Interestingly, these significant haplotypes were located in different protein domains for the AA and EA samples. In addition, we found two minor haplotypes in GABBR1 to be associated with ND in the EA sample. Finally, we demonstrated the presence of epistasis of SNPs in GABBR2 for both samples, as well as between GABBR1 and GABBR2 in the EA sample. We conclude that variants of GABBR1 and GABBR2 are significantly associated with ND. Our findings imply that both GABAB receptor subunit genes are involved in the etiology of ND, although the involvement of GABBR1 is primarily through its interaction with GABBR2, whereas GABBR2 has a direct effect on ND. (This study is being supported by NIH grants DA-12844 and DA-025095.).

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## 820/W/Poster Board #478

**Strong association between an ADRB2 haplotype and obesity in Mexican Population.** *M. Salas-Martínez[1,2], Y. Saldaña-Alvarez[1], S. Jiménez-Morales[1], A. Luckie-Duque[3], G. García-Cárdenas[4], H. Vicenteño-Ayala[5], A. Carnevale-Cantoni[6], L. Orozco[1,2].* 1) Instituto Nacional de Medicina Genómica-INMEGEN; 2) Posgrado en Ciencias Genómicas-UACM; 3) Hospital Regional 1° de Octubre-ISSSTE; 4) Clínica de Diagnóstico Automatizado-ISSSTE; 5) Hospital Regional López Mateos-ISSSTE; 6) Coordinación de Medicina Genómica-ISSSTE. Mexico City, Mexico.

Introduction. Beta-2-adrenergic receptor gene (ADRB2) is involved in several mechanisms related with obesity, like thermogenesis regulation and lipolysis activation. Several single nucleotide polymorfisms (SNPs) located in this gene have been identified as genetic risk factors to obesity in several populations, such as Caucasians and Asian. Objective. The aim of this study was to investigate the association of the Gly16Arg (rs1042713 G/A), Glu27Gln (rs1042714 C/G) and Leu84Leu (rs1042717 G/A) polymorphisms located in the ADRB2 gene with obesity in Mexican population. Methods. We performed a case-control association study in 423 obesity cases with a BMI>30 and 268 unrelated non-obese subjects with a BMI<25. Cases and controls were recruited from two tertiary Hospitals in Mexico City. Genotyping was carried out by the 5' exonuclease assay (TaqMan). The association test, Hardy-Weinberg equilibrium (HWE) and haplotypes were determined using EPIDAT, FINETTI and HAPLOVIEW softwares, respectively. Results. All genotypes distribution in patients and controls were in HWE. We did not observe significant differences in the genotype and allelic distribution between obese and non-obese individuals. However, the haplotype analysis reveled five common haplotypes ( frequencies higher than 1%: ACG, GCA, GGG,GCG and ACA). Interestingly, haplotype frequencies distribution analysis between cases and controls showed a susceptibility obesity haplotype (GCG) harboring all ancestral alleles, showing a higher frequency in cases than in controls [7% Vs 1,7%; OR= 4.16; 95% CI=(1.96-9.09), p=0.000017 ]. Conclusion. Our results suggest that ADRB2 haplotypes could be a genetic factor conferring one of the highest risk to develop obesity. Further studies are necessary to understand the role of ADRB2 gene in obesity.

## 821/W/Poster Board #479

**SNPs associated with C-reactive protein levels in a cohort of young Filipino adults.** *G. Curocichin[1,2], T. McDade[3], C. Kuzawa[3], J. Borja[4], L. Qin[1], D. Croteau-Chonka[1], A. Marvelle[1], E. Lange[1,5], L. Adair[6], K. Mohlke[1], L. Lange[1].* 1) Department of Genetics, University of North Carolina, Chapel Hill, NC; 2) Department of Family Medicine, Moldova State Medical and Pharmaceutical University, Chisinau, Moldova; 3) Department of Anthropology, Northwestern University, Evanston, IL; 4) Office of Population Studies, University of San Carlos, Cebu City, Philippines; 5) Department of Biostatistics, University of North Carolina, Chapel Hill, NC; 6) Department of Nutrition, University of North Carolina, Chapel Hill, NC.

C-reactive protein (CRP) is an important risk factor for cardiovascular disease. However, the effect of genetic variants on risk in young adults and in Asian populations is not clear. Studies in European midlife-to-older adult populations have identified SNPs rs1205 in the 3'UTR of CRP and rs1169288 (Ile27Leu) in HNF1A as associated with serum CRP levels. We tested these SNPs for association with plasma CRP levels in young adults (21-23 years) from the Cebu Longitudinal Health and Nutrition Survey (CLHNS) and their mothers both separately, using analysis of variance, and in a combined analysis, using general linear mixed models. A total of 1,692 young adults and 1,782 mothers (35 to 69 years) were analyzed. Median CRP levels (inter-quartile range) were 0.2 (1.0) mg/L in the young adults (range 0 to 85.9) and 0.9 (2.5) mg/L in the mothers (range 0 to 122.6). CRP levels were log-transformed and genotype was modeled as an additive effect. Log-CRP was significantly lower in the young adults than the mothers (p<0.0001). Both SNPs were strongly associated with log-CRP in the young adults and their mothers analyzed separately (all p<2x10-7) and in the combined sample (rs1205, p=6.3x10-15; rs1169288, p=2.6x10-11). In the young adults, rs1205 explains 1.7% and rs1169288 explains 1.1% of the variability in log-CRP levels. In the mothers, rs1205 explains 2.0% and rs1169288 explains 1.6% of the variability. There was no evidence for either a genotype-by-BMI or genotype-by-genotype interaction in either group. The role of hygiene remains to be analyzed. These results indicate that the CRP SNP rs1205 and the HNF1A SNP rs1169288 are associated with CRP levels in both the young adults and the mothers in this Filipino cohort, and confirm associations identified in European populations in a population of Asian descent. While CRP levels are significantly higher in the mothers versus the young adults, the effect sizes are similar in both subsets, suggesting a consistent effect of these genes on CRP levels across a wide age range. The strong evidence for association in the young adults suggests that younger populations may also be valuable in identifying genetic loci for cardiovascular disease risk factors.

## 822/W/Poster Board #480

**GENETICS OF COMITANT STRABISMUS: A STUDY BASED AT CHILDREN'S HOSPITAL BOSTON.** *C. Andrews[1, 3], S. Mackinnon[2], D.G. Hunter[2], E.C. Engle[1,2,3].* 1) Dept Neurology and Pediatrics (Genomics), Children's Hosp, Boston, Boston, MA. USA; 2) Dept Ophthalmology, Children's Hosp, Boston, Boston, MA. USA; 3) Howard Hughes Medical Institute.

Strabismus is a misalignment of the eyes that includes various forms of horizontal and vertical deviations. The most common forms of strabismus are comitant, with a similar magnitude of deviation in all gaze positions. Comitant strabismus affects 2-4% of the population, and it can cause vision loss through amblyopia (vision loss in a structurally normal eye) or by reducing binocular vision and stereopsis. The etiology of comitant strabismus is generally unknown, although hyperopic refractive error is a recognized risk factor, and amblyopia may sometimes occur in the absence of strabismus as a result of refractive error. Comitant strabismus clusters in families and appears to segregate as a complex trait. To identify genetic contributions to comitant strabismus and amblyopia, we have undertaken a prospective study of Children's Hospital Boston Department of Ophthalmology patients with comitant strabismus or its risk factors, enrolling probands and all available family members, whether affected or unaffected. Participants undergo an ophthalmic examination, and provide a blood/salivary specimen as well as medical, birth, and family history. To date, 605 probands (51% male, 49% female; 70% Caucasian), 199 affected family members, and 1290 unaffected family members have been enrolled in the study. Of the affected probands, 382 have esotropia, 151 have exotropia, 62 have anisometropia, and 10 have high hyperopia. Within this population, 51% of the pedigrees report at least one additional affected first, second, and/or third degree relative (6 or more individuals affected in 9 pedigrees, 5 affected in 13 pedigrees, 4 affected in 28 pedigrees, 3 affected in 88 pedigrees, and 2 affected in 173 pedigrees). The calculated relative risk of strabismus to a sibling of an affected individual (lS) is 4, presuming an incidence of strabismus within the general population of 3%. These preliminary data support a genetic contribution to strabismus and a significantly high lS to support a search for strabismus genes in this population. We are now beginning linkage analysis of the pedigrees to the several potential loci identified to date, including STBMS1, and are continuing ascertainment for a comitant strabismus GWAS study.

## 823/W/Poster Board #481

**Heritable and lifestyle effects on sleep/wake patterns in the Old Order Amish.** *D.S. Evans[1], S.-H. Wu[1], A. Mody[2], A.R. Shuldiner[3], S. Snitker[3], W.-C. Hsueh[1].* 1) Department of Medicine, University of California, San Francisco, San Francisco, CA; 2) Department of Medicine, Duke University, Durham, NC; 3) Department of Medicine, University of Maryland, Baltimore, MD.

Sleep/wake patterns in humans are regulated by at least two factors: the biological circadian rhythm and entraining environmental stimuli. The goal of our study is to characterize both the non-genetic and heritable factors that are associated with sleep patterns in the Old Order Amish, whose agrarian lifestyle is more homogeneous than modern societies.

The study population of 868 healthy adults (53% men) had a mean age of 43.8 ± 14.0 years (range: 20-80 yrs). 81% of men were farmers or skilled tradesmen and 75% of women were home-makers. Subjects wore accelerometers 24 h/day for 7 days. The actigraphy records were analyzed to determine weekly averages for (1) the time of day when subjects fell asleep (sleep time), (2) wake-up time, (3) sleep duration, and (4) physical activity levels (total counts). Subject-specific sleep and wake-up times were validated in a substudy of 179 subjects who kept sleep diaries (r = 0.83 for wake-up time and 0.76 for sleep time, both P < 0.001). All analyses were performed conditioned on the relatedness of individuals using the SOLAR program.

On average, both men and women went to sleep at 10:00PM ± 44 min, but men woke up earlier than women (4:59AM ± 46 min and 5:12AM ± 44 min, respectively, P = 0.0001). Correspondingly, mean sleep duration was shorter in men compared to women (7.0 ± 0.9 hours and 7.2 ± 0.8 hours, respectively, P = 0.03). Seasonal and household effects were significantly associated with all three sleep parameters. In addition, the average wake-up time was significantly associated with sex, age, occupation, and physical activity levels, the average sleep time with age, and the average sleep duration with occupation and total activity levels. Subjects with higher activity levels had shorter sleep durations and earlier wake-up times. Older subjects had earlier wake-up times and sleep times, but sleep durations were not affected by age. After adjusting for the aforementioned factors, there was no significant heritability for sleep times or sleep duration, while the heritability for wake-up times was 0.19 ± 0.10 (P = 0.02). In conclusion, in the Amish, wake-up times appear to have a significant heritable component even after adjusting for household effects.

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## 824/W/Poster Board #482

**Genetic variations in genes of the axon guidance pathway associated with Adolescent Idiopathic Scoliosis (AIS) etiology and progression.** *R.A. Macina, J. Gu, L. Nelson, R. Chettier, K. Ward.* Axial Biotech, Inc., Salt lake City, UT.

Adolescent Idiopathic Scoliosis (AIS) is a complex, familial disorder characterized by a lateral deformity of the spine, affecting 2-3% of the pediatric population. Up to this date, no single causative factor for the development of AIS has been described. Even though neurological defects have been associated with scoliosis, it is not clear whether these observations are causative or secondary to the development of the disease. Genomic DNA samples from 700 severe AIS patients and 1500 controls were used in a genome-wide association study (GWAS)utilizing Affymetrix 6.0 HuSNP arrays.This genome-wide study identified, among others, 53 single nucleotide polymorphisms (SNPs) thoroughly validated in clinical settings to provide significant predictive value for AIS progression. These 53 genetic markers are the components of our AIS prognostic Test (AIS-PT). Two of these 53 genetic markers map to genes that belong to the axon guidance pathway. These SNPs are rs6693477 (P value=1.92E-.05, OR=1.331) in the proximity of EFNA1 and EFNA3, and rs7802114 (P value=9.21E-05, OR=0.4875) intronic to PLXNA4. To further investigate the role of this pathway in the etiology of AIS, we used a genomic pathway approach to mine the genome-wide association dataset to identify SNPs that are within axon guidance pathway genes. Preliminary results of this genomic pathway approach identified a number of SNPs with a significant association to the disease in an additional axon guidance gene, SLIT3 when comparing severe AIS patients to controls. We will show results of applying this genomic pathway approach to a large number of axon guidance pathway genes and we will use the SNPs that show association to the disease to determine if they have a predictive value for AIS progression and could enhance the current AIS-PT. Our results demonstrate that genetic variations in multiple genes in the axon guidance pathway are associated with the etiology of AIS and the progression of the disease. This is the first report of single nucleotide polymorphisms within genes involved in nervous system development contributing to the pathogenesis of AIS and its progression. Moreover, this discovery could lead to the development of additional novel diagnostics and prognostics tests as well as novel therapeutic approaches for adolescent idiopathic scoliosis.

## 825/W/Poster Board #483

**Differential Expression of Cytokines as an Indicator for Classification of Autism Phenotypes.** *T.L. Pawlowski[1], A.N. Allen[1], J.J. Corneveaux[1], J. Pruzin[1], A. Sekar[1], E. Salomon[1], C.J. Smith[2], S. Brautigam[2], J. Kirwan[2], S. Ober-Reynolds[2], D.C. Craig[1], M.J. Huentelman[1].* 1) Neurogenomics, TGen, Phoenix, AZ; 2) Southwest Autism Research and Resource Center, Phoenix,AZ.

Autism is a devastating neurobiological disorder affecting an estimated 1 out of every 150 children. The incidence of autism appears to be growing at a rate of approximately 10% per year, making it the most common developmental disorder. Children with the diagnosis of autism vary widely in the severity and presentation of their symptoms. Current theory in the field supports the idea of many sub-types of autism characterized by clusters of behavioral and physical traits. There is growing evidence that immune system dysfunction is one possible cause of autism. Through the use of quantitative antibody microarrays we investigated the correlations between specific autism physical and behavioral phenotypes and cytokine levels in two well phenotyped cohorts of autistic cases and controls. One cohort is from the Southwest Autism Research and Resource Center (SARRC), and the second from the Autism Genetic Resource Exchange (AGRE). The examination of the IgG levels of 33 autistics and their normal siblings from AGRE revealed significant differences in levels of IgD (p=0.03), IgE (p=0.03), IgM (p=0.009) and IgG1 (p=0.04). A measurement of the same immunoglobulins in 87 autistics and 73 age-matched, unrelated controls from SARRC did not find the same significant differences, but did find significant correlations with several physical features and ADI-R scores. A measurement of 40 cytokines in both cohorts found significant correlations between physical traits and behaviors. In particular, a significant negative correlation between BDNF (p=0.0054), E-Selectin (p=0.017), IGFBP-1 (p=0.019), IGFBP-2 (p=0.019), MIP-1b (p=0.007) and VCAM1 (p=0.03) levels and total ADI-R scores was found in the SARRC population. Also in this cohort a significant correlation between head circumference and IL-1a (p=0.03), IL-2 (p=0.02), IL-5 (p= 0.02), IL-6 (p=0.006), IL-8 (p=0.04), IL-10 (p=0.03), IL-13 (p=0.01), IL-17 (p=0.02), MIP-1a (p=0.02) and IFN-gamma (0.02) was noted. Eotaxin was significantly negatively correlated (p=0.025) with ADI-R scores in the AGRE cohort and other groups of cytokines were correlated with social and stereotyped behaviors. Better understanding of how dysregulated cytokines influence the brain could translate into pharmalogical targets for the treatment of some types of autism, help characterize sub-types of autism and ultimately may help to clarify the pathobiology behind autism.

## 826/W/Poster Board #484

**Thrombotic Storm: definition of an extreme clinical phenotype.** *T.L. Ortel[1], C.S. Kitchens[2], M.A. Pericak-Vance[3], D. Erkan[4], A.H. James[5], R. Kulkarni[6], R.W. Hoffman[7], L. Brandao[8], S.E. Hahn[3], J.M. Vance[3].* 1) Hemostasis & Thrombosis Center, Duke University Medical Center, Durham, NC; 2) Division of Hematology/Oncology, University of Florida, Gainesville, FL; 3) Miami Institute for Human Genomics and The Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami, Miller School of Medicine, Miami, FL; 4) Hospital for Special Surgery, New York, NY; 5) Department of Maternal-Fetal Medicine, Duke University, Durham, NC; 6) Michigan State University, East Lansing, MI; 7) Division of Rheumatology and Immunology, University of Miami, Miami, FL; 8) The Hospital for Sick Children, Toronto, Ontario, Canada.

Thrombotic storm (TS) was first described in 1998 as a self-perpetuating process in which an initial thrombotic event could lead to multiple thromboses and even death. 6 cases were initially used to demonstrate the features of this disorder: (1) an underlying hypercoagulable state; (2) provocation to initiate thrombosis; (3) rapid development of new thromboembolic events; (4) response to prompt intervention; and (5) good prognosis if the cycle of thrombosis is successfully interrupted. Collaborators representing eight sub-specialties (adult and pediatric hematology, immunology, maternal fetal medicine, laboratory medicine, neurology, genetics, and rheumatology) met in March 2009 to initiate a study to further elucidate this rare disorder. We hypothesize that individuals who develop TS have an underlying genetic predisposition to this severe, frequently life-threatening, response to an initial thrombotic stimulus. In order to search for this inherited risk factor, we have developed a set of inclusion/exclusion criteria that define this severe syndrome. Inclusion criteria include individuals age 55 years or younger who manifest 2 or more of the following: 1) acute, 2 or more arterial and/or venous thromboemboli, and/or thrombotic microangiopathy; 2) unusual location (e.g., cerebral sinus thrombosis); 3) progressive/recent unexplained recurrence; and 4) refractory to acute therapy and/or an atypical response to therapy. This must be in the absence of 1) active cancer, 2) advanced coronary artery disease; 3) cocaine use associated with symptom onset; 4) intravascular devices; 5) paroxysmal nocturnal hemoglobinuria or myeloproliferative syndromes; 6) subtherapeutic treatment; 7) multiple/severe trauma, and 8) pre-morbid clinical status. Using these criteria, we identified six new cases with TS, confirming the phenotype and clinical course, who represent the initial members of a patient cohort to investigate the genetics of TS through a nationwide recruiting strategy.

## 827/W/Poster Board #485

**Investigation of the genes PTCH1 and GLI2 in the etiology Anophthalmia and Holoprosencephaly in Brazilian cases.** *C. Bertolacini[1], AL. Petrin[2], LA. Ribeiro-Bicudo[1], AR. Costa[1], JC. Murray[2].* 1) Hosp of Rehabilitation of Craniofacial Anomalies, Bauru, SP., Brazil; 2) Department of Pediatrics, University of Iowa, Iowa City, IA, USA.

Craniofacial anomalies are alterations of the cranium and face that can be associated with central nervous system malformations. They represent a frequent cause of congenital anomalies in newborns. Craniofacial midline defects are directly related to the lack of closure of the neural tube and are among the most common of central nervous system malformations. These defects have both genetic and environmental etiological factors. We report the molecular analysis by direct sequencing of coding regions and exon-intron boundaries of the genes PTCH11 and GLI2 in 2 cohorts of Brazilian individuals with midline defects: 39 with anophthalmia/microphthalmia and 77 with holoprosencephaly spectrum (HPE). In the cohort with anophthalmia/microphthalmia we found 2 missense mutations in gene GLI2 and 6 variants in splicing site in gene PTCH1.In the cohort of HPE we found 3 missense and 2 nonsense mutations in the gene GLI2. For PTCH1 we found another 6 variants in splicing region, one of them was present in 7 unrelated individuals out of the 77 cases and was not present in 96 control samples. These genes belong to SHH (Sonic Hedgehog) pathway that plays a critical role in early midline craniofacial and central nervous system development that make them very strong candidate genes for midline defects studies. Mutations in the gene PTCH1 may affect the ability to bind SHH, or perturb the intracellular interactions of PTCH1 with other proteins involved in SHH signaling. The mutations can also enhance the repressive activity of PTCH1 on the SHH pathway and so decrease SHH signaling. GLI2 is a transcription factor implicated as obligatory mediators of SHH signal transduction, mutations on GLI2 have been previously reported on HPE individuals but we report it for the first time in anophthalmia cases. In conclusion our study shows the role of GLI2 and PTCH1 genes in the etiology of midline the cranium and face development that can be associated with the central nervous system malformations and report new mutations increasing the etiological knowledge of these anomalies.

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## 828/W/Poster Board #486

**An increase in the TLR7 gene dosage is associated with childhood-onset systemic lupus erythematosus in Mexican population.** *H. García-Ortiz[1,2], R. Velázquez-Cruz[2], F. Espinosa-Rosales[3], V. Baca[4], L. Orozco[2].* 1) Programa de Doctorado en Ciencias Biomédicas- UNAM; 2) Investigacion, Inst Natl de Medicina Genomica; 3) Servicio de Inmunología, Instituto Nacional de Pediatría; 4) Hospital de Pediatría, Centro Médico Nacional Siglo XXI, IMSS. Mexico City, Mexico.

Congenic dissection of lupus-prone mouse strains has provided important insights of the role of Toll-like receptor 7 gene (TLR7) in the pathogenesis of systemic lupus erythematosus (SLE). The BXSB male mice develop a severe form of murine lupus. The autoimmune enhancing-effect in BXSB males was attributed to a Y-linked autoimmune accelerator (Yaa) locus, resulting from a duplication and translocation to the Y-chromosome of a cluster of X-linked genes. This duplication in male mice carrying Yaa leads to a higher expression of several of the translocated genes, including Tlr7, the major candidate gene for causation of the Yaa-associated autoimmune phenotypes. In this context, we investigate if there is an increase in the number of TLR7 gene copies in childhood-onset SLE in Mexican population. We performed a case-control study in 326 (268 female, 58 male) unrelated patients with childhood-onset SLE and 326 sex matched controls recruited from Mexico City. We estimated the relative TLR7 gene copy number by real time PCR normalizing with two housekeeping genes (TBP and HPRT1) and using two pairs of specific primers for TLR7 (Xp22.3) within a distance of 1.8 kb. The TLR7 gene copy number was determined by the standard curve and ΔΔCt methods. Association analysis was performed using the Mann-Whitney U test. To calculate the Odds Ratio (OR), we used a multivariate logistic regression model. Additionally, a stratification analysis was performed in the SLE cohort to investigate the association of TLR7 gene copy number with renal disorder. We found a significantly increased in the relative TLR7 gene copy number in SLE pediatric patients compared with controls ( P=0.0015). However, when we stratified by gender, only female patients showed a significant association (P<0.0001). By logistic regression analysis we found an increased risk for development of SLE in female patients with three or more copies of TLR7 (OR = 2.94; 95% CI 1.72-4.94). Otherwise, we did not find a significantly difference in the TLR7 gene copy number in patients with lupus nephritis compared with those without renal disease. In conclusion, our data show that the gene dosage of TLR7 is an important risk factor for disease susceptibility in childhood-onset SLE in Mexican population. In addition, our results also support the notion that the female predominance of SLE may in part be explained by an X-chromosome gene dosage.

## 829/W/Poster Board #487

**Genome-wide screen for common and rare copy number variation in metabolic syndrome.** *K. Kristiansson[1], C. Barnes[1], M. Perola[2,3,4], A. Jula[5], V. Salomaa[6], S. Ripatti[2,3], M. Hurles[1], L. Peltonen[1,2,3,4,7].* 1) The Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 2) Dept. of Molecular Medicine, The National Institute for Health and Welfare, Helsinki, Finland; 3) FIMM, Institute for Molecular Medicine Finland, Helsinki, Finland; 4) Dept. of Medical Genetics, University of Helsinki, Helsinki, Finland; 5) Dept. of Health and Functional Capacity, The National Institute for Health and Welfare, Helsinki, Finland; 6) Dept. of Health Promotion and Chronic Disease Prevention, The National Institute for Health and Welfare, Helsinki, Finland; 7) The Broad Institute of MIT and Harvard, Boston, MA, USA.

Metabolic syndrome (MetS) is a common disorder characterized by a clustering of risk factors, such as impaired fasting glucose, insulin resistance, dyslipidemia, and central obesity. The prevalence of MetS, which is highly age-dependent, has greatly increased during the last few decades. The overlap between T2DM, CVD, and MetS suggests that the same genetic determinants may contribute to the complex pathophysiology, which even more emphasizes the significance of identifying the largely unknown genetic loci underlying MetS. We carried out a genome-wide screen for copy number variation (CNV) in the genome, with two distinct strategies to assess the contribution of common and rare CNVs as risk factors for MetS. Probe signal intensity data from Illumina human610 beadchip, which is enriched for CNV-targeted probes, was analyzed for 1,100 MetS cases and their matched controls using several CNV analysis programs (PennCNV, QuantiSNP, CNVtools) in parallel for improved quality assessment of our results. The search for common CNVs utilized two recent genomic maps of known CNVs: a study of 11 diverse HapMap populations, and a higher-resolution study of 40 individuals of European or African ancestry. We also identified CNV-tagging SNPs from a reference set of CNV and SNP genotypes in the same population from which the MetS cases and controls were sampled. This allowed us to use robust SNP genotypes to evaluate the role of a subset of CNVs in MetS. In parallel to our analysis of common CNVs, we searched for rare CNV associations, concentrating on those overlapping with genes or covering large genomic regions, with the hypothesis that these structural variants may have large effects on individual phenotypes. So far, we have identified common CNV loci with putative association to MetS as a whole, along with its component traits. One of these loci is 5p12-q11.2, where two CNVs show suggestive association with MetS (p<0.05), and SNPs spanning a 4 Mb region adjacent to insulin gene enhancer protein (*ISL-1*) gene associate with insulin levels (p=0.05-0.0000016). Furthermore, we detected a gene region (*ANKRD23*, 2q11.2), implicated in insulin resistance and energy metabolism, where few of our study subjects, who are located at the far ends of the population distribution of relevant phenotypes, carry large CNVs. These results provide ground for replication studies in large population cohorts and hopefully eventually elucidate the genetic background of MetS and its components.

## 830/W/Poster Board #488

**CETP, HDL and CVD: A detailed genetic analysis.** *I.M Stylianou, A.P Kent, A.C Edmondson, B.J. Keating, M. Wolfe, S. DerOhannessian, M.P Reilly, M. Li, D.J Rader.* ITMAT, Sch Med, Univ Pennsylvania, Philadelphia, PA.

Cholesteryl ester transfer protein (CETP) is known in humans to regulate high-density lipoprotein (HDL) cholesterol levels. Indeed, the association between common polymorphisms in CETP with HDL is among the most significant reported for any gene-phenotype to date. HDL is an independent risk factor for cardiovascular disease (CVD) and its physiological role in reducing atherosclerosis through reverse cholesterol transport is compelling. However, despite concerted efforts, there has been little to link CETP genetic polymorphisms with CVD, presumably due to underpowered efforts or an underlying complex genetic architecture which has thus far prevented a comprehensive assessment. Here we lay bare the detailed complex genetic architecture of CETP using the CVD 50K IBC array. We demonstrate at least 10 independent linkage disequilibrium (LD) groups that are significantly associated (genome-wide threshold) with plasma levels of HDL, replicated across several populations. Examining each of these LD groups independently and in combination against associations with CVD phenotypes is aiding in the unraveling of the CETP-HDL-CVD paradox.

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## 831/W/Poster Board #489

**Genetic prediction of diabetic nephropathy - A multi-factorial model.** *I. Blech[1], M. Katzenellenbogen[5], A. Katzenellenbogen[5], C. Norymberg[2], A.E. Buchs[3], I. Harman-Boehm[4], T. Pollin[6], B. Glaser[1], Israel Diabetes Research Group.* 1) Endocrinology & Metabolism, Hebrew Univ-Hadassah, Jerusalem, Israel; 2) Diabetes Department, General Health Services, Natanya, Israel; 3) Department of Internal Medicine "C" and Diabetes Unit, Assaf Harofe Medical Center, Zerifin, Israel; 4) Department of Internal Medicine, Soroka University Medical Center, Beer-Sheva, Israel; 5) The Mina & Everard Goodman, Faculty of Life Science, Bar Ilan University, Ramat-Gan, Israel; 6) Division of Endocrinology, Diabetes and Nutrition, University of Maryland School of Medicine, Baltimore, MD.

Diabetes-associated metabolic dysregulation causes devastating multi-organ complications, including nephropathy, the risk of which is partially genetically determined. **AIMS:** We hypothesize that combined analysis of multiple genetic markers will improve the ability to predict nephropathy. **METHODS:** We selected 27 SNPs in 15 genes based on previous publications and their association with relevant metabolic pathways, and genotyped them in 1274 well-phenotyped Ashkenazi or Sephardic patients with Type 1 or Type 2 diabetes of ≥10y duration. A backward stepwise logistic regression model was developed as described below. The model was validated by randomly dividing the primary population into "training" (75%) and "test" (25%) subgroups and by replication in an independent Ashkenazi Jewish sample set. **RESULTS:** Five SNPs in 5 genes (*HSPG2*, *eNOS*, *ADIPOR2*, *AGER*, and *RANTES*) showed nominally significant association with nephropathy. The logistic model based on these 5 SNPs and 4 conventional variables (age, sex, diabetes type and duration), and allowing for all possible 2 way interactions, predicted nephropathy in our initial population (C-statistic = 0.66) better than a model based on conventional variables only (C= 0.57). Re-modeling using the "training" subset of the population produced a model identical in structure and predictive ability in both the "training" (C= 0.65) and the "test" (C=0.68) subsets. While in the replication set of 906 Ashkenazi patients the C-statistic was lower than in the original population (C=0.57), it was still higher than for the model including only conventional risk factors (C=0.54). The lower predictive ability in the replication set may in part be related to differences in ethnicity and prevalence of T1DM. Association of the genetic model with nephropathy was highly significant in the replication set ($\chi 2$ =17.43, p<0.0001), whereas the conventional variable model was not ($\chi 2$=1.62, p=0.2). **CONCLUSIONS:** Using 5 SNPs and 4 conventional risk factors and allowing for 2 way interactions between all variables, we developed and validated a model that can predict nephropathy more efficiently than was previously possible using conventional risk factors. Similar models, based on a larger number of relevant SNPs are likely to provide even better predictive ability. Further refinement may permit creation of population-specific models.

## 832/W/Poster Board #490

**Comprehensive metabolic trait association analysis using gene expression and single nucleotide polymorphisms.** *M. Inouye[1,2], K. Silander[3], E. Hamalainen[1], V. Salomaa[3], K. Harald[3], P. Jousilahti[3], S. Mannisto[3], M. Perola[3], E.T. Dermitzakis[1,4], L. Peltonen[1,5,6].* 1) Department of Human Genetics, Wellcome Trust Sanger Institute, Cambridge, United Kingdom; 2) Department of Human Genetics, Leiden University Medical Centre, Leiden, The Netherlands; 3) THL-National Institute for Health and Welfare, Helsinki, Finland; 4) University of Geneva, Geneva, Switzerland; 5) Institute for Molecular Medicine, Finland (FIMM) and Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 6) The Broad Institute of MIT and Harvard, Cambridge, MA, USA.

Genome-wide association studies have been immensely successful in finding statistically significant associations of single nucleotide polymorphisms (SNPs) with complex traits. In particular, the genetic basis of metabolic traits and disorders has benefited with several dozen SNPs identified to date. However, studies of whether these SNPs drive gene expression and whether the expression of these or other genomic loci predict trait status has not been thoroughly studied. Here, we attempt to answer these questions using over 500 unrelated Finnish individuals from the Helsinki area recruited during 2007 as part of the Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic syndrome (DILGOM) study. For these samples, detailed metabolic trait measurements and lifestyle factors were available as well as genome-wide genotypes from the Illumina 610K array and genome-wide Illumina HT-12 expression profiles from total blood. While implementing stringent quality control filters and controlling for population structure, we use multiple regression to test the association of each expression probe with each trait and investigate different approaches to control for multiple testing, such as the Bonferroni correction and generating null distributions of P values by permutation. Initial analysis shows the presence of previously known associations, e.g. blood fasting glucose with the jun oncogene, insulin with alpha defensins, and free fatty acids with carnitine/acylcarnitine translocase, in addition to suggesting several novel findings including a shared loci between C-reactive protein and diastolic blood pressure. Further, genomic loci with significant or strong previous evidence of trait association are modeled as a function of both cis gene expression and SNP variants. With these loci models, we hope to better understand the functions and dynamics underlying metabolic traits.

## 833/W/Poster Board #491

**The Genetic Association of Copy Number Variation and Type 1 Diabetes.** *H.Q. Qu[1], Q. Li[1], J.T. Glessner[2], K. Wang[2], S.F.A. Grant[2], H. Hakonarson[2], C. Polychronakos[1].* 1) Pediatrics and Human Genetics, McGill University, Montreal, PQ, Canada; 2) Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA USA.

**Background:** Genome-wide association studies (GWAS) have revealed many genetic associations of type 1 diabetes (T1D) with common variants which, however, leave much of the familial clustering of T1D unexplained. Copy number variations (CNVs) are the attractive candidates to explain the unknown genetic susceptibility. This study aims to genotype deletion loci using the data of fluorescence signals from the *Illumina* Hap550 SNP genotyping arrays. **Methods:** The subjects were a family cohort with 466 T1D case-parents pairs, and a Case-Control cohort with 499 cases and 1000 controls. The genotype calling of a deletion locus was based on the normalized fluorescence signal intensity ($log_2R$) of pairs of consecutive SNPs. The genotype clustering was performed by the K means method, optimized by Silhouette scores. The genotyping quality was assessed by Silhouette scores, Hardy-Weinberg equilibrium test, Mendelian error in the 466 families, and one-copy genotype miscalled in the presence of heterozygous SNP. **Results:** Four quality criteria were well correlated in the assessment of the genotyping quality. The number of miscalled heterozygous deletion with heterozygous genotype (A/B) was sensitive to detect genotyping error. Altogether, 216 deletion loci tagged by 323 SNPs had Silhoutte score>0.64, Mendelian errors ≤3 in the family cohort, miscalled A/B≤3 in each cohort, and Hardy-Weinberg Equilibrium test *P*>0.01. Five deletion loci had *P*<0.01, and 10 deletion loci had *P*=0.01~0.05, in the combined results of the family cohort and the Case-Control cohort. The most significant association (*P*= $2.73x10^{-5}$) was tagged by the SNP rs743862, ~25kb upstream of the *HLA-DRA* gene, which effect may be due to the LD with HLA class II genes. The most interesting functional candidate is the deletion tagged by the SNP rs4529574 (*P*=$9.84x10^{-4}$), an intronic SNP on the spleen tyrosine kinase gene (*SYK*) at Chr9q22. SYK plays critical roles in T and B lymphocyte development. The deletion allele has frequency=0.046, OR (95%) = 0.573 (0.412, 0.798). **Conclusion:** The two neighboring $log_2Rs$ algorithm can genotype deletion loci with high genotyping quality and thus enables further association test of the deletion loci. The potential T1D genetic susceptibility from the deletions will be confirmed by direct genotyping (TaqMan) in independent T1D samples and other T-cell autoimmune diseases that may share common genetic susceptibility with T1D.

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## 834/W/Poster Board #492

**Functional classification based on gene expression data indicates that T2D genes play a role in liver, subcutaneous adipose tissue, and visceral adipose tissue.** *M.G.M. Wolfs[1], L. Franke[2, 3, 4], E.J. Bruin[1], S.S. Rensen[5], J.W. Greve[5], W.A. Buurman[5], T.W. van Haeften[1, 6], C. Wijmenga[2], M.H. Hofker[1].* 1) Department of Pathology and Medical Biology, Molecular Genetics Section, University Medical Center Groningen, Groningen, Netherlands; 2) Department of Genetics, University Medical Center Groningen, Groningen, Netherlands; 3) Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, London, UK; 4) Complex Genetics Section, DBG-Department of Medical Genetics, University Medical Center Utrecht, Utrecht, Netherlands; 5) Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Department of General Surgery, Maastricht University Medical Center, Maastricht, Netherlands; 6) Department of Internal Medicine, G 02-228, University Medical Center Utrecht, Utrecht, Netherlands.

Recently 19 common genetic variants associated to type 2 diabetes (T2D) have been identified through GWAS. Although the functions of the candidate genes located close to these genetic variants are largely unknown, preliminary evidence has been obtained for several of the identified T2D variants to be also associated to β-cell function. However, a systematic analysis of the novel diabetes genes is still lacking. This study has evaluated expression levels of the candidate diabetes genes in three different tissues that are involved in the development of insulin resistance, and thus are crucial in the development to T2D. Our study was carried out in 90 morbidly obese individuals (BMI over 35). During bariatric surgery, biopsies were obtained from human liver, visceral adipose tissue, and subcutaneous adipose tissue. mRNA from each of these tissues was hybridized to Illumina HumanHT12 BeadChips and quantile-normalized. We focused on 43 probes representing 24 genes that map to well established T2D loci. 18 out of these 24 genes are expressed throughout the tested tissues, indicating that they are likely to have a function within those tissues. Moreover, the expression of 9 of these genes was significantly correlated to metabolic traits of the patients, including levels of free fatty acids, HDL, and triglycerides. In order to further strengthen the evidence for a functional role of these tissues, co-expression networks were constructed. These networks were based on co-expression between the 43 probes and all the other probes present on the array (T2D genes were connected if they share at least 3 genes with a correlation coëfficient > 0.5). We found that in these networks strong correlations exist between T2D genes, of which many are tissue-specific. Remarkably, in both adipose tissues the expression levels of PPARγ are significantly correlated with WFS1 and CDKN2B expression. In visceral adipose tissue these genes also correlate with IGF2BP2, NOTCH2, and TSPAN8 whereas in subcutaneous adipose tissue these genes significantly correlate with IDE, FTO, and CDC123. Hence, on the basis of phenotypic correlations and network analysis, we predict that ADAMTS9 and IGF2BP2 play a role in liver, PPARγ, CDKN2B, WFS1, IGF2BP2, NOTCH2, and TSPAN8 play a role in visceral adipose tissue, and PPARγ, CDKN2B, WFS1, IDE, FTO, and CDC123 play a role in subcutaneous adipose tissue. The majority of these results could be replicated in a morbidly obese mouse model.

## 835/W/Poster Board #493

**Searching for mitochondrial mutations involved in age-related hearing impairment.** *E. Fransen[1], S. Bonneux[1], E. Van Eyken[1], L. Van Laer[1], HJM. Smeets[2], G. Van Camp[1].* 1) Center for Medical Genetics, University of Antwerp, Antwerp, Belgium; 2) Department of Genetics and Cell Biology, Clinical Genetics, Maastricht University, Maastricht, The Netherlands.

Age-related hearing impairment (ARHI) is a condition whereby hearing acuity declines with ageing. Although this occurs in every individual, some persons are more severely affected than others. About half of the phenotypic variance is attributable to genetic factors. We have previously identified SNPs within two nuclear genes (GRHL2 and GRM7) that are associated with ARHI, and reported significant linkage between the ARHI phenotype and chromosomal region 8q24, but much of the variance remains unexplained Mitochondrial (mt) DNA mutations have been implicated in several ageing processes, and certain forms of nonsyndromic hearing loss are caused by mitochondrial mutations. In this study we explore whether mt variation contributes to ARHI. We have fully sequenced the 16 kb mt genome in 199 good-hearing and 200 bad-hearing persons, using the GeneChip Human Mitochondrial Resequencing Array (Affymetrix). Of the 399 participants, 369 could be classified into one of the European mt Haplogroups. No association was found between Haplogroup and affection status. When accounting for known ARHI risk factors (occupational noise, smoking, bmi, alcohol consumption, solvent exposure), no association was found either. With regard to the revised Cambridge Reference Sequence (rCRS, Genbank Accession Number AC_000021), a total of 818 distinct mutations were found across the dataset. More than half of these mutations (451 = 55%) were found in only one individual. The average number of mutations per individual was not significantly different between cases and controls, even when weighting the mutations according to conservation. For the more common variants, a Fisher-exact test was used to screen for allele frequency differences between cases and controls. The most significant signal was observed for position 10876. Seven of the cases carry a G at this position instead of a C, which was not observed among any of the controls. (p=0.01). Apart from the C10876G variant, we found eight additional variants with p-values between 0.01 and 0.1, that were all specific to subhaplogroup U2e. Six of the 9 disease-associated variants are located within the protein-coding region, but none of them alters the protein sequence. One of the variants belongs to a tRNA gene, while the last variant is part of the control region (D-loop).

## 836/W/Poster Board #494

**Development of a lung disease severity phenotype in Cystic Fibrosis and its application in the Canadian CF Modifier study.** *C. Taylor[1,2], R. Dorfman[3], L.J. Strug[1,2], W. Li[1], A. Cojocaru[1], N. Anderson[4], A. Sandford[5], P. Paré[5], J. Zielenski[3], P. Durie[4], M. Corey[1,2].* 1) Child Health Evaluative Sciences, The Hospital for Sick Children, Toronto, ON, Canada; 2) Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada; 3) Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, ON, Canada; 4) Physiology and Experimental Medicine, The Hospital for Sick Children, Toronto, Canada; 5) James Hogg Research Centre, University of British Colombia, Vancouver, Canada.

To minimize non-genetic variation and maximize the ability to find the contribution of genetic variation, a phenotype measure must be accurate, precise, and informative. Ideally it will apply to all populations and ascertainment schemes, allowing valid comparisons between studies. In Cystic Fibrosis (CF), Forced Expiratory Volume in 1 second as a percent of predicted (Wang/Hankinson) based on height, age, and sex (FEV1pp) is a clinically useful and relevant measure of severity of lung disease. However, since CF is a progressive lung disease, average values of FEV1pp in older patients are lower than those in younger patients, making it difficult to define “mild” vs. “severe” across ages. Mortality selection further compromises the comparison. We propose a lung phenotype adjusted for age and survival censoring. Between 2000 and 2006, we recruited a representative sample of 2474 CF patients across Canada (approximately 75% of the Canadian CF population). FEV1pp was calculated and plotted against age the 1948 patients with available data. A local smoothing regression procedure (LOESS) was used to obtain age specific predicted FEV1pp values and residuals. Using the Canadian CF Patient Data Registry we determined survival probabilities to Dec 31, 2002 for each birth year cohort, and smoothed estimates of survival at decimal ages. Ranked FEV1pp residuals and age-specific survival estimates were combined to produce an adjusted cohort percentile for each patient relative to others who had lower lung function or had died. LOESS regression produced a flat pattern of age specific mean FEV1pp up to age 12, declining values to age 28, followed by a second plateau, due to mortality of those with the lowest lung function, leaving those with milder disease. Survival-correction increased percentile scores in all patients, with larger increases in older patients. For example, two patients with FEV1pp scores of 60% - one 11 years old and the other 30 years old - had age-adjusted FEVpp at the 10th percentile and 50th percentile, respectively. After adjustment for survival, the 11 year old remains at the 10th percentile while the 30 year old rises to above the 70th percentile. Correction for mortality attrition, after non-linear age-adjustment provides a phenotypic score that can be used in genetic studies for the entire age range of CF patients.

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## 837/W/Poster Board #495

**Evidence for different autophagy genes in Crohn's disease susceptibility.** *I. Cleynen, L. Henckaerts, W. Van Moerkercke, P. Rutgeerts, S. Vermeire.* Gastroenterology, KU Leuven, Leuven, Leuven, Belgium.

Variants in two autophagy genes, *ATG16L1* and *IRGM*, have been associated with susceptibility to Crohn's disease (CD), highlighting the role for autophagy in CD pathogenesis. Our aim was to identify other autophagy genes possibly involved in CD pathogenesis. All known human functional homologues of yeast autophagy genes were screened for their location near a known IBD locus, a region of linkage previously reported in the Belgian cohort, or a genomic region surrounding a hit from the CD genome-wide association study (GWAS) meta-analysis. In the thus prioritized 12 genes, 75 haplotype tagging SNPs (tSNPs) were selected. tSNPs were genotyped in an exploratory cohort (cohort A) of 911 CD patients, and 407 healthy controls, and in a replication cohort (cohort B) of 374 CD patients, and 196 healthy controls, using the Sequenom® platform. Groups were compared using Haploview 4.1. All p-values were corrected for multiple testing with the FDR method in R 2.7.1. A corrected p-value ≤0.05 was considered to be significant. In cohort A, significant associations were found in two of the 12 genes: *ULK1* and *MAP1LC3B*. The frequency of the T-allele of the intronic tSNP rs12303764 in *ULK1* was significantly lower in CD patients (36%) vs healthy controls (42%, p=0.03) of cohort A. This association was replicated in cohort B ($MAF_{patients}$=37%, $MAF_{controls}$=44%, p=0.05), with a p-value of 0.001 in the combined cohort (A+B). In addition, the T-allele of rs3923716 in *ULK1* had at a higher frequency in CD patients (10%) vs controls (5%, p=0.03) in group B, but not in group A, with a combined p-value of 0.005. In cohort A, three variants in *MAP1LC3B* (rs933717, rs7204722, and rs2241617) were found to be associated with CD (all p <0.005). None of these were replicated in cohort B, but they remained significant in the combined cohort (all p =0.009). None of the other tSNPs tested were associated with susceptibility to CD in this cohort. These results show that different autophagy genes are involved in CD pathogenesis. *ULK1* (*ATG1*) is the key signal in autophagy induction, and is known to intervene in cell growth, differentiation and survival. MAP1LC3B is 1 of 3 human orthologs of the yeast protein Atg8, an autophagosome membrane marker. Upon induction of autophagy, Atg8 (cytosolic LC3-I) is cleaved and lipidated to form LC3-II. Investigation of the influence of these polymorphisms on gene function or expression will be essential to clarify their specific role in CD pathogenesis.

## 838/W/Poster Board #496

**Gene Expression Profiles Associated with Lack of Response to Intravenous Corticosteroids in Children with Severe Ulcerative Colitis.** *B. Kabakchiev[1,10], D. Turner[9], J. Hyams[5], D. Mack[4], N. Leleiko[7], W. Crandall[8], J. Markowitz[6], A. Otley[3], W. Xu[10], P. Hu[2], A. Griffiths[2], M. Silverberg[1,10].* 1) SLRI, Mount Sinai Hospital, Toronto, Ontario, Canada; 2) The Hospital for Sick Children, Toronto, ON, Canada; 3) Izaak Walton Killam Hospital, Halifax, NS, Canada; 4) Children's Hospital of Eastern Ontario, Ottawa, ON, Canada; 5) Connecticut Children's Medical Center, Hartford, CT, USA; 6) Schneider's Children's Hospital, Long Island, NY, USA; 7) Brown University, Providence, RI, USA; 8) Columbus Children's Hospital, Columbus, OH, USA; 9) Shaare Zedek Medical Center, Jerusalem, Israel; 10) University of Toronto, Toronto, ON, Canada.

**Background**: Microarray analysis of RNA expression allows examination of specific pathways operative in inflammation. In the context of a multicenter pediatric study of outcomes and predictors of outcome in acute severe UC, we aimed to determine whether genes expressed in whole blood early following initiation of intravenous corticosteroid (IVCS) treatment can predict response. **Methods**: From a prospectively accrued cohort of 110 pediatric patients hospitalized for IVCS treatment of severe UC (mean PUCAI 73±12), we selected for analysis 10 steroid responsive (PUCAI<35 by day 5) and 10 steroid-refractory patients (persistence of PUCAI≥65 by day 5 and need for 2$^{nd}$ line medical therapy or colectomy by hospital discharge). Mean age of the twenty children was 14.5±2.2 yrs; mean disease duration 0.9±1.5 yrs.; mean PUCAI score at admission 75±11. Total RNA was extracted from blood samples collected on the 3$^{rd}$ day of IVCS therapy. The eluted transcriptomes were run on Affymetrix Human Gene 1.0 ST arrays and analysed by the LPE method for discovery of differentially expressed genes. False discovery rate (FDR) correction for multiple comparisons was applied to all p-values. **Results**: Our analysis returned 63 statistically significant genes whose transcript levels correlate with therapy outcome. A gene whose overexpression correlated strongly with IVCS refractoriness was *CD177*, a member of the *Ly-6* gene superfamily (FDR p = 3.29E-11). *CD177* is polymorphic and has at least two known alleles - *PRV-1* and *NB1* - encoding surface glycoproteins found on neutrophils. Other genes overexpressed in IVCS refractory subjects include defensin alpha 1 and alpha 3 (*DEFA1*, *DEFA3*, FDR p = 1.39E-07), genes also expressed primarily in neutrophils. By contrast, HLA DQ beta 1 (*HLA-DQB1*, FDR p = 0.002), a gene previously associated with UC, was more highly expressed in IVCS responsive subjects. Overall, at least one third of the 63 significant genes are known to be directly involved in the human inflammatory response and more specifically with neutrophils. Absolute neutrophil counts were similar among responders and non-responders (p = 0.98). **Conclusion**: Several genes involved in neutrophil function are associated with response to IVCS therapy early in the course of treatment. Gene expression profiles may be useful to predict response to IVCS in children with severe UC and assist with clinical management decisions. **Note**: B. Kabakchiev and D. Turner contributed equally to the abstract.

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## 839/W/Poster Board #497

**Genetics of Gene Expressions under Perturbation of Environments.**
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Complex phenotypes are the consequence of the interplay of genetic and environmental factors. Development of disease is a dynamic process of gene-gene and gene-environment interactions within a complex biological system which is organized into complicated interacting networks. Despite current enthusiasm for investigation of gene-environment interactions, published results that document these interactions in humans are limited, and the essential issue of how to define and detect gene-environment interactions remains unresolved. The concept of gene-environment interactions is often used, but rarely specified with precision. In this report, we developed longitudinal genetic models for investigation of dynamic gene-environment interactions influencing gene expressions under stimulus of environments. Specifically, gene expressions are taken as a quantitative trait. A locus influencing gene expression variation is referred to as eQTL. Time course gene expressions as responses to genetic and non-genetic perturbations are a function of time t. We can view the time-course gene expressions as a realization of a stochastic process and decompose them into mean function, and genetic additive effect and dominance effect functions using Fisher's factor analysis and variation method, which leads to a general genetic model of functional trait and formulates genetic effect functions as function of genotypic values and allele frequencies. As a proof of principle, the proposed models for studying dynamic gene-environment interaction were applied to 79 fibroblast strain obtained from our Tissue Culture Core of the Specialized Center of Research (SCOR) in Scleroderma. We use RT-PCR to measure expressions of the genes of COL1A2, COL3A1, CTGF, SPARC and TIMP3 with and without perturbation of 10 μg silica at 24-, 48-, 72-, 96- and 120-hour exposure. All 79 fibroblast strains were genotyped by Illumina 317K SNP array. After checking the quality of typed SNPs, total of 313,932 SNPs were left for analysis We identified 23, 9, 5, and 18 SNPs which interacted with silica to significantly affect the expression of genes CoL1A2, COL3A1, CTGF and TIMP3 under stimulus of silicon.

## 840/W/Poster Board #498

**Sequence variants in PPARG and TCF7L2 are associated with plasma calcium and glucose levels in an island population of the eastern Adriatic.**
*R.A. Karns[1], G. Zhang[1], N. Jeran[2], D. Havas[2], S. Missoni[2], G. Sun[1], H. Cheng[1], S.R. Indugula[1], J. Mallik[1], Z. Durakovic[2], R. Chakraborty[1], P. Rudan[2], R. Deka[1].* 1) Environmental Health, University of Cincinnati, Cincinnati, OH; 2) Institute for Anthropological Research, Zagreb, Croatia.

We performed an association analysis of 97 Single Nucleotide Polymorphisms (SNPs) in eight candidate genes (FTO, INSIG2, PPARG, MC4R, SLC30A8, CDKN2A, TCF7L2 and HHEX) with 17 metabolic traits in a sample of >950 unrelated adult individuals from the island of Hvar in the eastern Adriatic coast of Croatia. This population of Slavic origins, living in relative isolation for over 400 years, has a high prevalence (~26%) of metabolic syndrome despite maintaining traditional life style patterns and adherence to a Mediterranean diet. The traits include anthropometric, blood pressure measurements, fasting plasma glucose, insulin, total cholesterol, HDL-C, LDL-C, triglyceride, uric acid, calcium, creatinine, fibrinogen and HbA1c levels. SNPs were either selected from published GWAS (SNPs with genome-wide significance in FTO, INSIG2, MC4R, SLC30A8, CDKN2A, TCF7L2, and HHEX) or tagSNPs (FTO, INSIG2, and PPARG). Three SNPs maintain significance after permutation tests and Bonferroni adjustments (p values reported here are permutation p-values multiplied by the number of traits). Two SNPs in TCF7L2, rs7903146 and rs1225536, are significantly associated with fasting plasma glucose (p=.0042 and p=.0069, respectively) and HbA1c (p=.0031 and p=.0046, respectively). These results are consistent with previous findings that variants in TCF7L2 influence Type 2 diabetes related phenotypes. Our most important finding is an association between plasma calcium concentration and rs1152005, a tagging SNP downstream of PPARG (p=.011). Calcium dysregulation in platelets, reported in patients with diabetes, causes hyperactivity and increased aggregation of platelets. While variants in PPARG have been implicated in the development of obesity and diabetes, to our knowledge, this is the first reported association between a variant in or near PPARG and plasma calcium concentration. Eight SNPs, including five previously reported with genome-wide significance, within a 45Kb region in FTO show nominal significance with BMI and waist circumference. Although their significance do not hold after multiple test adjustments, the FTO region needs further investigation given the gene being strongly implicated in obesity related traits. The study is supported by NIH grant R01DK069845.

## 841/W/Poster Board #499

**Genome-wide haplotype association mapping in mice identifies a genetic variant in CER1 associated with bone mineral density and fracture in southern Chinese women.**
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**Introduction:** Osteoporosis is characterized by a decrease in bone mass, deterioration of bone tissue, impaired bone strength and increased fracture risk. It is a medically, socially, and economically important disease, especially among the aging population. Bone Mass Density (BMD) is a quantitative index of osteoporosis. Acquisition of bone mineral is a complex process involving genetics and environmental factors. **Methods:** A genome-wide Haplotype Association Mapping (HAM) approach was performed by using inbred mice strains which had been genotyped and phenotyped in the Mouse Phenome Project. In HAM, a dense SNPs map was first partitioned into blocks of three SNPs with an average length of 1Mb. Modified F-statistics were calculated for the whole genome to test if blocks exist where the haplotypes can partition inbred strains into high and low BMD groups. In this study, the candidate gene Cerberus 1 (Cer1) suggested from HAM analysis was eventually tested by a human case-control cohort of 1,083 subjects. **Results and conclusion:** In this study, we used a HAM approach to identify a haplotype block within Cer1 that partitions inbred mice strains into high and low BMD groups. A cohort of 1083 high and low BMD human subjects were studied and a non-synonymous SNP (rs3747532) in human CER1 was identified to be associated with increased risk of both low BMD in premenopausal women (OR 2.2; 95% confidence interval: 1.0 - 4.6; $p < 0.05$) and increased risk of vertebral fractures (OR 1.82, p=0.025) in the post-menopausal cohort. We also showed that Cer1 is expressed in mouse bone and growth plate by RT-PCR, immunohistochemistry and in situ hybridization, consistent with polymorphisms potentially influencing bone mineral density. Our successful identification of an association with CER1 in humans together with our mouse study suggests that CER1 may play a role in the development of bone or its metabolism.

## 842/W/Poster Board #500

**Negative association of CD244 with susceptibility and radiographic joint damage of rheumatoid arthritis in a Japanese population.**
*A. Tokita, K. Ikari, Y. Kawaguchi, A. Taniguchi, H. Yamanaka, S. Momohara.* Inst Rheumatology, Tokyo Women's Med Univ, Tokyo, Japan.

Recently, CD244 was reported to be associated with susceptibility to rheumatoid arthritis (RA) in a large Japanese RA cohort(1). To validate this association, we tested the association in another Japanese cohort. Further, since susceptible gene is often associated with disease severity, association between the CD244 SNPs and radiographic joint damage in the RA patients was studied. DNA samples of 1504 RA patients were obtained from the IORRA (Institute of Rheumatology RA cohort) DNA collection and 752 population-based controls were obtained from the DNA collection of Health Science Research Resources Bank (Osaka, Japan), which is entrusted by Pharma SNP consortium (Tokyo, Japan). All the RA patients fulfilled the American College of Rheumatology 1987 revised criteria for RA. Sharp/ van der Heijde score (SHS) of the hands at 5-year disease duration, which represents radiographic joint damage, were measured in 628 RA patients. Genotyping was performed using TaqMan assay for the CD244 SNPs, rs3766379 and rs6682654 (Applied Biosystems Japan). Associations of the SNPs with RA susceptibility were estimated by Chi-square test, and associations between the SNPs and SHS were analyzed using regression analysis. Genotype distributions for the SNPs were in Hardy-Weinberg equilibrium. The SNPs were not associated with RA in the tested populations. Odds ratio were 1.03 (P = 0.69) and 1.02 (P=0.72), respectively for rs3766379 and rs6682654. In addition, we could not detect the impact of the CD244 SNPs on SHS (P=0.71 and 0.43, respectively). We conclude that the CD244 SNPs is not associated with RA and radiographic joint damage in the tested Japanese population. The result indicates that more replication studies are needed to reveal the association between CD244 and RA susceptibility.

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## 843/W/Poster Board #501

**Identification of *KIAA1529* as a novel susceptibility gene for Behçet's disease.** *A.H. Sawalha[1,2,3], Y. Fei[1,2], R. Webb[2,4], B.L. Cobb[2,5], J. Wren[2], H. Direskeneli[6], G. Saruhan-Direskeneli[7].* 1) Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA; 2) Arthritis & Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; 3) US Department of Veterans Affairs Medical Center, Oklahoma City, OK 73104, USA; 4) College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA; 5) JK Autoimmunity, Inc., Oklahoma City, OK, USA; 6) Division of Rheumatology, Department of Internal Medicine, Marmara University Medical School, Istanbul, Turkey; 7) Department of Physiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey.

Behçet's disease is a chronic vasculitis characterized by recurrent orogenital ulcers, skin lesions, uveitis, recurrent venous thrombosis and arterial aneurysms. The disease also affects the brain, joints, and in some cases the gastrointestinal tract. Behçet's disease is poorly understood, and treatment options currently available are nonspecific and frequently ineffective. There is clear evidence to suggest a genetic contribution to the pathogenesis of Behçet's disease. The genetic association with HLA-B51 is the only established and repeatedly confirmed genetic association with this disease. The association in the HLA region, however, accounts for less than 20% of the genetic risk for Behçet's disease. We have performed the first genome-wide association scan (GWAS) in a cohort of 152 Behçet's disease patients and 172 ethnically-matched healthy controls using DNA pooling and the Affymetrix 500K arrays, followed by TaqMan® genotyping in individual samples in candidate susceptibility loci. Our results identified 5 novel candidate genes for Behçet's disease (*KIAA1529*, *CPVL*, *LOC100129342*, *UBASH3B*, and *UBAC2*). Cluster analysis of the GWAS data identified 14 SNPs in the *KIAA1529* locus (9q22) that are associated with Behçet's disease. Among the associated SNPs, the Behçet's disease risk allele in rs2061634 located within *KIAA1529* leads to the change in the amino acid at position 995 to cysteine (S995C) in the KIAA1529 protein (odds ratio= 2.04, 95%CI= 1.45-2.88, P value= 4.2X10-5). By identifying genes specifically and consistently co-expressed with *KIAA1529* over 3,651 human 2-color microarray datasets from NCBI's GEO, we used an algorithm to infer function using a "guilt by association" method. The 20 genes most closely matching *KIAA1529*'s expression trends (i.e., up and down-regulated with it) were analyzed for commonalities using a literature mining software (IRIDESCENT). Through these analyses, we predict that the transmembrane protein KIAA1529 is expressed on the nuclear membrane and is involved in nuclear trafficking.

## 844/W/Poster Board #502

**Psorasis prediction from genome-wide SNP profiles.** *S. Fang[1,2], X. Fang[2,3], M. Xiong[2].* 1) Breast Medical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, TX; 2) Human Genetics Center The University of Texas Health Science Center at Houston Houston, TX; 3) School of Mathematical Sciences, Bejing University, Beijing.

With the availability of high-throughput genotyping technology, data on hundreds of thousands of SNPs are available through genome-wide association studies (GWAS). One of main goals of GWAS is to identify an optimal set of SNPs that can predict disease status with greatest possible accuracy. Prediction of genetic risk will be increasingly useful in diagnosis, treatment, and prognosis of complex diseases. In this study, a new classifier, the sequential information bottleneck (sIB), was proposed to perform classification on a big dataset with 2,798 observations and 451,724 SNPs. We first reduced the GWAS feature set into a manageable one through an effective filter method and then performed a feature selection wrapped around a classifier to identify an optimal SNP subset for predicting psoriasis. To deal with an unbalanced distribution of positive and negative observations, HMSS was used as the performance measurement of the predictive models. This study aimed to select optimal subsets of SNPs with maximum classification performance for psoriasis prediction. Process for searching a set of SNPs to predict psoriasis consisted of two steps. The first step was to search top 1,000 SNPs with high accuracy for prediction of psoriasis from GWAS dataset. The second step was to search for an optimal SNP subset for predicting psoriasis. The sequential information bottleneck (sIB) method was compared with classical linear discriminant analysis for classification performance. A chi-square test was also performed to look at the relationship between each SNP and disease. The proposed methods were applied to a GWAS dataset of psoriasis with a total of 2,798 samples and 451,724 SNPs. The best test harmonic mean of sensitivity and specificity for predicting psoriasis by sIB was 0.6745. If the test accuracy in the case group or control group was required to be not less than 0.4, then the highest test accuracy of sIB in the other group (i.e., control or case) reached 0.8503 or 0.7494, respectively. The fact that a small set of SNPs can predict disease status with average accuracy of 0.68 is of great value in clinical practice.

## 845/W/Poster Board #503

**COPACETIC, a Genome-wide association study on chronic obstructive pulmonary disease (COPD).** *J. Smolonska[1], HM. Boezen[2], H. Groen[3], A.E. Dijkstra[3], D.S. Postma[3], M. Oudkerk[4], B. de Hoop[5], B. van Ginneken[6], W. Mali[4], J.W. Lammers[7], P. Zanen[7], C. Wijmenga[1], COPACETIC consortium.* 1) Department of Genetics UMCG, Groningen, Groningen, Netherlands; 2) Department of Epidemiology UMCG, Groningen, Netherlands; 3) Department of Pulmonology UMCG, Groningen, Netherlands; 4) Department of Radiology UMCG, Groningen, Netherlands; 5) Department of Radiology, UMCU, Utrecht, Netherlands; 6) Image Sciences Institute, UMCU, Utrecht, Netherlands; 7) Department of Pulmonology, UMCU, Utrecht, Netherlands.

Chronic obstructive pulmonary disease (COPD) is characterized by persistent, progressive airway obstruction that is a result of emphysema and chronic bronchitis. The disease is responsible for almost 5% of all mortalities. COPD is a complex genetic disease which is triggered by tobacco smoking and/or air pollution. Alpha1-antitrypsin deficiency is responsible for 1-2% of COPD cases whereas the remainder of the genetic factors is largely unknown. The aim of our study is to identify novel genetic factors for COPD using a genome-wide association study (GWAS). Study participants were recruited from the Nelson study, a 4-year follow-up study on the incidence of lung cancer in heavy-smokers of Dutch descent (>20 pack years). A GWAS was performed on 1273 individuals with obstruction, 1389 individuals with emphysema and 1083 "supercontrols" (i.e. individuals without airway obstruction and emphysema). In addition we have access to 900 blood bank controls with unknown smoking history. All samples were genotyped using Illumina Human610-Quad arrays. Furthermore, from nearly 600 individuals we obtained RNA expression data from peripheral blood lymphocytes on 48,000 transcripts using Illumina HT12 arrays. Genotyping has been finished recently and we are currently at the stage of quality control of the data. Our GWAS study on COPD is unique since it includes both CT scan data and lung function for each participant and detailed smoking history. Therefore we expect to identify loci associated with COPD in heavy-smokers and possibly also loci that distinguish between the different COPD phenotypes emphysema and airway obstruction. Expression data will allow us to link genetic variation to differences in gene expression levels and may assist in identifying the culprit genes. We will next proceed with replication of the top 384 SNPs in five independent cohorts comprising 3687 COPD cases and 10491 controls.

## 846/W/Poster Board #504

**Studies of Genetic and Phenotypic Variations under Environmental Stimulation.** *X. Zhou[1], L. Luo[2], X. Guo[1], F. Tan[1], F. Arnett[1], M. Xiong[2].* 1) Deptartment of Internal Medicine, University Texas, Medical School at Houston, Houston, TX; 2) Department of Biostatistics, University of Texas School of Public Health, Houston, TX.

Despite extensive searches for genes causing complex diseases, connections between DNA variation and complex phenotypes, which is essential for unraveling the pathogeneses of complex diseases and predicting variation in human health, has proved to be elusive. In the past several decades, biologists have mainly focused on testing association of single markers or haplotypes with diseases attempting to find connections between the genotype and phenotype. However, health states of individuals remain a complex, multidimensional phenomena. Clinical manifestations arise from integrated actions of multiple genetic substracts(such as mutation, insertion/deletion, copy number variation, RNA regulation, etc) and environmental factors (such as infections, nutrition, stress and activity), through dynamic, epigenetic, and regulatory mechanisms. The analytic tools for this simple approach are unable to take comprehensive looks at entire systems that lead to changes from genomic and environmental variation to complex phenotypic variation. Holistic views of complex path from genotypes to phenotypes holds a key to unravel the mechanism of diseases. In this report, we developed longitudinal genetic models for the investigation of genetics of time-course gene expressions under the perturbation of environments. We genotyped 79 fibroblast strains with the genome-wide single nucleotide polymorphism (SNP) profiling (Illumina Human 317 K SNP panel). We examined gene expression changes of COL1A2, COL3A1, CTGF, SPARC and TIMP3 of these fibroblasts at five different time points (0, 1, 2, 3, and 5 days) in response to silica stimulation. We identified 38, 27, 19, and 15 eSNPs significantly affecting the expression of genes COL1A2, COL3A1, CTGF and TIMP3 after silica stimulation, respectively.

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## 847/W/Poster Board #505

**Small, hard macular drusen and peripheral drusen: Associations with *CFH* Y402H in the Inter99 Eye Study.** *J. Ek[1], I.C. Munch[2,3], L. Kessel[2], A. Linneberg[3], B. Sander[2], G.J. Almind[1], K. Brondum-Nielsen[1], M. Larsen[1,2].* 1) Med Genetics Lab Cen, Kennedy Center, Glostrup, Denmark; 2) Department of ophthalmology, Glostrup Hospital, University of Copenhagen, Denmark; 3) Research Centre for Prevention and Health, Glostrup Hospital, Copenhagen Denmark.

**Background:** Age-related macular degeneration (AMD) is the most common cause of legal blindness in elderly people. The hallmark of AMD is retinal drusen >125 μm consisting of extracellular deposits accumulated on Bruch's membrane below the retinal pigment epithelium. AMD is a complex disease with multiple environmental and genetic risk factors. Several single nuclear polymorphisms (SNPs) in genes encoding CFH, CFB, and LOC387715 are strongly associated to the development and progress of AMD. The presence of numerous small, hard retinal drusen < 63 μm is a highly hereditary feature that predisposes development of AMD but the association between this feature and AMD-related SNPs is unknown. **Objective:** To investigate the associations between the presence of ≥ 20 small, hard retinal drusen and other AMD-related retinal changes to known AMD-associated SNPs. **Participants:** The study included 1007 participants aged 30 to 66 years. **Methods:** Digital greyscale, red-free fundus photographs were graded for the presence of drusen and other fundus elements. The participants were genotyped for AMD-related polymorphisms in *CFH* (Y402H), *CFB* (R32Q and L9H), and *LOC387715* (A69S and rs11200638). **Main Outcome Measures:** Prevalence of ≥ 20 small, hard macular drusen per eye, stippling, macular drusen > 63μm, peripheral drusen and genotype associations. **Results:** The prevalence of ≥ 20 small, hard macular drusen, peripheral drusen and drusen > 63μm increased with age. Peripheral drusen were significantly associated with macular drusen > 63μm (OR 2.5 (CI95 1.2-5.4)) as was ≥ 20 small, hard macular drusen (1.7 (1.1-2.9)). The *CFH* Y402H polymorphism was associated with peripheral drusen (4.7 (1.5-15) for CC versus TT genotype) and macular drusen > 63μm (1.9 (1.1-3.1), for CC versus TT genotype) but not with ≥ 20 small, hard macular drusen. No further genotype associations were found. **Conclusions:** Although previous investigations have shown that the presence of many small, hard drusen is highly hereditary and related to future development of AMD, this study found no association between major genetic polymorphisms that predispose to AMD and the characteristic of having ≥ 20 small, hard macular drusen. This indicates the existence of additional, hitherto unidentified genotypes associated with AMD. Associations between peripheral drusen, macular drusen > 63μm and *CFH* Y402H were confirmed.

## 848/W/Poster Board #506

**The polygenic nature of open-angle glaucoma is supported by WDR36 acting as a modifier gene.** *V. Raymond[1], P. Belleau[1], K. Lebel[1], S. Dubois[1], R. Arseneault[1], J.L. Anctil[2], E. Shink[1], M.A. Rodrigue[1], G. Côté[2], M. Amyot[3], The Quebec Glaucoma Network.* 1) Biology of Sensory Systems and, Ocular Genetics & Genomics, CREMOG, Laval University Hospital (CHUL) Research Center, Québec City, PQ, Canada; 2) Department of Ophthalmology, Laval University, Québec City, PQ, Canada; 3) Department of Ophthalmology, Univ of Montréal, Montréal, PQ, Canada.

Open angle glaucoma (OAG) is primarily considered a common complex hereditary disease. More than 20 loci have been mapped for OAG but only three genes, *myocilin* (*MYOC*), *optineurin* and *WDR36*, have been implicated in mendelian forms of the disorder. To test for the polygenic nature of open-angle glaucoma we assessed if variations in *WDR36* changed the severity of OAG in heterozygotic carriers of *MYOC* mutations. To do so, we screened WDR36 variations and performed genotype/phenotype correlation studies in a large French-Canadian glaucoma family in which the primary disease-causing mutation was the MYOC K423E variation. One hundred and forty-two (142) MYOC K423E heterozygotes were investigated. Ninety-five (95) of them were diagnosed with either juvenile OAG (JOAG, a severe form of OAG diagnosed at or before 35 years old), primary OAG (POAG, diagnosed after 35 years of age), or intraocular hypertension (OHT) with treatment. Nineteen (19) were OHT without treatment, while the remaining 28 carriers were asymptomatic. All 23 exons of *WDR36* were screened for variations in these 142 heterozygotes. Our screening revealed that 24 heterozygotes carried simultaneously the MYOC K423E mutation plus one of five non-synonymous amino acid changes in WDR36. These individuals were named double variants. Since almost all K423E carriers had yearly ocular examinations, we determined age-at-onset (AAO) of the disorder in each one of them, and assessed for an effect of WDR36 variations on AAO by comparing AAO of glaucoma in the double variants versus the median of AAO of MYOC K423E carriers who were WDR36 wild-type and shared a kinship coefficient equal to or less than 0,0625 with their respective double variants. Of the 24 double variants detected, 18 had a neighborhood available for such comparison. Of these 18 double variants, 10 showed AAOs younger than the median AAO of their neighborhood while 5 of them displayed similar AAO. Surprisingly, 4 of these 10 double variants harbored the D658G WDR36 variation and showed an AAO 10 years or younger than the medians of their neighborhood. Two other double variants also displayed younger AAOs. We conclude that three WDR36 variants acted as susceptibility modifier genes by increasing glaucoma severity in heterozygotes for the MYOC mutation. Our findings support the polygenic inheritance of open-angle glaucoma and support *WDR36* as a modifier gene.

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## 849/W/Poster Board #507

**Comparative Haplotypic Congruence Analysis of Addison's Disease and Type 1A Diabetes.** *E.E. Baschal, J.C. Siebert, T.M. Triolo, T.K. Armstrong, J.M. Jasinski, K.N. Johnson, S.R. Babu, P.A. Gottlieb, P.R. Fain, J.M. Barker, M.J. Rewers, G.S. Eisenbarth.* Barbara Davis Center, University of Colorado Denver, Aurora, CO.

Addison's disease (AD) is a rare autoimmune disorder of the adrenal cortex that affects approximately 1:10,000 individuals. AD is associated with type 1 diabetes (T1D), with the highest risk HLA genotype for AD being DR3-DQB1*0201/DRB1*0404-DQB1*0302. We analyzed HLA-DR-DQ-stratified patients with AD (N=70) and T1D [Type 1 Diabetes Genetics Consortium (T1DGC): 2300 affected sibling-pairs and their parents genotyped for both SNPs and HLA alleles across the MHC region, N=2873 T1D patients]. The HLA-B8 allele is greatly increased in patients with AD compared to controls and to those with T1D [DR3+ individuals (%B8+): AD 51/56 (91%) vs. DR3/4 controls 174/271 (64%), p=1.9E-6, odds ratio=6.3; AD vs. T1D patients 1775/2873 (62%), p=2.9E-5, odds ratio=5.7]. In that 91% of all DR3+ AD cases had B8, AD may be associated with the DR3-B8-A1 extended HLA haplotype. The DR3-B8-A1 haplotype is conserved for millions of base pairs as revealed by high density MHC SNP typing. Data from the exon-centric Illumina SNP panel were analyzed for 35 of the AD patients, and 96% of those with DR3-B8 have SNPs matching the DR3-B8-A1 extended haplotype. AD patient chromosomes were phased by comparing genotypes to the DR3-B8-A1 consensus sequence established in homozygous DR3-B8-A1 AD patients and phased T1DGC chromosomes. We developed a computerized algorithm to evaluate long-range SNP congruence between chromosomes. Our congruence methodology attempts to find a long contiguous SNP identity (e.g., 100 contiguous SNPs) that characterizes a high percentage of the chromosomes. We inspected multiple overlapping 100-SNP sequences across the MHC, ranging from position 29,299,390 to 33,883,424 Mb. These analyses demonstrate that regions that approached or were at 100% congruence were similar for AD DR3-B8 chromosomes and T1DGC control DR3-B8 chromosomes. In addition, AD DRB1*0404 chromosomes showed a region of 100% congruence not seen in DRB1*0404 chromosomes from T1D patients or controls. We conclude that the DR3-B8 conserved extended haplotype is particularly and uniquely high risk for AD (DR3-B8-A1 is lower risk for T1D), and the DRB1*0404 haplotype in AD patients may be particularly congruent between 31,947,288 and 32,326,821 Mb, which encompasses the 21-hydroxylase gene (CYP21A2), a candidate gene for AD. The 21-hydroxylase molecule is the major adrenal autoantigen targeted by antibodies in AD.

## 850/W/Poster Board #508

**NPSR1 polymorphisms affect neuropeptide S receptor function and expression.** *M. D'Amato[1], F. Anedda[1], D. Schepis[2], G. McInerney[2], A. Hellquist[1], L. Corrado[3], A. Achour[4], M. Zucchelli[1], J. Kere[1].* 1) Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden; 2) Department of Microbiology Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden; 3) Department of Medical Sciences and IRCAD, University of Eastern Piedmont, Novara, Italy; 4) Center for Infectious Medicine, Karolinska Institutet, Stockholm, Sweden.

Background: The neuropeptide S (NPS) receptor gene, NPSR1, is mainly expressed in the brain and in enteroendocrine cells, but increased levels have been detected in lymphocytes and mucosal epithelia of asthma and inflammatory bowel disease (IBD) patients. Although still poorly characterized, the NPS-NPSR1 system appears to act along the gut-brain axis, and to contribute to the modulation of responses to stress, anxiety, nociception and inflammation. Remarkably, NPSR1 polymorphisms have been shown to associate with asthma, IBD and rheumatoid arthritis, but causative variations have not yet been identified. The NPSR1 gene spans 200 kb on chromosome 7p15, and more than 1400 SNPs have been described in different ethnic populations. To gain insight into the mechanisms leading to NPSR1 disease-predisposing effects, we performed functional analyses of all its promoter and coding SNPs occurring in Caucasians. Methods: NPSR1 promoter was predicted with bioinformatic tools. Five SNPs mapping within this region were studied by means of electrophoretic mobility shift assays (EMSAs), luciferase reporter assays on NPSR1-expressing Colo205 cells transfected with promoter variants, and Real-Time PCR quantification of NPSR1 expression in whole blood samples from genotyped healthy donors. Molecular modeling was used to evaluate the effect of four coding SNPs on receptor structure. NPSR1 coding variants were transfected in NPSR1-negative HEK293 cells, to evaluate their expression on the cell membrane by immunofluorescence and FACS analysis. Relative potency and specificity of NPSR1 downstream signaling was studied by microarray whole transcriptome analysis of HEK293 cells transfected with individual NPSR1 coding variants and stimulated with NPS. Kinetics studies (specific dose- and time-dependent responses to NPS) were performed on HEK293 cells co-transfected with NPSR1 coding variants and either cAMP response element binding (CREB-) or mitogen-activated protein kinase (MAPK/JNK- and MAPK/ERK-) driven luciferase reporter constructs. Results: One promoter SNP correlated with both altered luciferase activity and a significant effect on NPSR1 mRNA expression in white blood cells. Three NPSR1 coding variants were associated with considerable changes in receptor properties and signaling capacity. Conclusion: Specific SNPs at the NPSR1 locus affect receptor function and expression. Previous associations with disease(s) should be re-evaluated in the light of these findings.

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## 851/W/Poster Board #509

**Genetic predisposition to asthma in Mexican pediatric patients.** *S. Jimenez-Morales[1], N.E. Martínez-Aguilar[2], B.E. Del Rio-Navarro[3], J.L Jiménez-Ruíz[1], F. Cuevas[4], J. Gómez-Vera[5], G. Escamilla-Guerrero[6], E. Navarro-Olivos[7], R. Gamboa-Becerra[8], D.Y. López-Ley[8], Y. Saldaña-Alvarez[1], L. Orozco[1,9].* 1) Laboratorio de Enfermedades Complejas, Instituto Nacional de Médicina Genómica, Mexico City, Mexico Periferico Sur 4124, Torre Zafiro II, ExRancho de Anzaldo, Méx., D.F., Mex. CP 01900; 2) Servicio de Alergia, Hospital Regional 1° de Octubre, ISSSTE; 3) Departamento de Alergia, Hospital Infantil de México; 4) Departamento de Neumología y Cirugía de Tórax, Instituto Nacional de Pediatría; 5) Servicio de Alergia, Hospital Regional Adolfo López Mateos, ISSSTE; 6) Banco de Sangre, Instituto Nacional de Pediatría; 7) Programa de Maestría en Ciencias Médicas, Instituto Politécnico Nacional; 8) Programa de Licenciatura en Farmacobiología, Universidad Autónoma de Chiapas; 9) Programa de Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México.

Introduction: Asthma is an inflammatory chronic airway disease, which shows a high prevalence in Mexican population; actually the epidemiological studies suggest that nearby of 15% of the childhood population has asthma. Increasing evidences show that this disease has a highly complex genetic background and important ethnicity differences. At least one hundred candidate genes have been associated with this disease so far. Objective: The present study was undertaken to investigate the genetic risk factors associated with asthma in Mexican pediatric patients. Methods: Two hundred and forty one unrelated asthmatic patients from four tertiary Medical Centers in Mexico City and 400 healthy blood donor controls were included. All patients were diagnosed by a pediatric pneumologist or alergologist. Genotyping of 95 single nucleotide polymorphisms (SNPs) in 29 candidate genes was performed using TaqMan assay. False association due to stratification of the samples was discarded analyzing 10 informative markers which have been validated in previous analyses performed in Mexican population. To evaluate Hardy-Weinberg equilibrium we used FINETTI software. Stratification, haplotypes and statistical analyses were performed using STRUCTURE, STRAT, HAPLOVIEW and EPIINFO programs. Results: In this study we identified five genes associated to asthma: two as new genetic risk factors (CHRM2 and STAT1) and three previously reported (IL13, TNFa and MMP9). Four SNPs located in CHRM2 (rs324640:p= 0.04, rs324650: p= 0.03, rs8191992: p= 0.001 y rs6962027: p= 0.01), two in STAT1 (rs2280234: p= 0.0367 and rs2030171: p= 0.009), two in IL13 (rs1881457: p= 0.009 y rs1800925: p=0.05), one in TNFa (-308 GA: p= 0.014) and two in MMP9 (rs2274755: p= 0.002 y rs2274756: p=0.003).) showed significantly differences between cases and controls. On the other hand, some differences between cases and controls in the distribution of genotypes of CSF2, PDCD1, FCRL3 genes were observed. Conclusion: Complex genetic predisposition to asthma in pediatric Mexican patients was observed, inasmuch as only five of the 29 genes studied are associated with asthma in our sample. To know if these genes could be associated with severity of the disease, the number of patients is being increased.

## 852/W/Poster Board #510

**Role of IBD Risk Factor *MAST3* in TLR4 Signaling.** *C. Labbe[1], G. Charron[1], P. Goyette[1], C. Stevens[2], T. Green[2], M.K. Tello-Ruiz[2], Z. Cao[3], A.L. Landry[3], J. Stempak[4], V. Annese[5], A. Latiano[5], S. Brant[6], R. Duerr[7], K. Taylor[8], J. Cho[9], A.H. Steinhart[4], M. Daly[2], M. Silverberg[4], R. Ramnik[3], J.D. Rioux[1].* 1) Université de Montréal, Montreal Heart Inst, Montreal, PQ, Canada; 2) The Broad Institute of MIT and Harvard, Cambridge, MA; 3) Center for Computational and Integrative Biology and Gastrointestinal Unit, MGH, Harvard, Boston, MA; 4) Mount Sinai Hospital IBD Center, U. of Toronto, Toronto, On, Canada; 5) Gastrointestinal and Endoscopy Units, I.R.C.C.S. Hospital, San Giovanni Rotondo, Italy; 6) Meyerhoff Inflammatory Disease Center, Johns Hopkins University School of Medicine, Baltimore, MD; 7) School of Medicine, University of Pittsburgh, Pittsburgh, PA; 8) IBD center, Cedars-Sinai Medical Center, Los Angeles, CA, USA; 9) IBD Center, Department of Medicine, Yale University, New Haven, CT.

Inflammatory bowel disease (IBD) is a chronic disorder caused by multiple factors in a genetically susceptible host. Significant advances in the study of genetic susceptibility have highlighted the importance of the innate immune system in this disease. We previously completed a genome-wide linkage study and found a significant locus (IBD6) on chromosome 19p. We identified IBD risk factor *MAST3* through a two-stage association study of the region. We identified four *MAST3* coding variants, including a non-synonymous SNP rs8108738, associated to IBD. *MAST3* belongs to a gene family of serine/threonine protein kinases. To test whether *MAST3* was expressed in cells of interest, we performed expression assays which showed abundant expression of MAST3 in antigen presenting cells and in lymphocytes. The knockdown of *MAST3* specifically decreased TLR4 dependent NF-κB activity. In an effort to characterize the precise role of MAST3 in inflammation in general and in the TLR4 pathway leading to the activation of NF-κB specifically, we are studying: 1. the interactions of MAST3 with other proteins in the pathway (using a biotin/avi tag approach as well as a candidate protein approach) 2. the effects of MAST3 on phosphorylation of the partners in the TLR4-NF-κB pathway 3. the effects of the rs8108738 genotype on the expression of *MAST3* and pro-inflammatory cytokines downstream of the signaling cascade. In this genome-wide association studies era, one of the greatest challenges that geneticists are faced with is to understand the biological role of the numerous genes identified through association studies. Our findings highlight a role for MAST3 in the TLR4 pathway leading to the activation of NF-κB and provide additional proof of the pivotal role played by modulators of NF-κB activity in IBD pathogenesis.

## 853/W/Poster Board #511

**Association of polymorphisms in SPINK5 with atopic dermatitis is replicated in Korean.** *J. Namkung[1,2], J. Yang[1,3].* 1) Samsung Medical Center, Seoul; 2) Bioinformatics Program, Seoul National University, Seoul; 3) Department of Dermatology, Sungkyunkwan University School of Medicine, Seoul.

Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease that is characterized by intense itching and significantly deteriorates life quality. The importance of AD is growing as the prevalence increase in developed countries. Mutations in the gene encoding serine protease inhibitor Kazal-type 5 (SPINK5) has been reported to be responsible for Netherton syndrome, a severe recessive congenital skin disease associated with atopy. Recent studies reported that numbers of single nucleotide polymorphisms (SNPs) in SPINK5 are associated with AD in Japanese. Method: We genotyped 21 SNPs in SNPINK5 for 631 patients with AD and 459 normal controls. AD can be classified as extrinsic and intrinsic types based on the existence of manifest allergic reaction or not. Among our 631 AD patients, 417 presented extrinsic type of AD (ADe) and 214 presented intrinsic type (ADi). Association between SNPs and AD was tested using age, sex adjusted logistic regression. Haplotype association was also tested for haplotypes constructed across sliding windows of sizes 2-4 using haplo.stats in R package. Association test was conducted for severity for AD (SCORAD) index, total IgE level, eosinophil count, and eosinophilic cationic protein level. Result: Significant association was detected with rs17718511, rs17860502 (D106N), rs60978485, rs17718737 (A617A) in the single SNP analysis. None of the SNPs showed significant association with ADi. Three locus haplotype of rs60978485, rs6892205 (Q267R), rs2303064 (D386N) showed significant association with AD. This haplotype had OR 0.687 (95% CI: 0.501- 0.941) for ADe with the p-value of 0.02. Haplotypes of the three loci (rs2303067 (K420E), rs880687 (G518G), rs41291431) showed the most significant association with log-transformed eosinophil counts (p-value=0.003). Previously we have reported that SNPs in DEFB1 are associated with ADe. In order to find the most significantly associated interacting pair of SNPs from the two genes, we conducted gene-gene interaction analysis using imputeMDR in R packages. ImputeMDR allows the multifactor dimensionality reduction (MDR) analysis in the presence of missing values. The statistical significance and the effect size for the selected SNP pair via logistic regression. As a result, the alleles of the rs60978485 in SPINK5 and rs5743399 in DEFB1 act additively to increase the susceptibility of ADe. Conclusion: Our result replicates the association of SNPINK 5 with AD in Korean.

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## 854/W/Poster Board #512

**Genetic association studies of IL-13 gene in adult asthma in Japanese populations.** *M. Tamari[1], T. Hirota[1], M. Harada[1], Y. Hitomi[1], A. Miyatake[2], Y. Suzuki[3], Y. Nakamura[4].* 1) Lab Resp Dis, RIKEN Ctr Genomic Medicine, Yokohama, Japan; 2) Miyatake Asthma Clinic, Osaka, Japan; 3) Public Health Dept, Chiba Univ, Chiba, Japan; 4) Lab Mol Medicine, Hum Genome Ctr, The Univ of Tokyo, Tokyo, Japan.

(Background) Interleukin (IL-) 13 is a crucial mediator of allergic inflammation and induces airway hyperreactivity, epithelial cell damage, eosinophilia and goblet cell hyperplasia with mucus hyperproduction. Recent study has shown that *IL-13* polymorphisms are associated with allergic phenotypes. To assess genetic variants of *IL-13* related to susceptibility and clinical phenotypes in adult asthma, we conducted association analyses. Bronchial epithelial cells play a central role in the Th2-cell sensitization process by influencing the function of DCs and target cells of IL-13. The proinflammatory chemokines chemokine (C-C motif) ligand (CCL) 5 and chemokine CXC ligand (CXCL) 8 initiate airway inflammation by enhancing inflammatory cell recruitment, and a recent study has shown that poly(I:C) induces the synthesis of these chemokines by bronchial epithelial cells. We investigated whether IL-13 affects the poly(I;C)-induced chemokine expression in normal human bronchial epithelial cells (NHBE). (Method) After screening for polymorphisms, we identified a total of 14 variants and conducted linkage disequilibrium (LD) mapping. Pairwise LD was measured among 10 SNPs with a frequency of greater than 10%. Four variants (rs1881457, rs1800925, rs2066960, rs20541) were selected for genotyping, and these SNPs captured 10 of 10 alleles with a mean $r^2$ of 1.00. Genotyping was performed by the TaqMan allele-specific amplification method. The expression of *CCL5* and *CXCL8* was determined by real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) using SYBR Premix Ex Taq. (Results) We found significant associations between the SNPs of the *IL-13* gene and adult asthma susceptibility in two independent case-control studies. (P = 0.00086, OR=1.81; P = 0.00068, OR=1.80; meta-analysis). We also found that *IL-13* polymorphisms were significantly associated with susceptibility to aspirin-induced asthma and asthma severity. We could confirm that IL-13 enhanced mRNA levels of *CCL5* and *CXCL8*, which were induced by poly(I:C) in NHBE. (Conclusion) These findings suggest that the *IL-13* gene variants are involved in the development of adult asthma, asthma severity, and susceptibility to AIA through genetic polymorphism in the Japanese population, and IL-13 appears to influence chemokine induction in airway epithelial cells during viral infections.

## 855/W/Poster Board #513

**Caspase-12 and the inflammatory response to Yersinia pestis.** *B. Ferwerda[1], M.B.B. McCall[1,2], M.C. de Vries[3], J. Hopman[2], B. Maiga[4], A. Dolo[4], O. Doumbo[4], M. Daou[4], D. de Jong[5], L.A.B. Joosten[1], R.A. Tissingh[3], F.A.G. Reubsaet[3], R. Sauwerwein[2], J.W.M. van der Meer[1], A. van der Ven[1], M.G. Netea[1].* 1) Department of Internal Medicine, and Nijmegen Institute for Infectious inflammation & Immunity (N4i). Radboud University Nijmegen Medical Center, P.O.Box 9101, Geert Grooteplein 8, 6500 HB Nijmegen, The Netherlands; 2) Department of Medical Microbiology, Radboud University, Nijmegen Medical Centre, P.O.Box 9101, Geert Grooteplein 8, 6500 HB Nijmegen, The Netherlands; 3) Laboratory for Infectieus diseases and perinatal screening, Center for Infectieus Disease Conbtrol Netherlands, National Institute for Public Health and the Environment (RIVM), P.O. Box1, 3720 BA Bilthoven, The Netherlands; 4) Malaria Research & Training Centre, Faculty of Medicine, University of Bamako, Bamako, Mali; 5) Department of Gastroenterology and Hepatology, Radboud University Nijmegen Medical Center, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

Caspase-12 functions as an antiinflammatory enzyme inhibiting caspase-1 and the NOD2/RIP2 pathways. It has been proposed that due to increased susceptibility to sepsis in individuals with functional caspase-12, an early-stop mutation leading to the loss of caspase-12 has replaced the ancient genotype in Eurasia and a significant proportion of individuals from African populations. In African-Americans, it has been suggested that caspase-12 inhibits the pro-inflammatory cytokine production. We assessed whether similar mechanisms are present in African individuals, and whether evolutionary pressures due to malaria and plague may have led to the present caspase-12 genotype population frequencies in Africa and Eurasia. Whole blood was collected in two independent African populations. Whole blood stimulation with the TLR4 and TLR2 ligands lipopolysaccharide (LPS) and Pam3Cys was conducted. Stimulation with three pathogenic Yersinia species, namely Y. enterocolitica, Y. pseudotuberculosis and Y. pestis was done in one of the African populations. From all volunteers the caspase-12 genotype was also determined. To investigate if Yersinia spp recognition involved the NOD2/RIP2 pathway a total of 10 Dutch Caucasian controls and 5 Dutch Caucasian volunteers homozygous for the loss-of-function 3020insC NOD2 mutation were used for the stimulation experiments. No difference in cytokine induction through the caspase-1 and/or NOD2/RIP2 pathways was observed in two independent African populations, among individuals with either an intact or absent caspase-12. In addition, stimulations with Yersinia pestis and two other species of Yersinia were preformed to investigate whether caspase-12 modulates the inflammatory reaction induced by Yersinia. We found that caspase-12 did not modulate cytokine production induced by Yersinia spp., although we demonstrate for the first time the involvement of the NOD2/RIP2 pathway for recognition of Yersinia. Caspase-12 does not modulate innate host defense against Gram-negative bacteria and Y. pestis. Hereby it is unlikely that pathogenic selective pressure leading to sepsis caused the loss of caspase-12 in Eurasia. Therefore alternative explanations for the geographical distribution of caspase-12 should be sought.

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## 856/W/Poster Board #514

**Several of the genes showing association to SLE in the recent GWA studies are associated and show evidence of gene-gene interaction in the Finnish population.** *A. Hellquist[1], T.M. Jarvinen[1,2,3,4], M. Zucchelli[1], S. Koskenmies[3,5], H. Julkunen[6], M. D'Amato[1], U. Saarialho-Kere[2,7], J. Kere[1,3,4].* 1) Dept Bioscience & Nutrition, Karolinska Inst, Huddinge, Sweden; 2) Departments of Dermatology University of Helsinki, Helsinki, Finland; 3) Medical Genetics, University of Helsinki, Helsinki, Finland; 4) Folkhälsan Institute of Genetics, Helsinki, Finland; 5) Department of Dermatology, Helsinki University Central Hospital, Helsinki, Finland; 6) Peijas Hospital, Helsinki University Central Hospital, Vantaa, Finland; 7) Section of Dermatology and Department of Clinical Science and Education, Karolinska Institutet at Stockholm Söder Hospital, Stockholm, Sweden.

Systemic lupus erythematosus (SLE) is a complex autoimmune disease, characterized by production of pathogenic autoantibodies against self. This subsequently leads to the formation of immune complexes, followed by tissue inflammation in multiple organs. The genetic component in SLE is strong and in the last couple of years we have seen a dramatic increase of identified and confirmed SLE genes and loci by genome wide association (GWA) studies. We have analyzed SNPs in several genes shown to be associated in the recent GWA studies, including *STAT4*, *PXK*, *BANK1*, *ATG5*, *TNFAIP3*, *ICA1*, *IRF5/TNPO3*, *XKR6*, *BLK/FAM167A*, *LYN*, *KIAA1542*, *LRFN5*, *ITGAM*, *SCUBE1* and rs10798269 on chromosome 1q25.1 in a Finnish SLE cohort consisting of 275 SLE patients and 356 healthy controls. Strong associations were seen to several SNPs in STAT4 (best $P = 1.12 \times 10^{-6}$, OR = 1.92), *IRF5/TNPO3* (best $P = 2.06 \times 10x\ 10^{-7}$, OR = 1.95) and *ITGAM* (best $P = 8.29 \times 10^{-6}$, OR = 2.13). Furthermore, several SNPs in *TNFAIP3* (best $P = 4.50 \times 10^{-3}$, OR = 2.86) and *BLK/FAM167A* (best $P = 1.50 \times 10^{-3}$, OR = 1.52) showed significant but more modest association. Borderline significance was also seen for one SNP in *BANK1* (P = 0.04, OR = 1.30) and one SNP in *KIAA1542* (P = 0.02, OR = 1.35). None of the analyzed SNPs in *PXK*, *ATG5*, *ICA1*, *LYN*, *LRFN5* and *SCUBE1* showed significant association to SLE in our Finnish cohort, possibly due to low power. It has been suggested that gene-gene interaction may contribute to the risk of developing SLE and thus we wanted to investigate if any of the genes included in the present study have epistatic effects. A multiple logistic regression under a codominant model was used to test gene-gene interaction by adding an interaction term between the genotypes of interest using the R software v. 2.6.2 (www.r-project.org). Our preliminary results suggest epistatic interaction between SNPs in *STAT4* and *BANK1*, *STAT4* and *IRF5/TNPO3*, *BANK1* and *IRF5/TNPO3*, *ATG5* and *LYN*, *BLK/FAM167A* and *ITGAM*. In summary we have replicated several of the previously associated GWA genes in our Finnish SLE cohort, further supporting their importance in SLE pathogenesis. In addition we show preliminary evidence of epistasis, or gene-gene interaction between several of these genes.

## 857/W/Poster Board #515

**Replication of 5q22.1 region for Intraocular Pressure in Mongolian population by linkage and Association analyses: The GENDISCAN Project.** *M. Lee[1], J. Sung[1], S. Cho[1], J. Kim[2], J. Seo[2], H. Kim[2].* 1) Department of Epidemiology, Seoul National University, Seoul, Korea; 2) Department of Biochemistry and Molecular biology, Seoul National University College of Medicine.

Primary open-angle glaucoma (POAG) is the most common type of glaucoma, the second most frequent cause of visual impairment worldwide, and elevated intraocular pressure (IOP) is an important risk factor. A study using healthy population, particularly Asians, has academic and public health significance, since genetic studies of IOP have been mainly accomplished in glaucomatous families among Caucasians. The GENDISCAN study recruited large families from two isolated populations (Selenge and Dashibalbar) in Mongolia. IOP level, epidemiologic data, and clinical information were collected. Selenge population comprised 1451 individuals from 142 families and 390 short tandem repeat (STR) markers were genotyped. In Dashibalbar we collected 1490 individuals from 95 families and the genotyping is done with 1097 STR markers, and 610K genome-wide SNPs (Illumina) for 652 individuals. Candidate loci underlying IOP variation were investigated, using variance component-based genome-wide linkage analysis (SOLAR). We performed a family-based association analysis on chromosome 5, of which linkage evidence was replicated in both Mongolian populations. The heritabilities of IOP level among two populations were 0.48 and 0.50, adjusted for age, sex and age by sex interaction. The 5q22.1 region showed linkage signal: LOL score of 2.4 for Selenge and LOD of 2.05 for Dashibalbar. Genome-wide association analysis revealed two candidate genes, Zinc Finger protein 608 (ZNF608) and WD repeat domain 36 (WDR36), which are 1Mb apart. In test (Dashibalbar) we found closely-located 9 significant SNPs (p-value <0.0005) in ZNF608. The most significant SNP was rs12659606 (p-value=1.889e-07). We also detected a haplotype with 7 SNPs (p-value = 0.0009). In WDR36, associated with POAG in previous studies, we identified 4 SNPs and a haplotype with 8 SNPs with statistical significance. C alleles on rs7723819 increased IOP level by 7% and 2% for having one more C allele (WDR36, C allele frequency 0.39%). Our findings indicate that quantitative trait loci (QTL) regulating IOP in the general population are linked to regions harboring POAG genes, suggesting a QTL influence both normal variation and disease occurrence. This provides a new example showing that some genes may regulate both normal variation and the development of disease. In addition, the common genes shared in Whites and Mongolians suggest that the mutation causing high IOP and glaucoma should be old in the history of human evolution.

## 858/W/Poster Board #516

**Differential expression of microRNAs in peripheral blood mononuclear cells of Multiple Sclerosis patients.** *G. Solda'[1], E.M. Paraboschi[1], D. Gemmati[2], P. Zamboni[3], S. Duga[1], R. Asselta[1].* 1) Dipartimento di Biologia e Genetetica per le Scienze Mediche, Università degli Studi di Milano, Milan, Italy; 2) Center of Hemostasis and Thrombosis, University of Ferrara, Ferrara, Italy; 3) Vascular Diseases Center, University of Ferrara, Ferrara, Italy.

MicroRNAs (miRNAs) are a class of short (21-23 nucleotides) single-stranded RNAs that modulate the expression of multiple target mRNAs by inducing either translational repression or mRNA degradation. MiRNAs have emerged has key post-transcriptional regulators of diverse biological processes and their deregulation has been implicated in several complex human diseases, including neurodegenerative and inflammatory disorders. Multiple sclerosis (MS) is a multifactorial disease of the central nervous system characterized by chronic inflammation, demyelination, axonal damage, and progressive neurological dysfunction. In spite of extensive research, the molecular events involved in the initiation and progression of MS are still poorly understood. To be relevant for MS pathogenesis, candidate genes would be expected to be expressed either in tissues relevant for immune response, or in tissues affected by the disease process. Several miRNAs are known to be expressed specifically in the immune system and miRNA-dependent alterations in gene expression in hematopoietic cells are critical for mounting an appropriate immune response. Moreover, deregulation of hematopoietic-specific miRNA expression may result in defects in both central and peripheral tolerance. Therefore, we sought to explore the possible involvement of miRNAs in MS by monitoring for differential expression of specific miRNAs in peripheral blood mononuclear cells (PBMCs) of Italian MS patients and healthy controls. We selected a set of 22 candidate miRNAs that are expressed in the immune system and/or are transcribed from previously reported MS susceptibility loci. The differential expression of candidate miRNAs in cases versus controls was evaluated using a microbead-based technology. In a pilot experiment, performed on PBMC RNA of relapsing-remitting MS patients and controls, 4 miRNAs resulted >3 folds up-regulated in MS vs controls, whereas only the miR-150 resulted down-regulated (2-fold decrease). Interestingly, two of the most up-regulated miRNAs, mir-155 and mir-146a, have been reported to be altered also in rheumatoid arthritis and systemic lupus erythematosus, suggesting shared pathogenic mechanisms between different chronic inflammatory diseases. This is, to our knowledge, the first evidence that the expression of specific miRNAs is altered in MS.

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## 859/W/Poster Board #517

**Clinical features of mitochondrial encephalopathy, lactic acidosis and stroke-like episodes syndrome (MELAS) in 47 Chinese families.** *Y. MA[1], F. FANG[2], Y. CAO[1], Y. ZHANG[1], S. WANG[1], Y. YANG[3], Y. XU[1], P. PEI[1], Y. QI[1].* 1) Central Lab, Peking Univ First Hosp, Beijing, China; 2) Neurology Dept, Beijing Children's Hospital, Beijing, China; 3) Pediatrics Dept, Peking University First Hospital, Beijing, China.

Mitochondrial encephalopathy, lactic acidosis and stroke-like episodes syndrome (MELAS) is the most common form of mitochondrial encephalomyopathy. Molecular genetic studies have shown that 80% of MELAS patients have the A3243G mtDNA mutation. In order to further elucidate the symptoms and clinical course of MELAS patients and their relatives in larger population, the clinical symptoms, natural history, characteristics of inheritance, mitochondria-related complication of 47 Chinese families were analyzed. 47 MELAS patients known to harbor A3243G mutation and their 61 maternal relatives including proband's mothers and siblings were colleted and analyzed. Informed consent in accordance with the Helsinki Declaration was obtained from all participants. The age of probands ranged 2-36 years old (mean ± SD = 11.18 ± 6.51 years), and the male to female ratio is 1.35. The age of probands' mother ranged 27-57 years old (mean ± SD = 39.04± 6.96 years), and the age of probands' siblings ranged 3 months to 40 years old (mean ± SD = 17.09± 13.34 years) and the male to female ratio is 1.8. The lactic acidaemia (100%), exercise intolerance (97.5%), seizures (89.4%), short stature (89.4%), hairiness (89.4%) and weight loss (85.1%) are the most common clinical features in probands. 59.6% probands' mother have not any symptom, and lactic acidaemia (40.4%), exercise intolerance (36.2%), short stature (21.3%), weight loss (34.0%) are the most common clinical features in their mothers. And they have no symptoms of stroke-like episodes, seizures and hairness. A3243G mutation ratio of probands was 11.1~70.0% (40.45 ± 12.65) in blood and 48.8~91% (67.47 ± 12.65) in urine. Positive A3243G mutation was found in blood in 32 mothers with the ratio of 2.7~50% (14.23±10.85), in urine in 42 mothers with the ratio of 4.6~76.1% (35.63±17.10). A3243G were not detected in 5 mothers. In MELAS patients and their mothers A3243G mutation ratio is significantly higher in urine than in blood (p<0.01). 3 siblings whose mother carries A3243G mutation have no A3243G mutation. In conclusion, (1) lactic acidaemia, exercise intolerance, seizures, short stature, hairiness and weight loss are the most common clinical feature, but not the "stroke" in younger patients; (2) measurement of A3243G mutation ratio in urine is more powerful than that in blood; (3) the probands' A3243G mutation ratio is higher than their mothers but not linear correlated; (4) the "mutant mother" can have "healthy baby".

## 860/W/Poster Board #518

**Associations of functional polymorphisms in NLR genes with susceptibility to food-induced anaphylaxis.** *Y. Hitomi[1], M. Ebisawa[2], T. Hirota[1], M. Harada[1], Y. Suzuki[3], Y. Nakamura[4], M. Tamari[1].* 1) Laboratory for Respiratory Diseases, Center for Genomic Medicine, RIKEN, Yokohama, Japan; 2) Clinical Research Center for Allergy and Rheumatology, Sagamihara National Hospital, National Hospital Organization, Sagamihara, Japan; 3) Department of Public Health, Graduate School of Medicine, Chiba University, Chiba, Japan; 4) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

(Background) Anaphylaxis is a life-threatening allergic reaction, and food is one of the most common responsible allergens. Although anaphylaxis is a severe side effect of both the food challenge test and oral desensitization therapy, there is no completely reliable method to predict which subjects have a high risk for food-induced anaphylaxis. Furthermore, little is known about the genetic factors of food allergies or anaphylaxis. The nucleotide-binding domain, leucine-rich repeat-containing (NLR) family controls the activity of inflammatory caspase-1 by forming inflammasomes, which leads to cleavage of the proinflammatory cytokines IL-1$\beta$ and IL-18. To clarify the pathophysiology of food-induced anaphylaxis, we performed an association study between the polymorphisms in NLR family genes and food-induced anaphylaxis in a Japanese population. (Method) We selected tag SNPs of NLR genes using the HapMap Japanese data set and conducted association analyses using a total of 309 pediatric patients with food allergies diagnosed by oral food challenge tests (included 95 patients who had experienced anaphylaxis) and a total of 234 control children. Genotyping was performed by the TaqMan allele-specific amplification method. We further performed functional analyses of the susceptible SNPs. (Results) Two SNPs of a NACHT, LRR and pyrin domain- containing protein (NALP) gene were significantly associated with susceptibility to food-induced anaphylaxis (P = 0.00086, OR=1.81; P = 0.00068, OR=1.80). A haplotype of the gene also showed a significant association with susceptibility to food-induced anaphylaxis (P = 0.000098, OR=2.21). Since we could not find any SNP that was in strong linkage disequilibrium with these SNPs by resequencing and searching the dbSNP database, we focused on the functional effects of the two SNPs. Luciferase and electrophoretic mobility shift assays for these SNPs using human monocyte cell line THP-1 revealed that the risk alleles of two SNPs increased the enhancer activity of NLRP expression and mRNA stability, respectively. (Conclusion) Our results indicate that the NALP SNPs might play an important role in the development of food-induced anaphylaxis. Further research on the NLR family will contribute to the development of novel diagnostic and therapeutic methods for food-induced anaphylaxis.

## 861/W/Poster Board #519

**Positive association for FOXE1 and loci in 8q24 in CL/P cases.** *M.A. Mansilla[1], S. Patte[1], K. Ryckman[1], M.L. Feldkamp[2], R.G. Munger[3], J.C. Murray[1].* 1) Deparment Pediatrics, Univ Iowa, Iowa City, Iowa; 2) Utah Birth Defects Network and University of Utah , Salt Lake City, Utah; 3) Department of Nutrition and Food Sciences, Utah State University, Logan, Utah.

Non syndromic cleft lip with or without cleft palate (NSCL/P) is a common multifactorial birth defect caused by the interaction of genetic and/or environmental factors. To date a number of genes have been described positively associated with this trait, including IRF6, MSX1, FGFR2 and FOXE1. For the environmental role, smoking and vitamin intake have been extensively studied. The purpose of this study was to test SNPs known to be associated with NSCL/P as well as some involved in the folate pathway in a case control study of oral cleft birth defects conducted in Utah during 2000-2005 in collaboration with the Utah Birth Defects Network (UBDN). This study collected samples from 510 cases and 565 controls and their parents; a total of 29 SNPs in different genes including IRF6, FOXE1, BMP4, FGFR2, MTHFR, MTRR, MTR, MTHFD1, DHFR, NNMT, FOLR1, RFC1 and GART, were tested for association. Two markers in the 8q24 region recently described as associated with NSCL/P in a genome wide study were also analyzed (Birnbaum et al 2009). The Transmission Disequilibrium Test (TDT) showed significant results for SNPs in FOXE1 (rs3748249, p value=0.00457) and in 8q (rs987525, p value=5.63E-06), these results were also confirmed by case-control analysis of the probands, allelic p values 0.002 and 0.0001, respectively. Our study replicates the association found in FOXE1 (9q22.3) and the 8q24 region by TDT and is the first confirmation of these results in a case control study, therefore reinforcing the important role of these two loci in the etiology of NSCL/P.

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## 862/W/Poster Board #520

**Mapping of genetic risk for severe dengue virus infection to the *NFKB1-MANBA* region on chromosome 4 (4q21).** *M. Yasunami[1], TPL. Nguyen[1,2], N. Okuda[1], H. Horie[1], S. Kurata[1], TQH. Vu[2], TTN. Vu[2], TT. Tran[3], MT. Ha[3], VT. Vo[4], VD. Tran[4], M. Kikuchi[1], K. Morita[1], H. Hirayama[1].* 1) Immunogenetics,Inst Trop Med, Nagasaki Univ, Nagasaki, Japan; 2) Pasteur Institute in Ho Chi Minh City, Vietnam; 3) Nhi Dong Hospital No.2, Ho Chi Minh City, Vietnam; 4) Center for Preventive Medicine, Vinh Long Province, Vietnam.

Dengue fever is acute febrile disease caused by infection of dengue virus. A small fraction of the patients with dengue fever (DF) develop more severe form with hemorrhagic tendency, dengue hemorrhagic fever (DHF) or that with symptoms of profound plasma loss in addition, dengue shock syndrome (DSS). Multiple factors have been proposed for the development of these severe forms, and excessive or inappropriate host response would contribute to pathogenesis to significant extent. Therefore we explored genetic polymorphisms in the immune-related candidate genes, which may account for individual variations in responsiveness to pathogens, including the gene for a subunit of nuclear factor kappa B (NFKB1) on 4q21. Microsatellite polymorphism D4S3043 in the downstream region of NFKB1 exhibited bimodal distribution of allele frequency in the population and the shorter alleles were associated with the risk for disease progression. Because the microsatellite locates in the 12th intron of the gene encoding lysosomal beta mannosidase (MANBA), we further examined the association of two non-synonymous coding SNPs in MANBA as well as two SNPs in NFKB1. As a result, polymorphisms in downstream of NFKB1 gene form a LD block and exhibited significant association with severe forms of dengue virus infection. Variations in the function of these two gene conferred by these polymorphisms may influence host-pathogen interaction upon the infection of dengue virus.

## 863/W/Poster Board #521

**Prognostic significance of FTO genotype in the development of obesity in Japanese: the J-SHIPP study.** *Y. Tabara[1, 6], H. Osawa[4, 6], R. Kawamoto[3], H. Onuma[4, 6], I. Shimizu[5], Y. Takata[4], W. Nishida[4], H. Makino[4], H. Guo[2], K. Kohara[2,6], T. Miki[2,6].* 1) Department of Basic Medical Research and Education, Ehime University Graduate School of Medicine, Ehime, Japan; 2) Department of Geriatric Meidicne, Ehime University Graduate School of Medicine, Ehime, Japan; 3) Department of Community Meidicne, Ehime University Graduate School of Medicine, Ehime, Japan; 4) Department of Molecular and Genetic Medicine, Ehime University Graduate School of Medicine, Ehime, Japan; 5) Department of Internal Medicine, Ehime Prefectural Hospital, Ehime, Japan; 6) Ehime ProteoMedicine Research Center.

**Objective:** Susceptibility of fat mass and obesity-associated (FTO) gene polymorphisms to obesity has been reported in various populations. Polymorphisms in melanocortin 4 receptor (MC4R) gene were recently explored as another susceptible locus. However, prognostic significance of these genetic variations has not been fully elucidated. Here, we investigated the involvement of FTO rs9939609 and MC4R rs17782313 polymorphisms in a development of obesity. Association with type 2 diabetes (T2DM) was also investigated. **Subjects;** We analyzed 2,806 community-dwelling middle-aged to elderly subjects (61±14 years). Basic clinical parameters were obtained from the subjects' personal health records, evaluated at their annual medical check-up. Genomic DNA was extracted from peripheral blood and all SNPs were analyzed by TaqMan probe assay. **Results;** FTO but not MC4R genotype was significantly associated with current body mass index (BMI; TT 23.2±3.2, TA 23.7±3.2, AA 24.4±3.2 kg/m2, p=2.5*10$^{-6}$) and frequency of obesity (BMI >25kg/m$^2$) (26.6, 32.0, 43.0% respectively, p=2.0*10$^{-4}$). Age and sex adjusted odds ratio for obesity was 1.30 (p=0.004) in TA and 2.07 (p=0.002) in AA genotype. To clarify the prognostic significance of FTO genotype in the development of obesity, we retrospectively analyzed the association between SNP rs9939609 and the development of obesity with 9.4 years follow-up. During 9.4 years follow up period, 214 new cases of obese were diagnosed among 1,718 subjects whose retrospective data was available, and who were not obese at baseline (BMI less than 25 kg/m2; TT (n=1191), 21.8±2.0; TA (n=485), 22.1±1.8; AA (n=42) 22.6±1.6 kg/m$^2$, p<0.001). A-allele frequency of the FTO genotype was significantly higher in obese-developed subjects (22.2, 15.8%, p=0.001). Multiple logistic regression analysis identified A-allele of the FTO genotype as an independent determinant for the development of obesity (odds ratio 1.46 (95±37; confidence interval 1.04-2.04), p=0.031) after adjusted for age, sex and initial BMI. However, association studies and meta-analysis for T2DM did not actively support the involvement of FTO genotype. **Conclusion;** FTO genotype is an independent risk factor for future development of obesity. Since body weight is a trait which is modifiable by dietary and exercise interventions, early detection of at-risk populations using genetic information may be useful in preventing future obesity-related diseases.

## 864/W/Poster Board #522

**Genetic diagnosis of rheumatoid arthritis using one major and several minor risk gene markers.** *W. Li, J. Freudenberg, P.K. Gregersen.* Genomics & Hum Genet, Feinstein Ins Med Res, Manhasset, NY.

Rheumatoid Arthritis (RA) is a complex disease caused by genetic and environmental risk factors. Standard parameters describing genetic associations, such as the odds-ratio (OR) and p-value are not informative regarding the percentage of RA cases and controls that are explained by the presence of absence of the associated alleles. Towards this goal of predictive genetic test, we distinguish the classification rate of case group (sensitivity) and that of control group (specificity). Using a dataset with 908 RA patients and more than 1000 controls, we constructed single-gene and multiple-gene classifiers using the HLA-DRB1 (the major RA risk gene, shared-epitope (SE)=0,1,2), and SNP data for PTPN22, BLK, TRAF1, as well as other minor risk genes. The sensitivity of the classifier with DRB1-SE alone under the dominant model reaches 98%, and the specificity under the recessive model is 94%. However, the average classification rate ((sen+spe)/2) is reduced to 76% and 67% under the dominant and the recessive models, versus the 50% baseline classification rate. The receiver operating characteristic (ROC) curve shows the dominant model for the major gene to be only slightly better than the recessive model. Adding minor genes to the classifier does not increase the classification rate notably (only a few percent) for various types of classifiers including logistic regression, recursive partitioning, and random forests. We further used a threshold model that considers the total number of risk alleles in both major and minor RA genes. Multi-dimensional scaling showed graphically, for DRB1-SE=1 samples, that RA cases and controls overlap in the projected space. This conclusion that SE=1 group is the hardest to classify group is confirmed by the recursive partitioning result that for SE=1 samples, other minor risk genes can only achieve classification rates around the 60% range.

## 865/W/Poster Board #523

**Host Genetic Susceptibility Factors for Severe West Nile Virus Infection.** *K.J. Buckingham[1], A.W. Bigham[1], S. Husain[1], J. Kriesel[2], A. Rutherford[2], N.M. Astakhova[3], A.A Perelygin[3], M.P. Busch[4], K. Murray[5], M.A. Brinton[3], M.J. Bamshad[1].* 1) Dept. of Pediatrics, University of Washington, Seattle, WA, USA; 2) Dept. of Medicine, University of Utah, Salt Lake City, UT, USA; 3) Dept. of Biology, Georgia State University, Atlanta, GA, USA; 4) Blood Systems, San Francisco, CA, USA; 5) Dept. of Medicine, University of Texas Health Sciences Center at Houston, Houston, TX, USA.

West Nile Virus (WNV), a category B pathogen representative of the *Flavivirus* family, is the etiologic agent of WNV disease, and is endemic in parts of Africa, Asia, and Europe. In 1999, WNV emerged in Eastern North America, and over the past decade, WNV infections have spread across the U.S. The outcome of infection with WNV ranges from asymptomatic to severe neuroinvasive disease manifested as encephalitis, paralysis, and/or death. Severe WNV disease occurs in fewer than 5% of cases and risk of disease may be influenced by host genetic factors, although the identity of these genetic factors remains largely unknown. In animal models, host innate and adaptive immune networks are both essential for protection against severe WNV disease. Accordingly, we tested 360 common haplotype tagging SNPs and/or functional SNPs in 58 genes that encode key regulators of immune function in 760 WNV infection cases including 439 patients with mild or severe disease, and 321 viremic blood donors with asymptomatic infections. It is important to note that individuals with asymptomatic infection served as "controls" and this is presumably more robust than using individuals with unknown WNV exposure as controls. Comparison of WNV disease vs. asymptomatic infections identified several significant associations, after applying a false discovery rate correction for multiple tests, with SNPs in three genes using a dominant model (*IRF3*; OR 2.06 (p<0.005), *TLR8*; OR 2.17 (p<0.05), and *TBK1*; OR 2.06 (p<0.05)) and a suggestive association with a SNP in *CD40LG* (OR 1.81 (p<0.1)). This is consistent with the observation that mice lacking *Irf3* are uniformly susceptible to WNV infection (Daffis et al. *PLOS Pathogens* 3: e106 (2007)). To look for alleles associated with severe WNV disease, we compared mild WNV cases combined with asymptomatic infections to severe WNV disease cases. None of the SNPs associated with risk of disease were associated with disease severity. This suggests that genetic factors influencing the host immune responses underlying development of symptoms might differ from those influencing risk of severe neuroinvasive disease. Identification of candidate SNPs associated with WNV susceptibility and host response will help stratify risk, identify novel disease-related pathways, and discover new potential therapeutic targets.

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## 866/W/Poster Board #524

**Functional SNPs in CD244 gene associated with rheumatoid arthritis in a Japanese population.** *A. Suzuki[1], R. Yamada[1, 2], Y. Kochi[1], Y. Okada[2], T. Sawada[3], K. Matsuda[4], Y. Kamatani[4], M. Mori[3], K. Shimane[1, 3], A. Takahashi[1], K. Yamamoto[1, 3].* 1) Ctr for Genomic Medicine, Kanagawa, RIKEN, Yokohama City, Japan; 2) Laboratory of Functional Genomics, Human Genome Center, Institute of Medical Science, University of Tokyo, Japan; 3) Department of Allergy and Rheumatology, Graduate School of Medicine, University of Tokyo, Japan; 4) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Japan.

Rheumatoid arthritis (RA) is well-known as an autoimmune disease and is a chronic inflammatory disorder characterized by the destruction of multiple joints. Many genome wide association studies were performed and multiple RA-susceptibility loci and autoimmune-susceptibility loci have been identified. These studies suggested that multiple genes and its functions were related with disease causing and development. These studies also indicated an important factor regarding genetic factors of RA and autoimmune diseases; some of the RA-susceptible polymorphisms also increase the risks of other autoimmune diseases as reported for e.g., STAT4 (ref1) and FCRL3 (ref2) with RA and SLE. One of the mechanisms of the inflammation in autoimmune diseases associated with signal transduction via signaling lymphocytic activation molecule (SLAM). It was reported that SLAM family gene, e.g., Ly108 is also associated with systemic lupus erythematosus (SLE). We studied whether variants of the SLAM family gene in the chromosome 1q region are associated with susceptibility to RA. The association peak in the block was observed at two functional SNPs (rs3766379 and rs6682654) in CD244 in two independent RA cohorts from Japan (P= 3.23 x 10-8 and P=7.45 x 10-8). We found a Japanese cohort of systemic lupus erythematosus (SLE) that had the similar genotype distribution with RA cohorts. A correlation between CD244 expression and genotype was observed in lymphoblastoid cell lines and these disease-associated SNPs, rs3766379 and rs6682654 have been shown to increase their expression in luciferase and allele-specific transcript quantification assays. Furthermore, we indicated that rs6682654 locates on the binding site of USF-1 in CD244 gene and affect on the regulation of CD244 expression via USF-1. CD244 also expresses in NK cell marker positive cells in RA synovial tissues. We supposed that up-regulation of CD244 by transcription factors including USF-1 affect on RA and other autoimmune diseases, including SLE. Thus, CD244 is a novel genetic risk factor for RA and may have a role for autoimmunity in RA. 1) Remmers, E.F. et al. N Engl J Med 357, 977-86 (2007). 2) Kochi, Y. et al. Nat Genet 37, 478-85 (2005).

## 867/W/Poster Board #525

**Replication and trinucleotide repeat instability at the Myotonic dystrophy type 1: tissue-specificity and the contribution of *cis*-elements.** *J. Cleary[1,2], K. Hagerman[1,2], A. López Castel[1], S. Tomé[3], L. Foiry[3], I. Paradis[4], M. Dorschner[5], H. Sroka[6], D. Chitayat[6,7], J. Stamatoyannopoulos[8], R. Drouin[4], G. Gourdon[3], C. Pearson[1,2].* 1) Genetics & Genome Biology, Hospital for Sick Children, Toronto, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, Canada; 3) Inserm U871, Hopital Necker-Enfants Malades, Paris, France; 4) Department of Pediatrics, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, Canada; 5) Department of Medicine, University of Washington, Seattle, WA, USA; 6) Department of Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, Canada; 7) Department of Pediatrics, Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, Canada; 8) Department of Genome Sciences, University of Washington, Seattle, WA, USA.

Myotonic dystrophy type 1 (DM1), as well as other trinucleotide repeat diseases, are often characterized by complex patterns of inherited and ongoing tissue-specific instability and pathogenesis. However what regulates the tissue-specific and age-dependent patterns of repeat instability is unknown. Repeat instability is observed in proliferating and non-proliferating tissues suggesting contributions from both DNA repair and DNA replication. In DM1 patient cells, where instability is proliferation-dependent, treatment with drugs that alter replication fork progression affects the DM1 repeat expansions. An analysis of replication in the presence of active instability and in different tissues may yield clues to the unique molecular mechanisms of repeat instability. Replication initiation, replication direction and fork progression through the DM1 (CTG)n repeat was determined in DM1 patient cells and transgenic mouse tissues actively undergoing expansions. The replication profile was determined using quantitative competitive PCR of newly replicated DNA from both patient cells and mouse tissues, while the contribution of flanking *cis*-element was assessed via 2D gel, bisulfite and DNase hypersensitive site analysis.

Replication analysis reveals that the DM1 repeat tract is flanked by two replication origins, such that replication of the expanded repeat proceeds from the downstream origin. Both tissue-specific and age-dependent variations in replication and CpG methylation were observed from analysis of the DM1 transgenic mice. The replication profile orients the CAG repeats as the lagging strand template and positions flanking CTCF sites between the repeat tract and replication origin. Analysis of *cis*-elements revealed that the flanking CTCF binding sites contribute to replication fork pausing at the expanded DM1 repeat tract. Alteration at these flanking sites affected replication efficiency, pausing and repeat instability. These results coupled with the coincidence of replication origins, CTCF binding sites and DNase hypersensitive sites with disease-linked trinucleotide tracts suggest these *cis*-elements mark the DM1, and other repeat loci, for tissue-specific and age-dependent instability.

## 868/W/Poster Board #526

**Using ontology fingerprint derived gene network to infer polygenic model from GWA study.** *W. Zheng, T. Qin, L. Tsoi, A. Lawson.* Dept. Biostat, Bioinfo & Epid, Med Univ South Carolina, Charleston, SC.

Contrary to general believe that human diseases are caused by single genetic variant, more and more evidences show that many human diseases are caused by cumulative effect of many genetic variants. This polygenic model poses a significant challenge to current Genome-wide Association (GWA) study, as variants in a polygenetic model do not show strong association with the disease phenotype individually. Therefore, additional biological evidences are needed to identify polygenic model from genes that are marginally associated with disease phenotypes from GWA study. We have developed the concept of ontology fingerprints a list of ontology terms overrepresented in the PubMed abstracts linked to a gene or a disease along with their corresponding enrichment p-value, to characterize genes and diseases. We further have quantified the relationship between a gene and a disease by comparing their ontology fingerprints - the more similar the ontology fingerprint between a gene and a disease, the more likely the gene will play a role in the disease. Such quantified biological relevance has been applied to prioritize genes identified from GWA study to be associated with the plasma concentrations of HDL, LDL and TG. Here we report a new approach to derive novel gene networks by performing pairwise comparison of ontology fingerprints of human genes. In this network, genes are nodes and the similarity scores between genes are weighted edges. We further developed a Bayesian hierarchical model to use exiting pathway information to infer biologically relevant threshold for the weighted edges in the network. Applying the identified threshold and algorithms based on graph theory, we are able to identify network modules. While using this approach to analyze genes associated with LDL, HDL and TG as identified from a GWA study, we are able to identify network modules that show strong biological relevance to disease phenotype. One module contains APOE and LDLR, consistent with their polygenic effect on lipid concentration supported by previous study. Additional genes in the same module point to a potential multi-gene model that influences lipid concentration. Such network modules have significant implication for the genetic bases of the polygenic model. Our methodology provides a novel way to analyze GWA study to infer novel polygenic model that may contribute to human disease.

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## 869/W/Poster Board #527

**Interaction between periodontitis and arthritis involves immunological functional interferences and shared genetic susceptibility, with the possible involvement of NRAMP1/SLC11A1.** *G.P. Garlet[1], A.P. Trombone[2], M. Claudino[1], G.F. Assis[1], C.E. Repeke[1], E.M. Silveira[1], P. Colavite[1], M.J. Avila-Campos[3], A.P. Campanelli[1], M. de Franco[4], P.C. Trevilatto[5], W. Martins Jr[6], J.S. Silva[2], C.R. Cardoso[7].* 1) Biological Sciences, School of Dentistry of Bauru, São Paulo University, Bauru, SP, Sao Paulo, Brazil; 2) Department of Biochemistry and Immunology, School of Medicine of Ribeirão Preto, São Paulo University - FMRP/USP; 3) Department of Microbiology, Institute of Biomedical Sciences - ICB/USP; 4) Laboratório de Imunogenética, Instituto Butantan, São Paulo, Brazil; 5) Center for Health and Biological Sciences, Pontifícia Universidade Católica do Paraná - PUCPR; 6) Department of Periodontics, Dentistry School of University of Ribeirão Preto - UNAERP; 7) 3 Department of Biological Sciences, Federal University of Triângulo Mineiro - UFTM;.

Periodontitis (PD) and arthritis (AR) share characteristics such as the chronic nature of the inflammatory reaction associated with bone resorption activity, and have been found to be clinically associated. However, the mechanisms underlying such association are unknown. Therefore, we examined the basis of A. actinomycetemcomitans(AA)-induced PD and pristane-induced AR interaction in genetically selected mice strains (AIRmin and AIRmax) presenting distinct NRAMP (SLC11A1) alleles, and also AR-associated NRAMP SNPs in PD patients. AIRmax strain (that present R NRAMP allele) was found to be susceptible to the development of both experimental AR and PD, while AIRmin strain (that present S NRAMP allele) was found to be resistant to AR development, and to develop PD at a low severity. The increased tissue destruction in AIRmax mice was associated with higher levels of TNF-alpha, IL-1beta, IL-17, and also of MMP13 and the osteoclastogenic factor RANKL. When AIRmin and AIRmax mice were submitted to both AR and PD induction, higher scores of PD (but not AR) severity were seen in AIRmax but not in AIRmin strain. Increased tissue destruction in AIRmax mice submitted to both PD and AR was associated with increased IL-1beta, IFN-gamma, IL-17, RANKL and MMP13 levels, while similar levels of IL-4 and IL-10 were verified. Such functional interference of AR in PD development was associated with alteration in the patterns of T cell subsets transcription factors expression in submandibular LNs, where T-bet and RORgamma were found to be upregulated, while GATA3 levels were unaltered, when compared to mice only submitted to PD induction. Complementarily, NRAMP 5'(GT)n (rs34448891) SNP 3/3 genotype, previously associated with RA, was found to be significantly more frequent (p=0.0540, OR= 2.046, CI=0.98/4.21) in PD susceptible patients (N=178) than in control group (N=343) when compared to the 2/3 genotype, while no differences were found in comparison with 2/2 genotype (p=0.2528, OR=1.308). Interestingly, both murine R NRAMP allele and human 3 NRAMP 5'(GT)n allele were found to be associated with increased NRAMP mRNA expression in macrophages; and also with increased (human and experimental) PD and (experimental) RA severity. Our results demonstrate that the interaction between experimental periodontitis and arthritis involves functional immunological interferences and shared genetic susceptibility, with the possible involvement of NRAMP1.

## 870/W/Poster Board #528

**Glucose homeostasis Genes and Spina Bifida Risk.** *K.S. Au[1], K.S. Weymouth[1], C.M. Davidson[1], A.C. Morrison[2], J.-I. Lin[2], J.M. Fletcher[3], K.K. Ostermaier[4], G.H. Tyerman[5], S. Doebel[6], H. Northrup[1,7].* 1) Dept Pediatrics, Univ Texas Med Sch, Houston, TX, USA; 2) Human Genetics Center, Univ Texas Sch Public Health, Houston, TX, USA; 3) Dept Psychology, Univ Houston, Houston, TX, USA; 4) Texas Children Hospital, Houston, TX, USA; 5) Shriners Hospital for Children, Los Angeles, CA, USA; 6) The Hospital for Sick Children, Univ Toronto, Toronto, ON, Canada; 7) Shriners Hospital for Children, Houston, TX, USA.

Neural tube defects (NTDs) are a leading cause of infant mortality and morbidity involving the central nervous system. Spina bifida (SB) is a common NTD with the defect occurring in the developing spinal cord resulting in various degree of lower extremities paralysis. Glucose imbalance and deficiency of folate during pregnancy are two major risk factors for NTDs. We identified three single nucleotide polymorphisms (SNPs) in three glucose metabolic pathway genes showing association with SB risk in an earlier report. The current validation study investigated an additional 140 SNPs on the same simplex patient family set plus 103 additional patient families. The total number of simplex family included in the study is 608. Reconstruction-combined transmission disequilibrium test (RC-TDT) results verified association of a synonymous SNP coding for Pro196 (p=0.009) in the glucose transporter 1 (*GLUT1*) gene to occurrence of SB. Strikingly, nine additional SNPs in glucose transporters 1 (*GLUT1*), two SNPs in glucose transporter 3 (*GLUT3*), one SNP in glucose transporter 8 (*GLUT8*), one SNP in hexokinase 1 (*HK1*), two SNPs in insulin receptor (*INSR*), two SNPs in leptin (*LEP*), and two SNPs in superoxide dismutase 1 (*SOD1*) gene loci showed significant association to SB with nominal p-values range from 0.0039 to 0.049. The results in the present study strongly support a critical role of glucose homeostasis maintaining genes as contributory to SB susceptibility. Many of these genes have been associated with diseases (including NTDs) due to diabetes or glucose imbalance. The current findings support the hypothesis that maintenance of glucose homeostasis during pregnancy as a vitally important for normal neural tube development and implicate specific genes in this process.

## 871/W/Poster Board #529

**Haplotype-level candidate gene association analysis of *DPYSL2* and *COMT* in a European schizophrenia sample.** *M.E.S. Bailey[1], P.C.D. Johnson[2], B.J. Morris[1,4], J.A. Pratt[3,4], R. Hunter[2,4,5], C.L. Winchester[1,3,4].* 1) Molecular Genetics, and Neuroscience and Molecular Pharmacology, FBLS, University of Glasgow, Glasgow, UK; 2) Robertson Centre for Biostatistics, University of Glasgow, Glasgow, UK; 3) SIPBS, University of Strathclyde, Glasgow, UK; 4) Psychiatric Research Institute of Neuroscience in Glasgow (PsyRING), Glasgow, UK; 5) NHS Greater Glasgow and Clyde, Glasgow, UK.

When a gene harbours multiple functional variants, haplotype analysis may reveal genetic associations invisible at the single SNP level, particularly where confounding interaction or haplotype evolution effects are operating. Single SNP studies can thus be misleading. We are conducting a haplotype-level association study of two candidate genes in schizophrenia, *COMT* and *DPYSL2*. Five SNP markers spanning *COMT* were chosen to test the hypothesis that it harbours multiple functional variants, in addition to the widely studied exon 4 coding SNP, rs4680 (p.V158M), that influence schizophrenia risk via effects on transcription or mRNA stability. Eleven SNPs were chosen spanning *DPYSL2*, which had emerged as a physiological candidate from a microarray study of gene expression in prefrontal cortex in a phencyclidine (PCP) rat model of schizophrenia. *DPYSL2* maps to chr.8p21, a region believed to harbour one or more schizophrenia genes. We used a UK sample of 500 DSM-IV-diagnosed SCZ patients and 500 ethnically similar normal controls. ABI TaqMan assays were used to genotype SNPs. Haplotypes were predicted and analysed for association using the R module, haplo.stats, and using Haploview v.4.1. Five SNPs encompassing a region in the 3' half of *DPYSL2* previously implicated in schizophrenia showed modest associations with SCZ in our sample (best $P = 0.021$ for rs13277175 under a G allele-dominant model; O.R. = 1.35 [1.045-1.752, 95% C.I.]). Preliminary analysis of *DPYSL2* haplotypes in the sample suggest that the haplotype containing all 5 associated risk alleles is rare in the population and larger sample sizes may be required to demonstrate any haplotype-level association, but further analysis is ongoing. A qRT-PCR study of transcript levels in SCZ patient and control post-mortem brain showed that transcript levels are associated with genotype at several of the associated SNPs. Only one *COMT* SNP showed association with SCZ (rs4680; p.V158M; $P = 0.011$; O.R. = 1.44 [1.085-1.911] under a model in which the ancestral 'V' allele confers risk in dominant fashion). In a preliminary analysis, *COMT* haplotypes centred around rs4680 and two other nearby SNPs in a single LD block did not show any evidence for association with SCZ. Our modestly sized study supports the candidacy of *DPYSL2* in schizophrenia, but does not support a hypothesis of multiple SNPs near exon 4 of *COMT* influencing schizophrenia risk via local effects on mRNA stability.

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## 872/W/Poster Board #530

**Association between the SNAP25 gene and the extremes in intellectual performance; mental retardation and high IQ.** *G. Beunders[1,2], P. Rizzu[1,2], E.A. Sistermans[1], J.W.R. Twisk[3,5], W. van Mechelen[4,5], J.B. Deijen[6], P. Heutink[1,2], H. Meijers-Heijboer[1,2], D. Posthuma[1,2,7].* 1) Department of Clinical genetics, VU medical centre, Amsterdam, The Netherlands; 2) Neuroscience Campus Amsterdam, Department of Clinical Genetics, VU medical centre, Amsterdam The Netherlands; 3) Department of epidemiology and biostatistics, VU medical centre, Amsterdam, The Netherlands; 4) Department of Public and Occupational Health, VU medical centre, Amsterdam, The Netherlands; 5) EMGO institute, VU Medical centre, Amsterdam, The Netherlands; 6) Department of Clinical Neuropsychology, VU University, Amsterdam, The Netherlands; 7) Department of Biological Psychology, VU University, Amsterdam, The Netherlands.

*Introduction* Intelligence in the normal range is shown to be highly heritable. For high cognitive ability a recent twin study has shown a substantial heritability of 0.50 as well. Low intelligence, mental retardation, defined by an IQ below 70 and a deficit at two or more adaptive skills starting at a childhood age (American Psychiatric Association, DSM-IV 1994), is thought to have a heritable cause in about 50% of the cases. Genome wide and candidate gene association studies have identified some loci and genomic variants associated with variation in intelligence in the normal range. High IQ is thought to be caused by the same genomic variants, but until now there is no direct prove for this hypothesis. For mental retardation however, most genetic causes seem to be rare and monogenetic. *Aim* We set out to test whether the SNAP25-gene, which was recently linked to variation of intelligence in the normal range (Gosso et al., 2006), is also linked to variation in intelligence at both the lower (mental retardation) and the upper range (high IQ). *Methods* We genotyped two SNP's in SNAP 25 (rs363039 and rs363050), previously linked to variation in intelligence in the normal range (Gosso, et.al. 2006), in two samples by use of a Taqman assay. The first sample contained DNA of 644 children with mental retardation aged 4-16 that had been tested negative for fragile-X syndrome and had a normal karyotype. The second sample contained DNA of 360 participants of the Amsterdam growth and health study, which were recruited at age 13-14 from two secondary schools; all were following the highest level of education. *Results* Using a case-cohort association test, we found significant association of both SNP's with the extremes of intelligence. The previously identified increaser alleles (Gosso et al. 2006) had a significant ($p<0.05$) higher allele frequency in the high IQ sample compared to the mental retardation sample. *Conclusion* We found evidence for association of two non-coding SNP's (rs363039 and rs363050) in SNAP25 with variation in the lower and upper extremes of intelligence. This suggests that, at least for SNAP-25, the same genomic variants affect both variation in normal intelligence and mental retardation as well as high IQ, an often heard but hardly tested statement. *reference* M.F. Gosso, et al. (2006) The SNAP-25 gene is associated with cognitive ability: Evidence from a family based study in two independent Dutch cohorts. Mol Psychiatry 11: 878-886.

## 873/W/Poster Board #531

**SNP and expression analyses of candidate genes for orofacial clefts.** *J. Kim[1], M. Dunnwald[2], J.C. Murray[1,2].* 1) Department of Anatomy and Cell biology, University of Iowa, Iowa City, IA; 2) Department of Pediatrics, University of Iowa, Iowa City, IA.

Association studies based on single nucleotide polymorphism (SNP) have substantially advanced our understanding of the genetic basis of complex human diseases. However, it remains a challenge to establish a functional link between associated variants and disease traits. Variation in gene expression is important in mediating disease susceptibility, and the identification of polymorphic genetic variants that affect gene expression may contribute to a better understanding of disease etiology. In order to assess the relationship between sequence variants and gene expression, we analyzed SNPs in selected candidate genes for orofacial clefts, one of the most common birth defects with a complex etiology, and correlated these with transcript expression levels. A total of 145 human foreskin samples were genotyped for 34 SNPs in 10 candidate genes for clefting including *BMP4*, *FGFR1*, *FGFR2*, *FOXE1*, *IRF6*, *MSX1*, *PDGFC*, *SOX9*, *TFAP2*, and *TGFB3*. Quantitative real-time PCR (qRT-PCR) was performed to evaluate the expression levels of each gene at the mRNA level in the same tissue samples. Of the 10 genes, eight were highly expressed in the foreskin tissue and were included in the analysis to correlate DNA variations with gene expression levels. There were no SNP variants found that are associated with a twofold or greater difference in gene expression. One variant in *FGFR2* and two variants in *SOX9* exhibited less than a twofold, but statistically significant difference (one-way ANOVA, $p<0.05$). The results suggest that a combination of multiple SNPs may produce effects that would be difficult to determine by looking at one SNP at a time, and that larger sample sizes might identify more subtle effects. Further studies of the interactions among multiple polymorphisms are needed to elucidate the genetic basis of variation in gene expression. Interrogation of the effects of SNPs on gene expression may prove to be useful in unraveling the causes of complex phenotypes.

## 874/W/Poster Board #532

**Genetic and functional characterization of GRIP1 sequence variants in autism spectrum disorders.** *R. Mejias-Estevez[1], A. Adamczyk[1], V. Angonno[2], S. Yang[1], C.E. Schwartz[3], R. Stevenson[3], C. Skinner[3], M.D. Fallin[4], D. Valle[1], R.L. Huganir[2], T. Wang[1].* 1) Institute of Genetic Medicine and Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD; 3) Greenwood Genetic Center, Greenwood, SC; 4) Department of Epidemiology, Johns Hopkins University School of Public Health, Baltimore, MD.

Abnormal synaptogenesis and imbalance between the excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmission are implicated in autism spectrum disorders. Glutamate receptor interacting protein 1 (GRIP1) is a neuron-enriched scaffolding protein with seven PDZ domains. GRIP1 is abundantly expressed in both glutamatergic and GABAergic synapses, and plays an important role in receptor trafficking and synaptic organization. The PDZ4-6 domains of GRIP1 mediate a direct interaction with the AMPA glutamate receptor 2/3 (GluR2/3), GABAA receptor interacting protein-associated protein and gephyrin, key components of the postsynaptic complex at GABAergic synapses. A case-control study of a known SNP in the PDZ4-6 genomic region implicated GRIP1 gene in autism. By screening exon-containing regions of the GRIP1 gene in a large cohort of patients with autism (n=480) and a cohort of ethnically matched controls (n=480), we identified seven missense variants involving highly conserved amino acid residues. All seven were transmitted from parents and six were found only in the autism cohort. Four variants are located in domains PDZ4-6 and at least three have altered interactions with GluR2/3 based on coimmunoprecipitation and/or a yeast two-hybrid assay. Immunofluorescence and receptor trafficking assays on the variants are being conducted in primary neurons to delineate their roles in both glutamatergic and GABAergic synapses. A positive correlation of the severity in the behavioral phenotype was noted in a proband family with two affected brothers, one homozygous and one heterozygous for the M794R variant. These studies suggest that functional GRIP1 variants could have a pathogenic role in a small fraction of autism subjects by altering synaptic trafficking of glutamatergic and/or GABAergic receptors leading to an imbalance between the excitatory and inhibitory circuitries in specialized regions of the brain.

## 875/W/Poster Board #533

**Genes in Folate and Glucose metabolism Associate with attention-deficit/hyperactivity disorder risk in patients with Spina Bifida.** *A.C. Morrison[1], J.-I. Lin[1], K.S. Au[2], K.S. Weymouth[2], C.A. Martinez[2], P.T. Cirino[3], J.M. Fletcher[3], K.K. Ostermaier[4], S. Doebel[5], M. Dennis[5], H. Northrup[2,6].* 1) Human Genetics Center, Sch Public Health, Univ Texas, Houston, TX, USA; 2) Dept Pediatrics, Univ Texas Med Sch Houston, Houston, TX, USA; 3) Dept Psychology, Univ Houston, Houston, TX, USA; 4) Texas Children Hospital, Houston, TX, USA; 5) The Hospital for Sick Children, Univ Toronto, Toronto, ON, Canada; 6) Shriners Hospital for Children, Houston, TX, USA.

**Objective:** Spina bifida (SB) is a common NTD with the defect occurring in the developing spinal cord resulting in various degree of paralysis of the lower extremities. An approximately 6-fold increased incidence of attention-deficit/hyperactivity disorder (ADHD) subtypes has been associated with SB and shunted hydrocephalus. Previous studies indicate that single nucleotide polymorphisms (SNPs) in genes belonging to the glucose metabolic pathway and folate/homosysteine metabolism are associated with SB. This study examined whether SNPs in genes from these pathways were associated with presence of ADHD among SB patients. **Study Design:** The study included 253 unrelated simplex SB patient families. A total of 81 SB patients met the criteria for ADHD based on DSM-IV in Swanson, Nolan, and Pelham-IV (SNAP-IV) survey and 172 patients were determined not to have ADHD. We evaluated 32 SNPs in the methylene-tetrahydrofolate reductase (*MTHFR*) and the glucose transporter 1 (*GLUT1*) genes for an association with ADHD using the reconstruction-combined transmission disequilibrium test (RC-TDT). **Results:** Significant association was identified with three SNPs (rs2274976, rs3737965 and rs4846049) in *MTHFR* with ADHD with p-values of 0.0414, 0.0003 and 0.0475, respectively. Two SNPs (rs2229682 and rs1385129) in *GLUT1* also demonstrated a significant association with ADHD with p-values of 0.0433 and 0.0386, respectively. **Conclusion:** Increased risk of ADHD among patients with SB may be associated with transmission of specific SNPs alleles in *MTHFR* and *GLUT1*. This is the first study to show that genetic variation in genes from the glucose metabolic pathway and folate/homosysteine pathway may increase risk of attention problems and executive functions in children with SB.

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## 876/W/Poster Board #534

**Evidence for interactive effects of smoking and asthma status on associations between *NOS1*, *NOS2A*, and *NOS3* genetic variants and $Fe_{NO}$ or eosinophil levels in the EGEA study.** *R. Nadif[1], F. Monier[1], M. Boussaha[2], N. Le Moual[1], R. Matran[3], J. Bousquet[1], I. Pin[4], M. Lathrop[5], F. Kauffmann[1], F. Demenais[2], E. Bouzigon[2].* 1) Inserm U780, Univ Paris-Sud, IFR69, Villejuif, France; 2) Inserm, U946, Paris, France; 3) Univ Lille Nord de France, CHU, Lille, France; 4) Inserm U823, Univ Joseph Fourier, Grenoble, France; 5) CEA, CNG, Evry, France.

Asthma is a complex and heterogeneous disorder, resulting from both genetic and environmental factors. Blood eosinophil count and exhaled nitric oxide ($Fe_{NO}$) are markers of inflammation in asthma. Nitric oxide is produced endogenously by nitric oxide synthases (NOS). We investigated associations of $Fe_{NO}$ level and eosinophil count with 37 tag SNPs belonging to three candidate genes: *NOS1* (n=25), *NOS2A* (n=5) and *NOS3* (n=7), in current asthmatics and non asthmatics, and whether smoking habits modify these associations. Association analyses were conducted using GEE regression-based method in 172 families (482 adults, 39.2 yrs, 47% current asthmatics) from the French Epidemiological study on the Genetics and Environment of Asthma. Overall, $Fe_{NO}$ levels were positively associated with asthma (geometric mean: 20.4 *vs.* 15.9 ppb, P<0.0001) and with eosinophil count (*r*=0.28, P<0.0001) and negatively associated with smoking (trend P<0.0001). In asthmatics, *NOS1* polymorphisms were associated with both a decreased $Fe_{NO}$ level and an increase of eosinophil count (P ranging from 0.02 to 0.003), while no association was found in non-asthmatics ($P_{Interaction} \leq 0.03$). Regarding $Fe_{NO}$ level, we also found a strong interactive effect between *NOS3* (rs743507) and current asthma status ($P_{Int}<0.0001$). In asthmatics, TT genotype increased $Fe_{NO}$ (29.8 vs. 19.3 ppb, P=0.002), whereas this genotype was associated with a decrease in $Fe_{NO}$ in non-asthmatics (10.8 *vs.* 15.6 ppb, P=0.01). We also detected an interaction between *NOS2A* polymorphisms and smoking influencing $Fe_{NO}$ levels ($P_{Int}$ = 0.002). Indeed, non-asthmatic smokers carrying AA genotype at rs3730013 had a decreased $Fe_{NO}$ level (8.8 *vs.* 15.1 ppb, P=0.002), whereas no association was found in non-smokers. In asthmatics, this latter polymorphism was also associated with an increase of eosinophil count (P=0.002). No interactive effect between smoking and *NOS1* or *NOS3* genes on $Fe_{NO}$ level or eosinophil count was observed. Our findings suggest that 1) associations of *NOS1* and *NOS3* with $Fe_{NO}$ level and eosinophil count differed according to current asthma status and 2) smoking modify the effect of *NOS2A* variants on FeNO level.

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## 877/W/Poster Board #535

***ARMS2* A69S and *CFH* Y402H polymorphisms are not associated with choroidal neovascularization in highly myopic eyes of the Japanese population.** *H. Nakanishi[1,2], N. Gotoh[1,2], R. Yamada[3], K. Yamashiro[1], A. Otani[1], H. Hayashi[1,2], I. Nakata[1,2], A. Tsujikawa[1], N. Shimada[4], K. Ohno-Matsui[4], M. Mochizuki[4], M. Saito[5], K. Saito[5], T. Iida[5], F. Matsuda[2], N. Yoshimura[1].* 1) Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan; 2) Center for Genomic Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan; 3) Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; 4) Department of Ophthalmology and Visual Science, Tokyo Medical and Dental University Graduate School, Tokyo, Japan; 5) Department of Ophthalmology, Fukushima Medical University, Fukushima, Japan.

Choroidal neovascularization (CNV) is one of the most vision-threatening complications in high myopia. Clinical findings such as patchy chorioretinal atrophy and lacquer cracks are important predisposing signals for the development of CNV in highly myopic eyes. CNV is a complication which is observed in both high myopia and in age-related macular degeneration (AMD); this raises a hypothesis that the process involved in the development of AMD also contributes to the development of CNV in highly myopic patients. To examine this hypothesis, we studied genetic association of two major genetic determinants for AMD, namely, *ARMS2* and *CFH*, with the development of CNV in Japanese population. For this purpose, DNA samples of 569 high myopia patients (axial lengths > 26.00 mm or a refractive errors > -6.0 diopters) consisting of 281 patients with CNV (mean age ± standard deviation of 58.8 ± 13.6 years, ranged from 21 to 88 years; male : female, 22.8% : 77.2%) and 288 patients without CNV (55.1 ± 13.9 years, ranged from 21 to 88 years; male : female, 43.1% : 56.9%) were recruited. We compared genotype distribution of rs10490924 (A69S) of *ARMS2* and rs1061170 (Y402H) of *CFH* between these two groups using the Taqman technology. The differences in the genotype distributions for these two SNPs were tested with the Trend $\chi^2$ test. We failed to obtain significant difference in the genotype distribution of both SNPs between the two groups (*P*=0.49 for rs10490924 and *P*=0.38 for rs1061170). The P-values were not improved with adjustments for age and gender using logistic regression analysis (*P*= 0.50 for rs10490924 and *P*=0.78 for rs1061170). Although functional roles of *ARMS2* and *CFH* in the onset and progression of AMD, particularly CNV in the disease, are yet to be elucidated experimentally, genetic alteration of these two genes is not likely to contribute to the development of CNV in high myopia in Japanese.

## 878/W/Poster Board #536

**Common Variants in the Pregnane X Receptor Gene may contribute to the Genetic Susceptibility to Nonalcoholic Fatty Liver Disease.** *S. Sookoian[1,2], G. Castaño[2], A. Burgueño[1], T. Fernández Gianotti[1], M. Rosselli[1], C. Pirola[1].* 1) Laboratory of Clinical and Molecular Hepatology and Department of Molecular Genetics and Biology of Complex Diseases. Institute of Medical Research A Lanari-IDIM, University of Buenos Aires- National Council of Scientific and Technological Research. CONIC; 2) Department of Medicine and Surgery. Hospital Abel Zubizarreta. Ciudad Autónoma de Buenos Aires, Argentina.

Background: Nonalcoholic fatty liver disease (NAFLD), a disease characterized by fat accumulation in the liver, is an emerging epidemic disease affecting 10 to 40 percent of the general population in Western countries, and up to 80% of obese and diabetic individuals. While it is well known that environmental risk factors influence the development and progression of NAFLD, the contribution of the individual genetic variation to disease predisposition is still uncertain. Pregnane X receptor (PXR) is a member of the nuclear receptor superfamily, whose primary function is the regulation of an entire network of genes involved in the detoxification and elimination of xenobiotics from the body. Emerging evidence has also pointed to an equally important role of PXR as an endobiotic receptor by impacting on lipid homeostas.Objective: To explore the contribution of gene variants and derived haplotypes of the PXR to the severity of NAFLD. Methods: 290 individuals were evaluated in a case-control association study, including 188 NAFLD patients with different stages of disease severity and 102 healthy individuals. Four tag SNPs (rs12488820 C/T; rs2472671 C/T; rs2461823 A/G; rs1054191 A/G) encompassing 36 kb in chr.3 and representing 33 polymorphic sites (r2 >0.8) were genotyped. Besides, 4 additional SNPs (rs3814055, rs3814057, rs6785049 and rs7643645) were included because they showed previous evidence about functionality. Results: Genotypic tests for single SNP showed that rs7643645 and rs2461823 were significantly associated with disease severity by ordinal multinomial analysis (p=0.0011 and 0.038, respectively). A significant association was also observed under the additive model for both variants (p=0.00037 and 0.011, respectively). Consistent with the analysis of individual markers, we observed that the multimarker composed by the rs2461823/A-rs7643645/G was significantly associated with the disease severity (p=6.8X10$^{-5}$, beta 0.45). In addition, the rs7643645/G variant was significantly associated with ALT level (p= 0.025), a surrogate marker of severe liver injury. Finally, in univariate analysis the rs7643645/G was significantly associated with fatty liver disease (p value=0.039), odds ratio, 1.457; 95% confidence interval, 1.018-2.086. Conclusion: Our study suggests that PXR polymorphisms and related haplotypes may contribute to the disease severity in NAFLD by influencing the individual susceptibility to progress to more severe stages of the disease.

## 879/W/Poster Board #537

**Mitochondrial DNA polymorphisms in metabolic syndrome.** *M. Lu[1], R. Bai[3], L. Wong[3], S. Juo[1,2].* 1) Graduate Institutive Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Kaohsiung Medical Univerisity Memorial Hospital, Kaohsiung, Taiwan; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, USA.

Metabolic syndrome is characterized by hyperglycemia, dyslipidemia, hypertension and diabetes. Growing evidence indicated that mitochondrial dysfunction is involved in the development of type 2 diabetes mellitus and metabolic syndrome. We hypothesized that mtDNA variants may be involved in the development of metabolic syndrome. We proposed a case-control study to investigate the association between mtDNA polymorphisms and metabolic syndrome. A total of 537 cases and 1106 controls were recruited in our study. We selected 82 mtDNA SNPs and 18 Asian-specific haplogroups in the initial screening test (case n=141; control n=506). The initial screening showed that six mtSNPs (249delA, C3970T, A10398G, A12361G, G13928C, T12126C) and three haplogroups (D4, F and M10) were related to susceptibility to metabolic syndrome. Among these mtDNA polymorphisms, we selected the most significant genetic variant, mtSNP A10398G, for the validation test in another dataset. We found the G variant of mtSNP A10398G mildly increased the risk (OR=1.25, 95% C.I=1.00~1.57, p=0.05) for metabolic syndrome. However, the G variant was not found to be significantly related to any of the risk components of metabolic syndrome. Our results indicated that mtDNA polymorphisms may participate in the development of metabolic syndrome in the Chinese population.

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## 880/W/Poster Board #538

**HFE variants do not contribute to the high prevalence of diabetes in the Jordanian population.** *A. Alkhateeb, A. Uzrail.* Dept Biology, Jordan Univ Science & Tech, Irbid, Jordan.

Diabetes mellitus has different etiologies and different frequencies across populations. Prevalence in the United States is around 8%. In Jordan, a recent estimate of diabetes prevalence was 17.1%, about twice that of the United States. Some studies showed that mutations in HFE gene, the gene mutated in hemochromatosis, might play a role in increasing the risk of developing diabetes. In this study, we sought to examine whether HFE variants might partially explain the increased risk of diabetes in the Jordanian population. For that purpose we genotyped 89 patients with type 2 diabetes and 204 controls for the two main variants in HFE gene, C282Y and H63D. We extracted DNA from all participating individuals, used polymerase chain reaction to amplify exons 2 and 4 of the HFE gene and restriction fragment length polymorphism method to genotype the variants. We did not find any C282Y variant in either patients or controls. H63D heterozygous in diabetics were 23.60% and in controls 22.55%. Allelic frequency of the mutant H63D allele was 11.80% in diabetics and 11.27% for controls. Genotype and allele frequencies were compared using Freeman-Halton extension of Fisher's exact test for a 2*3 contingency table and Chi squared test, respectively. We found no significant difference between the two groups. Our results suggest that HFE variants do not play a major role in the increased frequency of diabetes in the Jordanian population and, consequently, other factors might be involved.

## 881/W/Poster Board #539

**Detailed physiologic characterization of novel genetic loci implicated in fasting glucose homeostasis.** *E. Ingelsson[1], M-F. Hivert[2,3], I. Prokopenko[4,5], V. Lyssenko[6], C. Langenberg[7], J. Dupuis[8,9], N.J. Wareham[7], M. Walker[10], J.B. Meigs[2,3], R.M. Watanabe[11,12], M. McCarthy[4,5], L. Groop[6], J.C. Florez[3,13] on behalf of the MAGIC investigators.* 1) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; 2) General Medicine Division, Massachusetts General Hospital, Boston, USA; 3) Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA; 4) Wellcome Trust Center for Human Genetics, Oxford, UK; 5) Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK; 6) Department of Clinical Sciences, Diabetes and Endocrinology, Lund University, University Hospital Malmö, Malmö, Sweden; 7) MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK; 8) Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts, USA; 9) National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, Massachusetts, USA; 10) Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK; 11) Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California, USA; 12) Department of Physiology and Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, California, USA; 13) Diabetes Research Center (Diabetes Unit) and Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts, USA.

Quantitative genetic studies have indicated that genetic factors underlie a substantial component of both insulin sensitivity and insulin secretion. Recently, the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) identified 9 novel genetic loci associated with fasting glucose (FG; in or near *ADCY5, MADD, ADRA2A, CRY2, FADS1, GLIS3, SLC2A2, PROX1* and *FAM148B*) and one novel locus associated with fasting insulin (*IGF1*). In the present study, we have genotyped the lead SNPs from these 10 loci as well as 7 additional loci previously associated with FG, in non-diabetic individuals from the general population assessed with 3- or 4-point oral glucose tolerance tests (OGTT; n=15,530), fasting proinsulin levels (n= 13,734), and/or euglycemic clamps (n=2,106). M/I from euglycemic clamps, Si from frequently-sampled intravenous glucose tolerance tests, and several OGTT-derived insulin sensitivity indices were used to reflect insulin sensitivity, whereas insulin response was approximated with the insulinogenic index (at 30 minutes).

Of the 17 examined loci, the FG-raising alleles at *MADD* and *FAM148B* were exclusively associated with higher proinsulin (~1.1 pmol/L per FG-raising allele at the *MADD* locus, $p=3.1*10^{-65}$ after adjusting for age, sex, fasting insulin and BMI), but not with the insulinogenic index, suggesting a defect in insulin synthesis or processing. The FG-raising alleles at *TCF7L2*, *SLC30A8* and *PROX1* were associated with higher fasting proinsulin as well as a lower insulinogenic index, indicating an effect on both insulin processing and secretion. The FG-raising alleles at 4 of the examined loci (*GCK*, *FADS1*, *DGKB*, *MTNR1B*) were associated with lower insulin response to glucose load, while 2 were associated with higher insulin response (*G6PC2* and *SLC2A2*). Finally, 3 of these loci previously associated with FG or fasting insulin were associated with lower insulin sensitivity measured with euglycemic clamps (*G6PC2*, *ADCY5*, and *TCF7L2*) or OGTT surrogate measures (*GCKR* and *IGF1*).

These loci have been suggested to affect FG levels through various biological functions, including signal transduction, cell proliferation and development, glucose-sensing and circadian regulation. Our findings extend the physiological knowledge about these loci and provide important mechanistic insights into their role in glucose and insulin regulation.

## 882/W/Poster Board #540

**Sex Hormone Binding Globulin (SHBG) $TAAAA_n$ polymorphism is associated with measures of fetal growth in a Hispanic cohort.** *C. Ackerman[1], M.G. Hayes[1], L.P. Lowe[2], H. Lee[1], R. Freathy[3], A.R. Dyer[2], B.E. Metzger[1], W.L. Lowe[1], M. Urbanek[1], HAPO Study Cooperative Research Group.* 1) Division of Endocrinology, Dept of Medicine, Northwestern University, Chicago, IL; 2) Dept of Preventive Medicine, Northwestern University, Chicago, IL; 3) Genetics of Complex Traits, Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter, UK.

Prenatal exposure to testosterone has been implicated in fetal growth and subsequent insulin resistance in animal models. Furthermore, prenatal testosterone exposure in humans, from maternal or fetal sources, is linked to low birth weight, head circumference and length. SHBG is a glycoprotein that binds the sex hormones testosterone and estradiol and regulates their biological activity. Therefore, genetic variation in SHBG may impact birth outcome in humans by changing the amount of biologically active testosterone *in utero*. The $TAAAA_n$ repeat in the SHBG promoter affects transcription of the gene and serum SHBG levels. We used the multi-ethnic Hyperglycemia and Adverse Pregnancy Outcome (HAPO) cohort, comprised of pregnant mothers and their offspring, to evaluate the impact of genetic variation at the SHBG $TAAAA_n$ repeat on fetal growth. This cohort is ideal for these studies due large subject number and consistently assessed measures of fetal growth. We tested for association between fetal genotype and fetal traits (cord C-peptide, cord and 2-hour plasma glucose, birth weight, fat mass, head circumference, length, and skinfolds) in four ethnic groups (3518 Caucasian babies, 2129 Thai babies, 1237 Afro-Caribbean babies; and 792 Hispanic babies). Alleles were re-coded into a biallelic system and associations were assessed through linear regressions with single trait outcomes under an additive model and adjusted for covariates. SHBG A07 (7 TAAAA repeats) was strongly associated all measures of fetal growth and adiposity in the Hispanic population. For example, SHBG A07 was negatively associated with birth weight ($\beta$= -76.24gm, [95%CI: -120.5 to -31.98] per allele, *P*=0.0008), fatmass ($\beta$= -29.79, [95%CI: -47.74 to -11.84], *P*=0.001), head circumference ($\beta$= -0.22cm, [95%CI: -0.35 to -0.1], *P*=0.0006), length ($\beta$= -0.22cm, [95%CI: -0.41 to -0.04], *P*=0.02), and sum of skinfolds ($\beta$= -0.52mm, [95%CI: -0.86 to -0.19], *P*=0.002). These associations were not seen in the non-Hispanic populations. Our results indicate that genetic variation at the SHBG promoter $TAAAA_n$ repeat may be involved with fetal growth. The fact that all the associations with SHBG A07 were negative suggests a protective effect, perhaps preventing the offspring from excessive growth. Because these associations were not seen in non-Hispanic populations it is unlikely that the $TAAAA_n$ repeat is the causal variant, but is in linkage disequilibrium (LD) with the causal variant in the Hispanic population.

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## 883/W/Poster Board #541

**Genetic risk factors for post-infectious irritable bowel syndrome (IBS) vs. sporadic IBS: the involvement of distinct pathways.** *A.-C. Villani[1], Y. Saito[2], M. Lemire[3], M. Thabane[4], J.J. Larson[2], E.J. Atkinson[2], J. Zimmerman[2], A.E. Almazar Elder[2], S.M. Collins[4], D. Franchimont[1,5], J.K. Marshall[4].* 1) McGill University, Montreal, QC, Canada; 2) Mayo Clinic, Rochester, MN, USA; 3) Ontario Institute for Cancer Reseach, Toronto, ON, Canada; 4) McMaster University, Hamilton, ON, Canada; 5) Erasme University Hospital, Brussels, Belgium.

Acute gastroenteritis (GE) is the strongest risk factor for development of irritable bowel syndrome (IBS), the most common and costly gastrointestinal disorder in Western society. In May 2000, over 2300 residents of Walkerton (Canada) developed GE from microbial contamination of the municipal water supply and a third of them subsequently developed post-infectious (PI)-IBS. We have previously identified *TLR9*, *IL6*, and *CDH1* regions as novel PI-IBS susceptibility loci in the unique and only known PI-IBS population cohort (Walkerton Health Study). We aimed to 1) defining whether these associations were independent from the known PI-IBS clinical predictors, 2) pursue functional experiments, and 3) evaluate whether these risk factors contributed to the development of IBS without an infectious onset since it's unknown whether sporadic IBS share a common disease trigger. Methods: The clinical predictors of IBS after acute GE were evaluated in the 1253 patients who provided DNA for the genetic analysis and variants at the 3 most significantly associated loci were integrated in the analyses. Quantitative RT-PCR, Elisa, and sequencing methods were used to assess gene-expression and protein level associations in monocytes derived from 32 healthy individuals. For the Mayo Clinic validation cohort, we compared 473 sporadic IBS cases to 464 healthy controls matched for age, gender, ethnicity and region. Associations between variants and traits were assessed using mixed effects logistic regression models and chi-square test. Results: The identified susceptibility variants in the *TLR9*, *IL6* and *CDH1* regions are independent risk factors for developing PI-IBS when compared to the previously identified clinical risk factors (i.e. younger age, female gender, bloody stools, abdominal cramps, weight loss). Carriers of the rs2069861 risk allele were associated with increased *IL6* mRNA expression ($p$=0.0366) and decreased, LPS-stimulated IL-6 production ($p$=0.0269). None of the *TLR9*, *IL6*, and *CDH1* functional and tagging variants were significantly associated with sporadic IBS. Conclusion: The 3 susceptibility loci are all independent risk factors for developing PI-IBS when controlling for previously reported PI-IBS clinical risk factors in the Walkerton population. This is the first study comparing genetic risk factors for PI-IBS and sporadic IBS. The results suggest that different pathways and distinct genetic risk factors contribute to PI-IBS and sporadic IBS susceptibility.

## 884/W/Poster Board #542

**Interaction between SNPs in the *ESR1* gene and estrogen exposure in age-related macular degeneration.** *J.A. Ayala Haedo[1], D.R. Velez[1], M. Polk[1], P.J. Gallins[1], P.L. Whitehead[1], A. Agarwal[4], E.A. Postel[5], S.G. Schwartz[2], J.L. Kovach[2], G. Wang[1], J.L. Haines[3], W.K. Scott[1], M.A. Pericak-Vance[1].* 1) Miami Institute of Human Genomics. University of Miami, Miller School of Medicine, Miami, FL; 2) Bascom Palmer Eye Institute. University of Miami, Miller School of Medicine, Miami, FL; 3) Center Human Genetics Research Medical Center, Vanderbilt University, Nashville, TN; 4) Vanderbilt Eye Institute, Vanderbilt University, Nashville, TN; 5) Duke University Eye Center. Duke University, Durham, NC.

Estrogen exposure is a strong protective factor for age-related macular degeneration (AMD). In addition, SNPs in estrogen receptor 1 (*ESR1*) correlate with estradiol levels and increase the risk for AMD. The purpose of our study was to assess association of AMD with SNPs in *ESR1* and their interactions with exogenous estrogen exposure. We genotyped 22 tagSNPs ($r^2$>0.60) in the *ESR1* gene using a Sequenom assay in 778 related and unrelated female subjects. Individuals were classified for AMD using a modified version of the Age related eye disease study (AREDS) classification, where grades 1 & 2 were considered controls (n=257) and grades 3, 4 and 5 were considered AMD cases (n=521). Estrogen exposure was determined through a series of questions that included hormonal replacement therapy (HRT) and oral contraceptive usage (OC) history. Binary outcome variables [yes (1)/ no (0)] were constructed for each category (OC and HRT). Association between AMD, tagSNPs and estrogen exposure through HRT and OC was examined using generalized estimating equations (GEE), adjusting for age and cigarette smoking. Significant interactions ($p<0.05$) between SNPs and estrogen exposure were examined by a stratified analysis of genotype by estrogen exposure. We found a protective effect of HRT (OR=0.67, 95% CI [0.45, 0.98], p=0.039) and OC (OR=0.60, 95% CI [0.41, 0.90], p=0.013) on AMD. No association was found with SNPs in *ESR1* and risk of AMD. There were no interactions detected with OC and *ESR1* SNPs. However, when considering all cases (grades 3-5, n=521) and controls (grades 1-2, n=257), a significant SNP/estrogen exposure (HRT) interaction ($p< 0.05$) was detected for RS3020375, RS9341052, RS2813543, RS2813544, RS827423, and RS2234693 . Of these SNPs, only the latter two are in strong linkage disequilibrium ($r^2$=0.78). Only RS2234693 has a putative functional effect, reportedly altering *ESR1* mRNA levels by eliminating transcription factor binding sites. The stratified analysis demonstrates that the protective effect of HRT is found only in carriers of at least one copy of the minor allele at SNPs, RS3020375, RS9341052, RS2813543, RS2234693, RS827423 and in carriers of two copies of the minor allele for RS2813544. These results suggest that the inverse association of AMD and HRT is dependent on *ESR1* SNP genotypes.

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## 885/W/Poster Board #543

**Angiopoietin-like protein 4 (*ANGPTL4*) gene polymorphisms and risk of brain arteriovenous malformations (BAVM).** *B. Mikhak[1], S. Weinsheimer[1], L. Pawlikowska[1,2], P.-Y. Kwok[2,3], S. Sidney[4], C.E. McCulloch[5], W.L. Young[1,6], H. Kim[1,2,5].* 1) Center for Cerebrovascular Research, Department of Anesthesia and Perioperative Care, University of California, San Francisco (UCSF), San Francisco, CA; 2) Institute for Human Genetics, UCSF, San Francisco, CA; 3) Cardiovascular Research Institute, UCSF, San Francisco, CA; 4) Division of Research, Kaiser Permanente Northern California, Oakland, CA; 5) Department of Epidemiology and Biostatistics, UCSF, San Francisco, CA; 6) Departments of Neurology and Neurosurgery, UCSF, San Francisco, CA.

**Background**: Brain arteriovenous malformations (BAVM) are high-flow vascular lesions with direct shunting of blood from the arterial to venous circulation and are an important cause of intracranial hemorrhage (ICH). The etiology of BAVMs is unknown, but abnormal angiogenesis is hypothesized to play a role. Angiopoietin-like protein 4 (*ANGPTL4*) has been implicated in lipid metabolism and in angiogenesis; it can show anti-angiogenic activity in tumorigenesis by inhibiting vascular permeability, tumor cell motility and invasiveness and pro-angiogenic activity during ischemia. Therefore, we investigated the association between common polymorphisms in *ANGPTL4* and risk of BAVM. **Methods**: We conducted a case-control study of 216 BAVM patients and 246 healthy controls among Caucasians. Using HapMap CEU data, minor allele frequency ≥5% and pairwise $r^2 > 0.8$, four haplotype-tagging single nucleotide polymorphisms (SNPs) in *ANGPTL4* captured variation over a 10kb region: rs2278236 *C/T* (intron 3), rs1044250 *C/T* (exon 5, Thr266Met), rs11672433 *G/A* (exon 6, Pro389Pro), and rs1808536 *A/G* (3' UTR). Allelic, genotypic, and haplotypic tests of associations were performed. **Results**: The minor allele (*A*) of rs11672433 was associated with an increased risk of BAVM (P=0.006) and the association persisted after Bonferroni correction for 4 tests (P=0.02). Carriers of the minor *A* allele were at higher risk for BAVM compared to non-carriers (Odds Ratio (OR)=1.56; 95% Confidence Interval (CI)=1.01-2.41; P=0.046). Risk of BAVM increased with increasing copy of the minor *A* allele after adjusting for age and gender (OR=1.49, 95% CI=1.03-2.15; P=0.03). No other SNPs were associated with BAVM (P>0.05). Five common haplotypes (frequency >1%) were inferred; overall haplotype distribution differed between BAVM patients and controls ($\chi^2$=12.2, df=4, P=0.02). Only 1 haplotype (AG<u>A</u>G) contained the minor *A* allele of rs11672433, and was associated with increased BAVM risk (p=0.01), consistent with single SNP analysis. **Conclusion**: In this population of Caucasians, we found an association between rs11672433 (synonymous Pro389Pro) and risk of BAVM. These results suggest that polymorphisms in the *ANGPTL4* gene may be involved in BAVM pathogenesis, and warrant further investigation.

## 886/W/Poster Board #544

**Crohn's Disease molecular interaction network identifies novel risk locus.** *G.A. Pirela[1], S. KaraMohamed[1], D. Nicolae[1,2,3], T.C. Gilliam[1].* 1) Human Genetics Department, The University of Chicago, Chicago, IL; 2) Statistics Department, The University of Chicago, Chicago, IL; 3) Medicine Department, The University of Chicago, Chicago, IL.

**Purpose -** Crohn's disease (CD) genome wide association studies (GWAS) along with a recent meta-analysis identified 22 potential risk loci. We employed this set of "seed genes" to generate CD-specific molecular interaction networks. We show that 18 of these genes map to a single network. Five network "candidate" genes, not previously reported, showed significant allelic association in the Wellcome Trust Case Control Consortium (WTCCC) data set. Furthermore, 4 of the associated genes showed similar association in the NIDDK-IBD data set.

**Methods -** We used Ingenuity Pathway Analysis (IPA) software to explore molecular and biological networks among 22 previously implicated CD candidate genes. These seed genes were used to interrogate the IPA molecular interaction database and networks were generated algorithmically by maximizing connectivity among seed genes. The software generates and rank-orders the minimum number of networks (up to 35 molecules each) required to include all seed genes. We treat the connecting genes as CD candidates and examine their allelic association to CD in the WTCCC and NIDDK-IBD data sets.

**Results and Conclusions -** Network core analysis with IPA identified a highly connected CD-network by maximizing connectivity among seed genes. The network harbors 18 seed genes and 17 interconnecting, or CD candidate genes. Association analysis of these candidate genes in large CD case-control data sets showed significant allelic association with 7 genes in the WTCCC data set; four of which also showed significant association in the NIDDK-IBD data set. For one of these genes, an upstream SNP was found to be significantly associated in both data sets. Further functional analysis of the core network revealed that 2 of the GWAS associated genes have well-established roles in inflammatory response and immunological disease. These findings further illustrate the power of the network approach for validating candidate gene predictions and for identifying novel risk loci. We discuss the concept of gene-mapping potential of a "threshold" number of authentic candidate genes required to elucidate a disease-related molecular interaction network.

## 887/W/Poster Board #545

**Scanning the Regulatory Landscape of Transcription Factor 7-Like 2 (*TCF7L2*).** *D. Savic, M. Nobrega.* Human Genetics, The University of Chicago, Chicago, IL.

Several genome-wide association studies (GWAS) have pointed to non-protein coding regions in the genome as harboring variants underlying common disease traits. One possibility is that these variants disrupt long-range cis-regulatory elements that control the tissue and temporal expression patterns of neighboring genes. However, given the paucity of efficient platforms for interrogating non-coding sequences for function and subsequently elucidating the effects of variation within these intervals, this makes the experimental follow-up of GWAS at present considerably difficult. Here we describe a strategy to systematically identify regulatory elements and functionally evaluate the impact of mutations within them. A 92.1-kb block of strong linkage disequilibrium (LD) within the *TCF7L2* locus has consistently shown associations to type 2 diabetes (T2D) in diverse human populations. Coding variations within *TCF7L2* were not identified, raising the possibility that regulatory variations in *TCF7L2* distant enhancers may underlie the increased risk to type 2 diabetes. Using a combination of bioinformatics, comparative genomics and mouse genetic engineering, we have obtained a comprehensive map of the regulatory landscape of *TCF7L2*. We have identified several tissue-specific enhancers in the region spanning the association LD block. These enhancers recapitulate the endogenous pattern of *TCF7L2* expression. Interestingly, several enhancers show expression in tissues with established roles in maintaining glucose homeostasis, including pancreas, stomach, brain and bones. Deep sequencing of these enhancers in various human populations, including diabetics and non-diabetics, is undergoing, aiming to identify potential causal variants within these enhancers. In vivo and in vitro studies are being carried out to infer the functional role of these enhancers and will be used to establish the consequences of variants within them. Our results strongly support the notion that distant cis-regulatory elements are often present in regions associated with common diseases, making this a priority target of investigation as a follow-up to GWAS.

## 888/W/Poster Board #546

**Examination of all type 2 diabetes GWAS loci reveals *HHEX* as a locus influencing pediatric BMI.** *J. Zhao[1], J.P. Bradfield[2], H. Zhang[2], K. Annaiah[2], K. Wang[2], C.E. Kim[2], J.T. Glessner[2], E.C. Frackelton[2], F.G. Otieno[2], K.A. Thomas[2], M. Garris[2], R.M. Chiavacci[2], M. Li[3], R.I. Berkowitz[4,5], H. Hakonarson[1,2,6], S.F.A. Grant[1,2,6].* 1) Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA; 3) Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA; 4) Behavioral Health Center and Department of Child and Adolescent Psychiatry, Children's Hospital of Philadelphia, Philadelphia, PA; 5) Center for Weight and Eating Disorders, Department of Psychiatry, University of Pennsylvania, Philadelphia, PA; 6) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA.

A number of studies have found that body mass index (BMI) in early life influences the risk of developing type 2 diabetes (T2D) later in life. Utilizing data from an ongoing genome wide association study (GWAS) of pediatric BMI in our cohort, we investigated the association of pediatric BMI with 21 SNPs at 17 type 2 diabetes loci uncovered through GWAS, consisting of *ACDC*, *ADAMTS9*, *CDC123-CAMK1D*, *CDKAL1*, *CDKN2A/B*, *EXT2*, *HHEX*, *IGF2BP2*, the intragenic region on 11p12, *JAZF1*, *KCNQ1*, *MTNR1B*, *NOTCH2*, *SLC30A8*, *TCF7L2*, *THADA* and *TSPAN8-LGR5*. All subjects were consecutively recruited from the Greater Philadelphia area from 2006 to 2009 at the Children's Hospital of Philadelphia. BMI percentiles were defined using the standard Center for Disease control (CDC) growth chart z-scores. All subjects were biologically unrelated and were aged between 2 and 18 years old. All subjects were between -3 and +3 standard deviations of CDC corrected BMI i.e. outliers were excluded to avoid the consequences of potential measurement error or Mendelian causes of extreme obesity. We partitioned our cohort exactly in half such that we had a 'discovery' cohort (n=3592) and a 'replication' cohort (n=3592). Our data show that the minor allele of rs7923837 at the *HHEX* locus was associated with lower pediatric BMI in both the discovery ($P$=0.0013; survived correction for 21 tests) and replication ($P$=0.023) sets (combined $P$=1.01x10$^{-4}$). However, association was not detected with any other known type 2 diabetes loci uncovered to date through GWAS. One hypothesis could be that rs7923837 is primarily associated with birth weight, as reduced birth weight is often reported to be associated with BMI and type 2 diabetes later in life; although we agree with previous studies that *CDKAL1* is a birth weight associated gene, we have not observed significant association between *HHEX* and this trait. So in summary, our data show that the same genetic *HHEX* variant that is associated with type 2 diabetes from previous studies also influences pediatric BMI.

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## 889/W/Poster Board #547

**Variants in the Regional Paralogs and Receptors of IL10 Gene and HIV-1 Susceptibility in African American Adolescents.** *S. Shrestha[1], H.W. Wiener[1], W. Song[1], A. Shendre[1], C.M. Wilson[1], R.A. Kaslow[1], B. Aissani[1], J. Tang[2].* 1) Dept Epidemiology, UAB, Birmingham, AL; 2) Dept Medicine, UAB, Birmingham, AL.

Cytokines in the interleukin-10 (IL10) gene family have broad immunomodulatory function and several SNPs in IL10 have been reported to influence HIV-1 pathogenesis or infection. While most genetic studies of IL10 have focused on its promoter region, we hypothesize that several genes in the IL10 family likely act in concert to modulate HIV susceptibility. We conducted a case-control study on 250 HIV-1 seropositive and 106 high-risk seronegative African American adolescents who were recruited into the Reaching for Excellence in Adolescent Care and Health (REACH) study in 13 US cities between 1996 and 1998 and followed until 2001. All subjects designated uninfected for this study had a history of either sexual intercourse or injection drug use, had negative HIV enzyme linked immunosorbent assay (HIV-ELISA) performed within 30 days of enrollment into the REACH study, and remained seronegative until the end of the study period. All infected subjects had a positive ELISA test, with a confirmatory Western blot performed before REACH enrollment. We screened 84 single nucleotide polymorphisms (SNPs) in the coding and UTR regions and haplotype tagging SNPs in introns of IL10 and five related genes, paralogs (IL19, IL20 and IL24), and receptors (IL10RA and IL10RB). In univariate (additive) model using logistic regression analysis, 8 SNPs in IL20, IL24, IL10RA and IL10RB resulted to be statistically significant (<0.0003-0.05). In the multivariate model, rs9610 (p=0.0005) in IL10RA 3'UTR, rs2981572 (p=0.02) in IL20 5'UTR, rs6517158 (p=0.003) and rs8178565 (p=0.01) both in IL10RB 3'UTR were independently associated with HIV-1 infection. Further, the haplotype analysis of these two SNPs in the IL10RB 3'UTR showed that one haplotype with 7% frequency in HIV-positives seemed very rare (0.5%) in HIV-negatives.Multiple studies have extensively examined the association between SNPs in the IL10 gene and HIV disease pathogenesis or infection, but the evidence for involvement remains inconclusive. Our study suggests that variants in several members of the IL10 gene family might influence susceptibility to HIV infection in African Americans. Involvement of the IL10 molecule in immunity to HIV infection could be modulated through more complex interactions among related genes. Investigation of the role of the IL10 gene family in HIV infection deserves a comprehensive systems biology approach.

## 890/W/Poster Board #548

**Impact of host APOBEC3 retroviral restriction family genes on HIV-1/AIDS.** *P. An[1], J.J. Goedert[2], G.D. Kirk[3], S. Donfield[4], S. Buchbinder[5], J. Phair[6], C.A. Winkler[1].* 1) Lab Genomic Diversity, SAIC-Frederick Inc, Frederick, MD; 2) Epidemiology Branch, National Cancer Institute, Bethesda, MD; 3) Department of Epidemiology, Johns Hopkins School of Public Health, Baltimore, MD; 4) Rho, Inc., Chapel Hill, NC; 5) San Francisco Department of Public Health, San Francisco, CA; 6) Northwestern University, Chicago, IL.

Human APOBEC3 cytidine deaminases are intrinsic resistance factors to retroviral infection including HIV-1. APOBEC3 proteins restrict the replication of vif-deficient HIV-1 by cytidine deamination on single-stranded DNA or by RNA binding. APOBEC3 family proteins (APOBEC3A-H) were encoded by seven genes (*APOBEC3A-H*) arrayed in a 150 kb region on 22q13.2, with near identity of several exonic sequences among APOBEC3 genes posing great challenge for genotyping. APOBEC3 genes may have arisen from gene expansion, domain duplication, gene conversion and recombination. In vitro.studies have shown that APOBEC3 proteins vary in their ability in restricting HIV-1 replication, vif resistance and deamination target motifs. Previously we have reported that a structural variation in *APOBEC3G* and a deletion in *APOBEC3B* were associated with HIV-1 disease progression. At the present study, we seek to evaluate the in vivo effect and interaction of all seven APOBEC3 genes on host susceptibility to HIV- acquisition and disease progression. Genetic association study was performed in five well-characterized HIV-1 natural cohorts consisting of more than 3000 HIV-1 infected and exposed but uninfected individuals of European and African Americans. Guided by phylogenetic and sequence homology analyses, gene-specific SNPs were selected for genotyping and the genotype distribution were found to conform to Hardy-Weinberg equilibrium. The association analysis was done by a logistic regression for the comparison of HIV-1 infected and uninfected groups and by Cox proportional hazards model for the rate of AIDS progression. Linkage disequilibrium blocks were formed for each gene, in consistence with the gene duplication origin. Variation in *APOBEC3A* and *APOBEC3C* had no effect on either infection or progression in both populations. On the order hand, in addition to effects of *APOBEC3B* and *APOBEC3G*, two amino acid-changing SNPs in *APOBEC3F* were significantly associated with slower rate of progression to AIDS in European Americans and a trend of protection in African Americans. Our epidemiological results are consistent with the in vitro observation that APOBEC3B, APOBEC3G and APOBEC3F have strong anti-HIV effect. Our comprehensive analysis provides insights into the in vivo role of APOBEC3 proteins and their interplay in host innate defense against retroviral infection. [Funded by NCI Contract HHSN261200800001E].

## 891/W/Poster Board #549

**Association of KIR gene polymorphism with malaria severity in Thailand.** *K. Hirayasu[1,2,3], J. Ohashi[4], K. Kashiwase[3], M. Minemoto[3], I. Naka[4], H. Hananantachai[5], A. Ogawa[3], M. Takanashi[3], K. Tokunaga[1], J. Patarapotikul[5], T. Yabe[3].* 1) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; 2) JSPS Research Fellow; 3) Tokyo Metropolitan Red Cross Blood Center, Tokyo, Japan; 4) Doctoral Program in Life System Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Ibaraki, Japan; 5) Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

**[Purpose]** Malaria is one of the most important infectious diseases, affecting 300 million people and causing more than 1 million deaths annually worldwide. Despite extensive studies on the critical roles of NK cells in malaria, epidemiological study concerning NK receptor polymorphism and malaria severity has not been described yet. In this study, we examined a possible association of killer Immunoglobulin-like receptor (KIR) gene polymorphism with severity of Plasmodium falciparum malaria.

**[Methods]** Subjects enrolled in this study were 203 mild malaria, 165 non-cerebral severe malaria, and 109 cerebral malaria patients living in northwest Thailand near the border with Myanmar. KIR ligand (HLA-A3, HLA-A11, HLA-Bw4, HLA-C1, HLA-C2) and KIR genotypes were determined by PCR-SSO and PCR-SSP method. The carrier frequencies of the KIR-HLA receptor-ligand pairs were compared between malaria patient groups using the Fisher's exact test based on a 2 x 2 contingency table.

**[Results]** In our study population, detected KIR-HLA receptor-ligand pairs were as follows: KIR2DL1-HLA-C2, KIR2DL2-HLA-C1, KIR2DL3-HLA-C1, KIR3DL1-HLA-Bw4, and KIR3DL2-HLA-A3, -A11. Among these pairs, KIR2DL3-HLA-C1 was significantly associated with cerebral malaria compared with non-cerebral severe malaria (odds ratio (OR) 3.44, P=0.001), and mild malaria (OR 2.90, P=0.004).

**[Conclusion]** KIR-HLA receptor-ligand combinations have been previously shown to influence differences in NK cell functional responses among individuals. Therefore, our result suggests that individuals carrying KIR2DL3-HLAC1 show NK cell response susceptible to cerebral malaria.

## 892/W/Poster Board #550

**Variants in Toll-like receptor 2 (*TLR2*) associate with pulmonary tuberculosis in Caucasians and West Africans.** *W.K. Scott[1], D.R. Velez[1], W.F. Hulme[1], J.L. Myers[1], M.E. Stryjewski[2], E. Abbate[3], R. Estevan[3], S.G. Patillo[4], R. Olesen[5], C. Wejse[5,6], G. Sirugo[7,8], A. Tacconelli[7,8], J.R. Gilbert[1], C.D. Hamilton[4,9].* 1) Dr. John T. Macdonald Foundation Department of Human Genetics and Miami Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) Department of Medicine, Centro de Educación Médica e Investigaciones Clínicas "Norberto Quirno" (CEMIC), Buenos Aires, Argentina; 3) Department of Medicine, Hospital F.J. Muñiz, Buenos Aires, Argentina; 4) Department of Medicine, Duke University Medical Center, Durham, NC; 5) Department of Infectious Diseases, Aarhus University Hospital, Skejby, Denmark; 6) Bandim Health Project, Danish Epidemiology Science Centre and Statens Serum Institute, Bissau, Guinea-Bissau; 7) Unità di Genetica Medica, Ospedale San Pietro FBF, Rome, Italy; 8) Dipartimento di Biopatologia e Diagnostica Per Immagini, Università di Tor Vergata, Rome, Italy; 9) Family Health International, Research Triangle Park, NC.

Tuberculosis (TB) is a global public health problem and a major cause of preventable death each year, with 8.8 million new cases of TB and 1.6 million deaths worldwide in 2005. Approximately ten percent of exposed individuals develop pulmonary or extrapulmonary TB, suggesting that host defense factors, assumed to be at least partly under genetic control, influence development of active disease. Polymorphisms in Toll-like receptors (TLRs) have been previously associated with regulation of TLR expression and development of active TB. In the present study 71 single nucleotide polymorphisms (SNPs) in *TLR1*, *TLR2*, *TLR4*, *TLR6*, and *TLR9* were examined in a case-control sample of TB cases, relatives, and close contact controls comprising 474 (295 cases and 179 controls) African-Americans, 381 (237 cases and 144 controls) Caucasians, and in a community-based case-control sample of 667 (321 cases and 346 controls) Africans from Guinea-Bissau. Association of SNPs with pulmonary TB was examined using generalized estimating equations and logistic regression. We also performed case-only tests of association for increased lung cavitation when data were available. Multiple statistically significant associations were observed across populations at *TLR9* and *TLR2*. The strongest evidence for association came at an insertion (I) /deletion (D) polymorphism (-196--174) in *TLR2* that strongly associated with TB in both Caucasians (II vs. ID&DD, OR=0.41 [95% CI 0.24-0.68], p=0.0007) and Africans (II vs. ID&DD, OR=0.70 [95% CI 0.51-0.95], p=0.023). Four SNPs in *TLR9* associated with TB in African-Americans with the strongest evidence for association coming from the additive model for rs352139 (OR=1.80[95% CI 1.29-2.50], p=0.001). One SNP in *TLR2* associated with increased cavitation in Caucasians (rs1816702, OR=3.00 [95% CI 1.27-7.13], p=0.013) and one SNP in *TLR1* associated with decreased cavitation in African-Americans (rs4624663, OR=0.40 [95% CI 0.17-0.96], p=0.040). Our preliminary findings in three independent population samples indicate that variations in *TLR2* and *TLR9* play important roles in both TB susceptibility and development of lung cavitation.

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## 893/W/Poster Board #551

**Obesity and diabetes genes are associated with being born small for gestational age: Results from the Auckland Birthweight Collaborative study.** *A.R. Morgan[1,2], D.Y. Han[1,2], W.J. Wam[1,2], J. Thompson[3], P.N Black[4], L. Ferguson[1,2], E.A Mitchell[3].* 1) Discipline of Nutrition, FM & HS, The University of Auckland, Auckland, New Zealand; 2) Nutrigenomics New Zealand; 3) Department of Paediatrics, FM & HS, The University of Auckland, Auckland, New Zealand; 4) Department of Pharmacology & Clinical Pharmacology, FM & HS, The University of Auckland, Auckland, New Zealand.

Individuals born small for gestational age (SGA) are at increased risk of rapid postnatal weight gain, later obesity and diseases in adulthood such as type 2 diabetes, hypertension and cardiovascular diseases. Although several hypotheses have been proposed, the mechanism underlying this association remains unknown. In this study, we have tried to determine genetic factors that may have a role to play. It has previously been estimated that about 46% of the predisposition to be delivered SGA is due to genetic factors. The fact that SGA births tend both to cluster in families and to recur in successive generations further supports the role of genetics.

In this study we tested the association between small for gestational age and polymorphisms in genes that have previously been associated with obesity and/or diabetes. We investigated 65 single nucleotide polymorphisms (SNPs) in 43 genes in samples from the Auckland Birthweight Collaborative study - a longitudinal study that is currently in phase 5. Data have been collected at birth, 1, 3.5, 7 and most recently 11 years of age. Approximately half the children were born small for gestational age (SGA) and half were appropriate for gestational age (AGA). DNA samples were available for 561 European children and genotyping of these samples was undertaken using the Sequenom genotyping platform.

To date 32 SNPs have been genotyped and from our preliminary analysis a number of these are showing evidence of association with SGA. This includes significant p values (less than 0.05) for the novel findings of the genes FTO (fat mass and obesity associated), KCNJ11 (potassium inwardly-rectifying channel J11) and BDNF (brain-derived neurotrophic factor) being associated with SGA at the allelic and/or genotypic level. Full results of all 65 genetic variants analysed for their association with SGA will be presented at the conference.

## 894/W/Poster Board #552

**Interaction of WDR36 & STI1 Mutations are Involved in Polygenic Inheritance of Primary Open-Angle Glaucoma.** *T. Footz[1], S. Dubois[2], V. Raymond[2], M. Walter[1].* 1) Medical Genetics, University of Alberta, Edmonton, Alberta, Canada; 2) Ocular Genetics and Genomics, CHUL Research Center, Quebec City, Quebec, Canada.

Glaucoma is a common neurodegenerative disease characterized by progressive irreversible blindness and frequently presents with increased intraocular pressure. A variety of loci have been linked to inherited forms of the most common subtype, primary open-angle glaucoma (POAG), including the rRNA-processing gene WDR36. The occurrence of rare non-synonymous WDR36 sequence variants in normal control subjects supported the theory that the genetics of POAG is complex, where the normally late-onset disease would result from gene:gene and gene:environment interactions. We previously demonstrated that WDR36 mutations encode functional defects that affect cell growth when expressed in the homologous UTP21 gene in haploid yeast carrying a null mutation in a second gene, the co-chaperone STI1. POAG patients carrying WDR36 variants have recently been screened for mutations in STI1, whose gene product also has neurotrophic properties. Using our yeast system, we investigated the functional consequences of the POAG-exclusive STI1(K434R) variant, which was found in a doubly-heterozygous patient also possessing the WDR36(L25P) variant. We created yeast strains carrying the null mutation of STI1 and a deletion of UTP21 but with low-copy plasmids expressing human STI1 and yeast UTP21 under the control of the corresponding yeast gene promoters. Strains co-expressing the POAG-associated mutation D621G in yUTP21 (homologous to D658G in hWDR36) and the K434R variant in hSTI1 exhibited slower growth than the strains with only wild-type or single-mutant constructs, indicating that hSTI1(K434R) is functionally deficient and synthetically interacts with UTP21. Therefore, WDR36 and STI1 also appear to be co-modifier genes in humans which affect POAG susceptibility through polygenic modes of inheritance. Furthermore, we propose that the WDR36/UTP21-STI1 interaction has been conserved throughout evolution and participates in the regulation of ribosome assembly and/or stress responses that normally prevent glaucoma.

## 895/W/Poster Board #553

**FOXP2 and CNTNAP2 Influence Phonology, Motor Praxis, and Reading in Individuals with Dyslexia.** *B. Peter[1], Z. Brkanac[2], M. Matsushita[3], M. Lisowski[3], T. Vu[3], V.W. Berninger[4], E.M. Wijsman[3,5], W.H. Raskind[2,3].* 1) Speech & Hearing Sciences, University of Washington, Seattle, WA; 2) Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA; 3) Department of Medicine, University of Washington School of Medicine, Seattle, WA; 4) Department of Educational Psychology, University of Washington, Seattle, WA; 5) Department of Biostatistics, University of Washington, Seattle, WA.

Mutations in the FOXP2 gene cause severe impairments in language comprehension and expression and concomitant apraxic speech deficits, but are rare among individuals with common forms of speech sound disorder (SSD) and language impairment (LI). Vernes et al. (2008) identified CNTNAP2 as a transcriptional target of FOXP2 and reported significant associations with a quantitative measure of nonsense-word repetition (NWR) and nine intronic CNTNAP2 SNPs in a sample of 184 family trios with common forms of LI. Because deficits in NWR are frequently reported for dyslexia (DYX), we hypothesized that CNTNAP2 might affect NWR performance in families with DYX. Also, because oral apraxia is often associated with oral reading fluency problems in DYX and FOXP2 mutations are associated with oral apraxia in LI, this gene may also influence motor praxis in both disorders. At issue is whether the apraxia affects both oral and finger motor planning. In the present study, we tested measures of NWR, oral and finger planning, and word reading for association with CNTNAP2, in a sample of 188 family trios ascertained through a child with DYX. Quantitative transmission disequlibrium testing was used. Two of the seven SNPs with significant association to NWR in Vernes et al.'s LI sample, rs2710102 and rs1922892, were associated with NWR in our DYX sample (p=.029). To extend the study to the FOXP2 gene, nine SNPs from FOXP2 were selected from the promoter and intronic regions and evaluated for associations with the same measures of interest. Strongest associations were found for rs7782412, located in the zinc finger region, for a finger succession task (p=.0076), word reading efficiency (p=.0074), word reading accuracy (p= .0351), and NWR (p=.0434). Two SNPs from the promoter region were associated with NWR (p=.0422) and finger succession (p=.0390), and two SNPs, rs10533005 and rs7785701, located between exons 1 and 3, were associated with oral praxis (p=.0196). These results suggest that CNTNAP2 affects NWR not only in LI but also in DYX, consistent with the facts that LI and DYX are frequently comorbid and share deficits in phonologic memory. The findings regarding the FOXP2 associations are consistent with the hypothesis that FOXP2 influences performance in motor planning tasks in individuals with DYX. This study shows the influence of CNTNAP2 and FOXP2 in a population (DYX) and for phenotypes (motor praxis) previously not evaluated for these genes.

## 896/W/Poster Board #554

**Association of Tumor Necrosis Factor-alpha Gene Polymorphisms with Overweight/Obesity in Korean Population.** *G. YU[1,2], SY. LEE[1,2], HK. SON[1,2], IS. CHUNG[1,2], JJ. LEE[1,2], MY. LEE[1,2], DH. SHIN[1,2].* 1) PREVENTIVE MEDICINE, KEIMYUNG UNIVERSITY SCHOOL OF MEDICINE, TAEGU, TAEGU, Korea; 2) INSTITUTE FOR MEDICAL GENOME RSEARCH KEIMYUNG UNIVERSITY , TAEGU, KOREA.

Obesity and excess weight are major risk factors for chronic diseases, including type II diabetes, cardiovascular diseases, gastrointestinal disorders and certain forms of cancer. Increased concentrations of tumor necrosis factor-alpha (TNF-alpha) might therefore play an important role in obesity-induced insulin resistance in humans. To this investigate the genetic association between TNF-alpha gene and the risk of overweight/obesity in Korean population, previously described three single nucleotide polymorphisms (SNPs;rs361525, rs1799274, rs1800630) in the TNF-alpha gene promoter region were analyzed with 123 control (body mass index, BMI 18 to less than 23) and 208 overweight/obesity (BMI more than 23) subjects. Among three SNPs, one SNP (rs361525) presented an association with overweight/obesity in the codominant model (p=0.0046).. In Haplotype analysis with three SNPs, one haplotype (ACC) presented protective effect with overweight/obesity (p=0.0052). There were significant differences in the clinical lipid biomarker and genotype among the groups compared to overweight/obesity and control subjects. Our results supported T carrier group of the rs1799724 in TNF-alpha gene were significantly associated with increasing TG. A carrier group of the rs1800630 in TNF-alpha gene were significantly associated with increasing TG and increasing LDL-C These result indicate that polymorphism of TNF-alpha gene may be associated with obesity and the clinical lipid biomarker in Korean population.

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## 897/W/Poster Board #555

**Are reporter gene and gel shift assays useful in assessing regulatory SNP function? *In vivo* assays to probe -308 TNF polymorphism function.** *L.J. Abraham[1], M. Karimi[1], L.C. Goldie[1], M.N. Cruickshank[1], E.K. Moses[2].* 1) Biomedical Biomolec & Chem Sci, Univ of Western Australia, Crawley, Western Australia, Australia; 2) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX.

One of the greatest challenges facing genetics is the development of strategies to distinguish functional from nonfunctional variation. Two first-pass functional assays, the reporter gene assay and the electrophoretic mobility shift assay (EMSA), both have substantial limitations. The reporter gene assay was originally designed to define transcriptional elements that have a substantial impact on expression; the differences due to a regulatory SNP are likely to be much smaller. Small differences in reporter activity can be due to a range of confounding factors rather than a true reflection of differences. Also, as chromatin context is likely to be important, the transient transfection assay is not a completely appropriate model to assess the influence of small transcriptional differences due to SNPs. To avoid these limitations, we have developed a novel system, which permits the comparison of transcriptional reporter gene activities following site-specific genomic integration and have validated its use by comparing G-308A variation in TNF expression. The second assay commonly used is the EMSA, which is a very sensitive assay to detect differences in transcription factor binding activity. Allele-specific binding is generally a good indicator of the likelihood that a SNP is functionally relevant. However, we have developed more in vivo approaches using the -308 TNF polymorphism as a model, to assess the use of two assays that measure allelic differences in tissues of defined genotype. The first is a chromatin accessibility assay to measure the effect of putative regulatory polymorphisms on chromatin arrangement. This is a very good measure of whether a region containing a SNP is relevant in regulating a gene. The second approach involves assessing whether allele-specific differential factor binding occurs in vivo by use of chromatin immunoprecipitation (ChIP). Haplo-ChIP (Knight et al., 2003) is able to measure differential transcription of each haplotype but requires the use of heterozygous material. We have developed an alternative ChIP-based method that is SNPcentric (SNP-ChIP). Once the transcription factors that interact are identified, an assessment of differential interaction of the factor with the polymorphic site can be made in vivo. Using genotyped material, we show by SNP-ChIP, that in vivo transcription factor interactions occur exclusively with the -308G SNP of the TNF gene reaffirming functionality.

## 898/W/Poster Board #556

**Novel Candidate Genes in Polycystic Ovary Syndrome: Harnessing Expression Data.** *M.R Jones[1,2], R. Azziz[3,4], M.O. Goodarzi[1,3,4,5].* 1) Endocrinology, Diabetes & Metabolism, Cedars-Sinai Med Ctr, Los Angeles, CA; 2) Graduate Program in Biomedical Sciences and Translation Medicine, Cedars-Sinai Med Ctr, Los Angeles, CA; 3) Department of Obstetrics & Gynecology, Cedars-Sinai Med Ctr, Los Angeles, CA; 4) Center for Androgen Related Disorders, Cedars-Sinai Med Ctr, Los Angeles, CA; 5) Medical Genetics Institute, Cedars-Sinai Med Ctr, Los Angeles, CA.

Familial aggregation and twin studies have established a genetic etiology for polycystic ovary syndrome (PCOS); however, few susceptibility genes are widely agreed upon. A number of expression studies have been performed in PCOS tissues (e.g. ovary, adipose) with remarkably few transcripts reported as differentially expressed in more than one study. Those transcripts that have been reported by more than one study represent a diverse group of proteins and include DKK1, an inhibitor of Wnt signalling and cellular growth repressor, and DNAJB1, a chaperone under transcriptional regulation by insulin. These represent novel pathways and candidates for PCOS. We genotyped 335 White women with PCOS and 198 White female controls for three single nucleotide polymorphisms (SNPs) in DKK1 and four SNPs in DNAJB1. These SNPs capture the majority of variation across these genes in the HapMap database. Association with PCOS was tested by logistic regression; association with quantitative traits in cases was tested by ANCOVA, adjusting for age and BMI. A modified Bonferroni approach was used to correct for multiple testing, such that a P value $<0.009$ was set as significant. We found highly significant associations of DKK1 SNP rs1569198 with total testosterone (AA/AG: 79.0 (34.8) vs. GG: 68.0 (26.0) ng/dl; P=0.0035). DNAJB1 SNP rs3962158 was significantly associated with fasting insulin (CC: 13.0 (15.4) vs. CT/TT: 18.0 (20.7) uIU/mL; P=0.003), and homeostatic model assessment measures of insulin resistance (HOMA-IR; CC: 1.72 (1.96) vs. CT/TT: 2.34 (2.39); P=0.006) and beta-cell function (HOMA-%B; CC: 145.2 (99.2) vs. CT/TT: 177.2 (108.6); P=0.004) [Data are median (IQR)]. We have demonstrated a novel approach to the genetics of PCOS. In an attempt to select candidate genes without being hindered by insufficient knowledge of PCOS pathophysiology, we have used mRNA expression data reported from several tissues to identify novel candidates. These results suggest that variation in gene expression may be an important factor in identifying relevant PCOS pathways. Of particular interest as a candidate was DNAJB1 as it lies in a previously identified PCOS linkage region on Ch19p13.2. Replication of these results in independent cohorts is warranted, followed by functional studies to elucidate the role of these genes in PCOS.

## 899/W/Poster Board #557

**Promoter SNPs in 5q22.1 associated with susceptibility to glaucoma.** *K. Kim[1], Y. Yun[2], C. Kim[2], C. Kang[1].* 1) Dept Biological Sciencesl, KAIST, Daejeon, Korea; 2) Dept of Ophthalmology, Chungnam National Univ Hospital, Daejeon, Korea.

Glaucoma is the second leading cause for optic nerve degeneration and irreversible blindness worldwide. Especially primary open-angle glaucoma (POAG), which constitutes the majority of glaucoma in most populations, is a multi-factorial optic neuropathy with a strong hereditary component. In this case-control study, candidate gene approach was used in order to discover the genetic polymorphisms associated with susceptibility to glaucoma. Genomic DNA was obtained from 176 unrelated Korean patients of glaucoma (including 108 patients of POAG, 31 patients of angle-closure glaucoma, and 37 patients of the other types) and 202 unrelated control subjects free of any eye diseases. All study subjects were successfully genotyped for 134 single-nucleotide polymorphisms (SNPs) located in 32 candidate genes and their associations with susceptibility to glaucoma were tested statistically. Our candidate genes were located within the previously identified glaucoma-susceptibility loci and have been differentially expressed upon elevation of intraocular pressures. We found that three SNPs located in the promoter region of an open-reading frame in 5q22.1 were significantly associated with glaucoma susceptibility. Effects of the promoter SNPs on gene expression level were determined using a luciferase reporter assay. A risk haplotype of two adjacent SNPs reduced the gene expression level significantly. Our results suggest that these two promoter SNPs control gene transcription level and reduced expression levels of an open-reading frame in 5q22.1 increases glaucoma susceptibility. [supported by a grant of the Korea Healthcare technology R&D Project].

## 900/W/Poster Board #558

**Association of *RBP4* gene variants and serum HDL cholesterol levels in the Newfoundland population.** *J.L. Shea[1], J.C. Loredo-Osti[2], H.W. Zhang[1], G. Sun[1].* 1) Discipline of Genetics, Memorial University of Newfoundland, St. John's, Newfoundland, Canada; 2) Dept of Math and Statistics, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

Objective: Retinol binding protein 4 (RBP4) is a novel adipokine that likely contributes to systemic insulin resistance and dyslipidemia. The role of genetic variations in *RBP4* on phenotypes of glucose and lipid metabolism is not clear in humans. The purpose of this study was to examine five SNPs in the *RBP4* gene to determine their relationship with markers of insulin resistance and serum lipids in the CODING Study. Research Design and Methods: The CODING Study consists of 1851 subjects recruited from the genetically homogeneous population of Newfoundland and Labrador (NL), Canada. Serum glucose, insulin, HOMAIR, HOMA$\beta$, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides were determined after a 12-hour fast. Five SNPs within the *RBP4* gene (rs3758539, G/A 5' flanking region; rs61461737, A/G intron; rs10882280, C/A intron; rs11187545, A/G intron; and rs12265684, C/G intron) were genotyped using TaqMan validated or functionally tested SNP genotyping assays. Results: We observed a significant association between rs11187545 (G allele) and higher HDL levels ($p = 0.016$; corrected $p = 0.050$, based on 10,000 permutations). Although serum HDL was also associated with rs61461737 and rs10882280, these did not survive correction for multiple testing (corrected $p = 0.096$ and $0.053$, respectively). An association was also evident between rs61461737 and triglyceride levels however this failed to remain significant after correction for multiple testing. We found no significant association between any variant sites and markers of insulin resistance. Conclusions: Our results suggest that genetic variations in *RBP4* may play a role in the differences in serum HDL levels in the NL population.

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### 901/W/Poster Board #559

**Genome-wide Analysis of Gene-Gene Interaction.** *Y. Zhu[1], G. Peng[1], H. Dong[1], X. Zhou[2], M. Ward[3], M. Weisman[4], M. Brown[5], J. Reveille[2], M. Xiong[2].* 1) Laboratory of Theoretical Systems Biology, School of Life Science, Fudan University, Shanghai, 200433, China; 2) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77225; 3) NIAMS, Bethesda, TX; 4) Cedars-Sinai Med Ctr, Los Angeles, CA; 5) Univ. of Queensland, Brisbane, Australia.

It is thought that complex genetic diseases are caused by multiple genes, primarily through nonlinear gene interactions and gene-environment interactions. Development of disease is thought to be a dynamic process of gene-gene and gene-environment interaction within a complex biological system which is organized into complicated interacting networks. Modern complex theory assumes that the complexity is attributed to the interactions among the components of the system, therefore, interaction has been considered as a sensible measure of complexity of the biological systems. We argue that interaction holds a key to dissecting the genetic architecture of complex diseases. Despite current enthusiasm for investigating gene-gene interactions, published results that document these interactions in humans are limited. Three issues(1) definition and measure of gene-gene interaction, (2) development of powerful statistics for testing gene-gene interaction, and (3) development of strategies and algorithms for genome-wide search of gene-gene interaction should be addressed are essential to the gene-gene interaction studies. In this report, we present a novel statistic for testing interaction between two linked loci. Its type 1 error rates are not appreciably different from the nominal levels. The statistic was applied to 35,206 SNPs in 501 pathways that were assembled from KEGG and BioCart pathway database, and investigated in a genomewide association study of susceptibility toankylosing spondylitis (AS). The AS dataset includes both UK/Australian and North American cohorts. In the North American cohort we have 762 cases and 1,616 controls. In UK/Australian dataset, we have 1,061 cases and 1,1117 controls. Illumina HumHap370 microarray platform was used to genotype 317,000 SNPs. We identified 392 interacted genes that were common in North American and UK/Australian datasets where each pair of interacted genes has at least one pair of interacted SNPs with P-values < 1.0x10-8. The maximum connected interaction network that was common in North American and UK/Australian studies includes 361 genes and 657 interactions.

### 902/W/Poster Board #560

**Searching susceptibility loci for nicotine dependence through a pathway-based association analysis.** *X.-Y. Lou[1], J.Z. Ma[2], T.J. Payne[3], G.-B. Chen[1], T. Niu[1], J. Wang[1], M.D. Li[1].* 1) Dept Psychiatry & NB Sci, Univ Virginia, Charlottesville, VA; 2) Department of Public Health Sciences, University of Virginia, Charlottesville, VA; 3) ACT Center, University of Mississippi Medical Center, Jackson, MS.

Following our previous genome-wide linkage scans for nicotine dependence (ND) in the Mid-South Tobacco family cohort that includes 2,037 subjects from 602 nuclear families of either African-American (AA) or European-American (EA) origin, we selected 3,072 single nucleotide polymorphisms (SNPs) from the five linkage regions based on several criteria including minor allelic frequency, distance between SNPs, location of SNPs, LD among SNPs as well as annotation information and then used to search susceptibility genes for ND. We first conducted family-based association test for each SNP with ND, which was assessed by smoking quantity, Heaviness of Smoking Index and Fagerström Test for ND, for EA, AA, and the pooled sample, respectively. In consideration of various numbers of SNPs being genotyped within each gene and their varying LD structure, we evaluated the significance of each gene (P-value) by conducting empirically permutation testing within each gene, and then used the P-values of 436 annotated genes which are retrieved from the genotyped SNPs to identify pathways associated with ND by using gene set enrichment analysis (GSEA) and other bioinformatics approaches. Such analyses reveal several important pathways, such as those involved in neuron differentiation, neurite development, and neuron development, to be significantly associated with ND phenotypes (FDR < 0.001). In summary, this study implies that pathway-based association analysis offers a valuable approach to identify susceptibility genetic variants with subtle effects for ND through comparing the distribution of genes in the dataset with the null distribution in a pathway library. (This study is being supported by NIH grants DA-12844 and DA-025095.).

### 903/W/Poster Board #561

**Comprehensive Genome-wide Association Studies of Psoriasis.** *M. Xiong[1], L. Luo[1], G. Peng[2], H. Dong[1,2], X. Wu[2], H. S[2], Y. Zhu[2], Y. Xiao[3], X. Zhou[4], C. Amos[5], L. Jin[2], J. Reveille[4].* 1) Dept Biostatistics, Univ Texas Hlth Sci, Houston, TX; 2) School of Life Science and Institute for Biomedical Sciences, Fudan University, Shanghai 200433, China; 3) Department of Computing and Information Technology, Fudan University, Shanghai 200433, China; 4) Division of Rheumatology, Medical School, University of Texas Health Science Center at Houston, Houston, TX 77030; 5) Department of Epidemiology, University of Texas, M. D. Anderson Cancer Center, Houston, TX 77030.

Despite their great success, the current GWAS can detect DNA variants that account for only a few percent of the genetic variance. This raised new questions about where and how we can find the remaining genetic variance. The current GWAS approaches have focused on single SNP analysis. However, common disease often arises from the joint action of multiple loci within a gene or joint action of multiple genes within pathways. If we only consider the most significant SNPs, the genetic variants that jointly have significant risk effects, but individually make only a small contribution, will be missed. Furthermore, the current statistics for GWAS have limited power to detect interactions among SNPs. Very few statistics exist for testing interactions among genes, where each contains a number of SNPs. We propose a novel analytical framework that will allow one to holistically unravel the complex genetic architecture of common diseases to gain insight into the biological processes and disease mechanism. Specifically, we develop novel concepts, statistical methods and algorithms for gene and pathway-based GWAS in which a gene or a pathway is taken as a basic unit of association analysis, genome-wide interaction analysis and genome-wide genetic studies of gene expressions, which lead to a network definition of complex disease. As a proof of concept, the developed new paradigm for GWAS was applied to two independent GWAS datasets and a whole genome-wide expression dataset of psoriasis. The preliminary results identify 50 significant loci in the MHC region, 40 significant genes on chromosome 6 and 5 significant non-MHC genes (e.g. LRP1B with P-value < 2.9E-6 by QT statistic, but the best SNP with P-value < 0.0004 by chi-square test) , 10 significant pathways such as Antigen processing and presentation (P-value < 4.5 E-11), Natural killer cell mediated cytotoxicity (P-value < 3.1 E-11), shared by GWAS, an interaction network with 626 interactions, and a comprehensive eQTL-expression network with 5 significant e-SNPs, 19 significant e-GENEs, 24 interacted ePSNPs, and 66 differentially expressed genes. These results were replicated in two independent studies.

### 904/W/Poster Board #562

**Regulation of tissue specific functions in susceptibility genes for schizophrenia.** *F. Macciardi[1, 2], E. Osimo[2], S.G. Potkin[1], J. Turner[1], F. Torri[2], J. Fallon[1], M. Vawter[1], B. Lerer[4], S. Gaudi[3].* 1) Dept of Psychiatry and Human Behavior, UCI, Irvine, CA, USA; 2) Dept of Science and Biomedical Technologies, University of Milano, Italy; 3) Istituto Superiore di Sanità, Roma, Italy; 4) Department of Psychiatry Hadassah - Hebrew University Medical Center Ein Karem, Jerusalem Israel.

It is increasingly clear that the complexity of the genome has little to do with the number and heterogeneity of its genes. Only 2% of the human genome codes for proteins, while 45% consists of Transposable Elements (TEs). The relative amount of TEs increases consistently with complexity (1) and the TEs domains of the regulatory genome (2, 3), and drives genome evolution (4), alters gene expression (5) and substantially contributes to the etiology and characteristics of complex diseases (6). The regulation of TE expression has been already associated with complex diseases; in particular cancer cells are characterized by high expression levels of LINE-1 (7), correlating transforming cancer cell to the reactivation of RT expression (8) that is barely present in differentiated non-pathological cells (9). Furthermore complex human diseases cannot be explained only by genetic factors and are likely to be the result of gene-environment interactions with the contribution of TEs. The detection of retroviral transcripts in the brains of schizophrenics suggests that activation or upregulation of distinct human endogenous retroviruses (HERVs) may play a role in the etiopathogenesis of neuropsychiatric diseases (10). Our aim is to investigate the functional relationship between TE and genes identified as "best" candidate from GWAS, validating them with exon-expression array and reconstructing their regulatory network with computational methods, and to propose a novel mechanism for gene dysregulation in schizophrenia, including differential methylation. We found preliminary evidence of alternative regulation of tissue specific gene expression in schizophrenic patients vs controls from genes that we identified as associated to schizophrenia from our GWAS and we present results for both tissue specific expression and alternative TSS in pivotal disease-related genes. REFERENCES 1. Taft R.J et al (2007). BioEssays. 29:288-299. 2. Caporale H. 2006. The implicit genome. Oxford University Press. 3. Slotkin, R.K., and Martienssen, R. (2007). Nat. Rev. Genet. 8(4), 272-285. 4. Kazazian HH Jr (2004). Science 303:1626-1632. 5. Goodier J.L. and Kazazian H.H. (2008). Cell. 135:23-31. 6. Sciamanna I et al. 2005. Oncogene 24:3923-3931. 7. Oricchio E, et al (2007). Oncogene. 26 (29): 4226-33 8. Sinibaldi-Vallebona et al 2006. Genes Chromosomes Cancer Jan;45(1):1-10. 9. Banerjee S. and Thampan R.V. (2000) Bioch Biophys Acta 1480:1-5. 10. Karlsson H et al. 2001. PNAS 98: 4634-4639.

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## 905/W/Poster Board #563

**Genetic Basis of Differential Transcriptional Aging and Cognitive Function.** *J.W. Kent[1], J. Charlesworth[1], J.E. Curran[1], M. Carless[1], T.D. Dyer[1], H.H.H. Göring[1], V.P. Diego[1], M.P. Johnson[1], M.C. Mahaney[1], L. Almasy[1], E.K. Moses[1], D.C. Glahn[2], J. Blangero[1], S. Williams-Blangero[1].* 1) Dept Genetics, SW Fndn Biomedical Research, San Antonio, TX; 2) Olin Neuropsychiatry Research Center, The Institute of Living and Dept of Psychiatry, Yale University, New Haven, CT.

Individual humans age at different rates, but the causes of heritable variation in biological aging are largely unknown. Our research group has previously examined genome-wide gene expression levels in lymphocytes from 1,240 Mexican Americans in the San Antonio Family Heart Study (SAFHS). Using these data, we have identified more than 600 gene expression phenotypes that are correlated with age at a 5% false discovery rate and exhibit polygenic genotype x age interaction (GxAI) at a nominal $p<0.05$. We searched OMIM for information on biological function for the top 25 of these by association with age; six (*LRRN3*, *FLNB*, *PLEKHG4*, *B3GAT1*, *PACSIN1*, *CAPN2*) are implicated in processes relevant to neurodegenerative disease including Alzheimer disease, Huntington disease, and spinocerebellar ataxia. Most show significant genetic and/or environmental correlation in bivariate analyses. We implemented a joint regression model in SOLAR that allows SNP genotype-specific regressions of phenotypes on age to test for equality of intercepts at mean age (association), equality of slopes (variance-type GxAI), and same sign for all slopes (correlation-type GxAI). We conducted genome-wide joint regression of the 6 expression phenotypes of interest on ~550K haplotype-tag SNPs in 858 SAFHS participants and ranked SNPs by likelihood ratio test (LRT) statistics. We performed network analyses (Ingenuity) on genes co-localized with a subset of SNPs with suggestive evidence (LRT > 20) of SNP-level GxAI. The largest network comprises 63 genes with known functional interactions, 34 of which are involved in neurological disorders, 18 in progressive motor neuropathy, and 21 in developmental disorders. Three hub genes in this network have known functional relationships with *PACSIN1* and *CAPN2*. Finally, we used our SNP subset in a joint regression analysis of a panel of cognitive phenotypes measured in 700 participants in the Genetics of Brain Structure and Function Study, including 458 former participants in SAFHS (~15y after they provided the gene expression data). Three SNPs showed suggestive evidence of GxAI effects on measures of processing speed, with the top hit in the thyrotropin-releasing hormone-degrading ectoenzyme gene *TRHDE*. Thus, our investigation of the genetic structure of differential transcriptional aging has revealed variants with possible prospective effects on cognitive function.

## 906/W/Poster Board #564

**Integrating Analysis of Genomic Variation and Transcriptional Profiling in Families with Childhood Atopic Dermatitis.** *A. Bauerfeind[1], J. Esparza-Gordillo[1,2], H. Schulz[1], K. Rohde[1], F. Ruschendorf[1], N. Hubner[1], Y.A. Lee[1,2].* 1) Max-Delbrück-Centrum, Berlin, Germany; 2) Pediatric Pneumology and Immunology, Charité, Berlin, Germany.

Atopic dermatitis (AD or eczema) is a common chronic inflammatory skin disorder with complex etiology. To identify genetic determinants of atopic dermatitis we have previously carried out a genome-wide association analysis for atopic dermatitis (1). As gene expression represents an important link between genetic variation and the phenotypic outcome that we diagnose as a clinical disorder, we performed gene expression profiling of more than 33,000 human genes in peripheral blood mononuclear cells representing key functional cells in the allergic immune response. Results were obtained in 236 individuals with atopic dermatitis including 47 nuclear families with affected children and 35 affected singletons in whom a genome-wide set of 440,794 SNPs have been genotyped. Heritability of expression traits was measured within a variance-component framework and the resulting distribution differ considerably from those under random conditions. On this evidence we identified cis and trans effects of genetic factors (eSNPs) on heritable gene expression traits. We found highly significant correlation with global gene expression levels measured in Epstein-Barr virus-transformed lymphoblastoid cell lines of children with asthma (2), another allergic disorder that is closely associated with atopic dermatitis. We identified expression quantitative trait loci that reflect genetic determinants of gene expression due to common genetic variation, but also found disease specific eQTLs, thus providing a functional annotation of genetic variants associated with allergic disease. (1 ) Esparza-Gordillo J et al. A common variant on chromosome 11q13 is associated with atopic dermatitis. Nat Genet 41[5], 596-601. 2009. (2) Dixon AL et al. A genome-wide association study of global gene expression. Nat Genet 39[10], 1202-1207. 2007.

## 907/W/Poster Board #565

**Deep MHC Haplotype Sequencing: An Integrated Approach to Common Disease.** *M.R. Hoehe[1], R. Horton[1], S. Schulz[1], S. Palczewski[1], H.v. Eberstein[2], S. Schreiber[2], T. Hübsch[1], E.-K. Suk[1].* 1) Vertebrate Genomics, Max-Planck-Institute for Molecular Genetics, Berlin, Germany; 2) University of Kiel, IKMB, Kiel, Germany.

**Purpose of project:** To sequence complete human major histocompatibility (MHC) haplotypes at the population level and in specific diseases. **Motivation:** The MHC has been recognized as the most important genetic region in relation to common diseases including inflammatory, infectious and autoimmune diseases as well as transplant medicine. Recent WGA studies confirmed associations between the MHC and numerous disease phenotypes of interest. To move from the regions of association to the causal variants, the complex nature of the MHC needs to be resolved. Copy-number variations, insertions, deletions and inversions, high levels of SNPs, differing degrees of recombination and LD extending over Megabases suggest that sequencing MHC haplotypes completely is the strategy of choice to capture and clinically harness the full variation content of the MHC. **Resources & technologies:** To this aim, we have established a unique 'Haploid Reference Resource' of 100 human fosmid libraries from a representative German population cohort (PopGen; 200 haploid genomes), and a SOLiD next generation sequencing (NGS) & MHC data analysis pipeline. **First results:** In the first phase of this project, presence and position of MHC haplotype-informative fosmids have been identified by high throughput SNP mapping (~2500 SNPs) of our fosmid libraries. SNP-based *in silico* assembly of the two MHC haplotypes of an individual showed that on average, 93,9% of the 4.6 MB mapped MHC was covered, and each haplotype was physically covered on average by 6,9 fosmids at any position. NGS of 10 BAC clones from the PGF MHC reference haplotype confirmed high quality performance of our SOLiD sequencing & data analysis pipeline (99,96% sequence identity). Entire fosmid pools (5000 and 15.000 fosmids per pool) have been sequenced to achieve an average 10x sequence coverage, which due to the physical redundancy of the libraries should allow a first assembly of MHC haplotype sequences. In addition, enrichment of distinct MHC fosmids is being tested with the HybSelect Oligo Array (Febit Inc.) and in-solution approaches, targeting complete 40 kb fosmids in the highly variable MHC regions, which is difficult to achieve with array-based enrichment technologies. To conclude, fosmid pool sequencing and sequencing of selectively enriched MHC fosmids are used as complementary approaches to generate complete MHC haplotypes at an unprecedented depth.

## 908/W/Poster Board #566

**Sequence variants of the CAST Gene are associated with Glaucoma related trait in a cohort of West Africans.** *Y. Chen[1], A. Adeyemo[2], G. Chen[2], A. Doumatey[2], H. Huang[2], J. Zhou[2], A. Amoah[3], K. Agyenim-Boateng[4], J. Oli[5], O. Fasanmade[6], C. Adebamowo[7], C. Rotimi[2].* 1) National Human Genome Ctr, Howard University, Washington, DC; 2) Center for Research on Genomics and Global Health, National Human Genome Research Institute, NIH, Bethesda, Maryland; 3) University of Ghana Medical School, Department of Medicine, Accra, Ghana; 4) University of Science and Technology, Department of Medicine, Kumasi, Ghana; 5) University of Nigeria Teaching Hospital, Department of Medicine, Enugu, Nigeria; 6) University of Lagos, College of Medicine, Endocrine and Metabolic Unit, Lagos, Nigeria; 7) University of Ibadan, College of Medicine, Ibadan, Nigeria.

Glaucoma is a chronic neurodegenerative disease of optic nerve characterized by degeneration of retinal ganglion cells and progressive loss of optic nerve axons, which is thought to be caused by Calpain induced apoptosis. Primary open-angle glaucoma (POAG), mainly caused by elevated intraocular pressure (IOP), is the most common adult form of glaucoma. Genetics play an important role in the pathogenesis of POAG with at least 20 genetic loci for POAG reported to date. In this study, we re-sequenced all the exons, their flanking regions and the promoter region of the CAST gene located in our linkage region on chromosome 5q in 48 West Africans (96 chromosomes). Identified SNPs were subsequently genotyped in a cohort of 630 West Africans with IOP measurements. The SNP rs9667 at exon 3 was significantly associated with elevated IOP with 30% of the cases (i.e., IOP ≥ 22mmHg) carrying the GG genotype compared to 18% of the controls (P=0.0024). A second SNP rs27524 in a different LD block was marginally associated with elevated IOP (P=0.0467). Considering that the CAST gene product, Calpastatin, is a highly specific activator of Calpains which plays a central role in glaucoma related retinal degeneration, we conclude that the CAST gene may play an important role in the pathological elevation of IOP, apoptosis of retinal ganglion cells and resulting retinal degeneration.

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## 909/W/Poster Board #567

**Integrative genomics and eQTL mapping in primary cells for the identification of disease-associated regulatory variation.** *B.A. Raby, A. Murphy, J. Lasky-Su, B.J. Klanderman, R. Kelly, S. Mazza, B. Himes, V.J. Carey, S.T. Weiss.* Channing Lab, Brigham & Women's Hospital, Harvard Medical School, Boston, MA.

Early studies using immortalized cell lines suggest that eQTL mapping in human populations combining genome-wide SNP and expression data is technically feasible, and can be used to identify disease-associated variation. We have previously demonstrated similar success using primary (non-immortalized) peripheral blood CD4+ lymphocytes collected in a population of well-characterized asthmatic adolescents (n=199), having mapped 6,799 cis-acting regulatory SNP (rSNP) in 1,831 genes. Here we demonstrate that these results are enriched for disease-susceptibility variants. We first compared our results with the Catalog of Genome-Wide Association Studies (Hindorff LA, PNAS 2009) and found that the set of rSNP includes regulatory variants for 45 candidate genes previously associated with clinical traits, including 18 examples where the rSNP represents the variant demonstrating the most significant association in the disease-GWAS scan. Though the list of disease-associated genes was itself enriched for immune-related diseases (inflammatory bowel disease, connective tissue disease, type I diabetes, asthma), disease-associated rSNP were also observed for other phenotype classes, including cancer, neuropsychiatric disorders, and anthropomorphic measures. We next assessed whether the set of rSNP could be used to identify novel asthma-associated variants. We found that 420 of the 6,799 cis-acting rSNP (6.2%) were nominally associated with asthma affection status in 395 non-Hispanic white parent-child asthma trios. , including one previously reported asthma gene - ORMDL3. Other asthma-associated SNP include variants on chromosome 12q for which we have previously demonstrated association with asthma in four independent populations. These variants reside on a 176kb haplotype block and are strongly associated with the expression of the IL4-related gene TFCP2. We next tested the remaining subset of asthma-associated rSNP for replication of the asthma associations in a hospital-based cohort of adult non-Hispanic white asthmatics (n=429) and healthy controls (n=853). 11 SNP in 8 genes were significantly associated in this second population, suggesting generalizability of results. Together, these findings illustrate the potential of eQTL mapping in human populations for the identification of regulatory variants that contribute to complex trait pathogenesis. Grant support: NIH/NHLBI RHL086601.

## 910/W/Poster Board #568

**Identification of TSLP as a Susceptibility Gene for Chronic Rhinosinusitis.** *L. Mfuna Endam[1], Y. Bosse[2,3], A. Filali-Mouhim[1], Z. Allakhverdi[4], G. Delespesse[4], M.P. Platt[5], K.M. Stankovic[5], R. Metson[5], P. Boisvert[6], L.P. Boulet[2], M. Desrosiers[1,7].* 1) Departement of Otolaryngology, CHUM Hotel Dieu , Montreal, PQ, Canada; 2) Institut Universitaire de Cardiologie et de Pneumologie de Quebec, Quebec, PQ, Canada; 3) Laval University Hospital Research Center (CRCHUL), Quebec, PQ, Canada; 4) Laboratory on Allergy, CHUM Research Center, Notre-Dame Hospital, Montreal, PQ, Canada; 5) Departement of Otolaryngology, Massachusetts Eye and Ear Infirmary, Havard Medical School, Massachusetts, USA; 6) Departement of Otolaryngology, Saint-Francois d'Assise Hospital, Quebec, PQ, Canada; 7) Departement of Otolaryngology - Head and Neck Surgery, Montreal General Hospital, McGill University, Montreal, PQ, Canada.

Background: Chronic rhinosinusitis (CRS) is a common disease characterized by inflammation of the paranasal sinuses of uncertain etiology. A pooling-based genome wide association scan (pGWAS) performed on individuals with or without CRS has suggested the Thymic Stromal Lymphopoietin (TSLP) gene as a promising candidate for CRS. TSLP is released by epithelial cells in response to certain microbial products and has been implicated in asthma and atopic dermatitis. Objective: To explore whether single nucleotide polymorphisms (SNPs) in the TSLP gene are associated with CRS. Methods: A set of 13 SNPs selected to capture most of the genetic variability in the TSLP gene were genotyped on individual samples from a population of 206 patients with severe CRS and 196 postal-code matched controls. A replication was carry out in a larger independent case-control data set of CRS patients recruited from the same location (n=407) and a shared control group (n=260). Functional validation was carried out by measuring gene expression in nasal biopsies taken from patients with or without CRS as well as by comparing TSLP-mediated IL5 production in cell lines isolated from CRS patients with different genotypes. Results: Four SNPs in the TSLP gene were significantly associated with CRS (rs3806932, OR=0.62, p=0.0009; rs2289276, OR=0.62, p=0.0032; rs11466741, OR=0.62, p=0.0033; rs10455025, OR=0.67, p=0.0050). Three of these SNPs were replicated in the second population (rs3806932, OR=0.62, p=0.00006; rs2289276, OR=0.67, p=0.0022; rs11466741, OR=0.67, p=0.0024). There was a 3.2-fold change in the expression of TSLP in tissue of CRS patients compared to controls (p adjusted =0.000066). Genotype-specific cell line assays suggest a greater TSLP-mediated IL-5 secretion in individuals with the risk allele. Conclusions: Polymorphisms in the TSLP gene are associated with CRS. Greater TSLP expression in CRS patients coupled with genotype-specific activity of this cytokine may explain the genetic association. The potential implication of TSLP in the development of CRS corroborates previous observations that demonstrate the role of this cytokine with other inflammatory disorders. This discovery is an important step in the understanding of the disease process and provides a novel therapeutic target for this condition.

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## 911/W/Poster Board #569

**Genomewide expression profiling suggests a role for granulocytes and B-cells in multiple sclerosis and reveals LILRA4 as a novel candidate gene for genetic studies.** *A.K. Kemppinen[1,2], E. Jakkula[1,3,4], V. Leppa[1,2,3], P. Tienari[5], K. Koivisto[6], J. Kaprio[7], I. Elovaara[8,9], T. Pirttila[10], M. Reunanen[11], A. Palotie[3,4,12], L. Peltonen[1,4,12], J. Saarela[1,3].* 1) Institute for Molecular Medicine Finland (FIMM), University of Helsinki and National Institute for Health and Welfare, Helsinki 00290, Finland; 2) Helsinki Biomedical Graduate School, University of Helsinki, Helsinki, Finland; 3) Institute for Molecular Medicine Finland (FIMM), Finnish Genome Center and Department of Clinical Chemistry, University of Helsinki, Helsinki 00290, Finland; 4) Program in Medical and Population Genetics, Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, MA 02142, USA; 5) Department of Neurology, Helsinki University Central Hospital and Molecular Neurology Programme, Biomedicum, University of Helsinki, Helsinki, Finland; 6) Department of Neurology, Seinäjoki Central Hospital, Seinäjoki, Finland; 7) Department of Public Health, University of Helsinki, Helsinki, Finland; 8) Department of Neurology, Tampere University Hospital, Tampere, Finland; 9) Medical School, University of Tampere, Tampere, Finland; 10) Department of Neurology and Neuroscience, Kuopio University Hospital, Kuopio, Finland; 11) Department of Neurology, Oulu University Hospital, Oulu, Finland; 12) Wellcome Trust Sanger Institute, Cambridge, UK.

Multiple sclerosis (MS) is a chronic inflammatory disease, leading to demyelination and axonal loss in the central nervous system. Associations with variants in MHCII, *IL7R* and *IL2R* suggest that immunological genes play a central role in MS. We conducted genomewide expression profiling in peripheral blood mononuclear cells (PBMCs) in 12 Finnish MS patients and 15 healthy controls using Affymetrix HG U133 Plus 2.0 array. 926 probesets were differentially expressed in MS patients versus controls by ≥1.5-fold with Benjamini-Hochberg corrected nonparametric P-value≤0.05. Of the most strongly upregulated genes, *CLC*, *DEFA1A3* and *LTF* are known to be highly expressed in granulocytes, possibly reflecting higher levels of granulocytes in MS patients. Other highly upregulated genes (≥4 fold) were *CD24*, *CD200*, *EBF*, *FCRL1*, *FOSL2*, *G0S2*, *HBA1*/*HBA2*, *HBM*, *IGHD*, *IGHM*, *IL8*, *MS4A1* (*CD20*), *MS4A3*, *NR4A2*, *TCL1A* and *TCN1*. We selected five genes for replication by RT-PCR in 30 MS patients and 30 controls and replicated the upregulation of *DEFA1A3*, and downregulation of *LILRA4* and *TNFRSF25*. *IGHM* and *IL8* showed a trend for upregulation, but were not statistically significant. Pathway analysis on all ≥1.2-fold differentially expressed genes (Ingenuity Pathway Analysis) identified B-cell receptor signaling as the most strongly enriched pathway and in total 27 pathways, including IL-2, IL-4, IL-8 and IL-12 signaling, were enriched with a P-value≤$10^{-5}$. Finally, we genotyped tagging SNPs in the identified candidate genes *LILRA4*, *TNFRSF25* and *IL8* in 803 cases and 1171 controls. Although no significant associations were observed in the entire sample, a SNP in *LILRA4*, encoding for leukocyte immunoglobulin-like receptor subfamily A member 4 located at 19q13, showed suggestive evidence for association in meta-analysis of three geographically matched case-control subsets (P-value=0.002). However, we did not observe significant correlation between the associated allele and LILRA4 transcript levels in 42 Finnish MS patients. Genotyping of this SNP is currently ongoing in other Nordic populations and, of note, has not been genotyped or tagged in previous genomewide association studies. In conclusion, our results suggest a role for granulocytes and B-cells in MS pathogenesis and provide initial evidence for association of *LILRA4* with MS.

## 912/W/Poster Board #570

**Integrative Genomics: Environmental Influences on Expression Quantitative Trait Loci.** *K.G. Tantisira[1,2], A. Murphy[1], J. Lasky-Su[1], Q.L. Duan[1], S. Niu[1], C. Anderson[1], B. Klanderman[1], V. Carey[1], B.A. Raby[1,2], S.T. Weiss[1,3].* 1) Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 2) Pulmonary Division, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 3) Partners Center for Personalized Genetic Medicine, Partners Health Care, Boston, MA.

Combining genome-wide association (GWAS) genotyping with genome-wide expression to identify regulatory variation in the form of expression quantitative trait loci (eQTL) has proven to be a powerful analytic strategy; many of the identified cis-acting eQTL explain a large proportion of the variability in expression. To date, eQTL studies have focused primarily on explaining variability related to basal expression characteristics. We hypothesized that integrative analysis of changes in cellular expression would identify eQTL that strongly influence the genomic response to environmental perturbation. We used immortalized B-cells and Illumina HumanHap550 genotyping data obtained from participants in the Childhood Asthma Management Program. Cells from 118 asthmatics were cultured and treated with $10^{-6}$M dexamethasone or sham, RNA isolated, and spotted on Illumina HumanRef8 v2 microarray BeadChips. Following background correction and normalization, the change in expression response related to environmental stimulation was quantified within each subject as $\log_2$(Dexamethasone) expression minus $\log_2$(Sham) expression. Cis-eQTL analysis, evaluating the region within 50 kb of a gene, was performed using the BioConductor package GGtools and a GLM analysis. There were 2755 cis-acting eQTL identified using a nominal p-value threshold of 0.01 and 355 using a p-value of 0.001. For the latter, the median heritability was 10.2% with an interquartile range of 9.5-11.6%. In addition, fourteen eQTL met criteria for multiple comparisons at an FDR of 0.1, with a median heritability of 16.8%. From the 355 eQTL meeting p<0.001 criteria, a large number of genes were immediately recognized as contributing to asthma pathogenesis and/or to corticosteroid modulation, including *ADRB2, CCL17, CFTR, IFNW1, IL17R, PDGFB,* and *TSLP*. Ontologic analyses, as well as analyses correlating the identified eQTL with clinical response to inhaled corticosteroids, are underway. We conclude that, in a fashion similar to basal expression, there are eQTL that contribute substantially to the expression response resulting from environmental exposures within a system.

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## 913/W/Poster Board #571

**Genetical genomic analyses of the nuclear factor-κ B signal transduction network: Detection of emergent system-level properties relevant to the aging process.** *V.P. Diego[1], A. Vinson[2], P.B. Higgins[1], J.M. Peralta[1], J. Charlesworth[1], S.A. Cole[1], T.D. Dyer[1], J.E. Curran[1], M.P. Johnson[1], E.K. Moses[1], H.H.H. Göring[1], L. Almasy[1], A.G. Comuzzie[1], D.L. Rainwater[1], M.C. Mahaney[1], J.W. MacCluer[1], J. Blangero[1], S. Williams-Blangero[1].* 1) Dept Genetics, SW Foundation Biomed Res, San Antonio, TX; 2) Dept Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR.

A promising theory of aging holds that the physiological decline associated with aging is caused by the dysregulation of transcription factors that govern central physiological processes. One such transcription factor is nuclear factor kappa B (NF-κB). From work on NF-κB in relation to aging, it has become clear that the NF-κB signal transduction network (STN) plays a crucial role both in the normative senescence associated with aging, and in the associated pathophysiology of complex diseases such as cancer, neurological disorders, and the metabolic syndrome which includes type 2 diabetes, obesity, and cardiovascular disease. To better understand the role of the NF-κB STN in aging, we adopted a systems genetics approach to studying the transcriptional profiling data available for the San Antonio Family Heart Study. Ingenuity Pathways Analysis (IPA) was used to identify published gene/gene product interactions between NF-κB and the genes detected in our transcriptional profiling. The genes of interest were overlaid onto a global molecular network developed from the literature on reported connectivity recorded in the IPA knowledge base. Principal components factor analysis was performed on a candidate sub-network of 19 genes directly related to the NF-κB STN, and produced 6 orthogonal factors with eigenvalues greater than 1. Using a maximum likelihood variance components approach, we performed multipoint genome-wide scans of these factors. For factor 1comprised of genes involved in the alternative NF-κB signaling pathway (*RelB*, *TRAF5*, and *TRIF*)we found a LOD score of 3.23 at 62 centiMorgans (cM) on chromosome 15, and for factor 5comprised of the genes *Rel* and *TRADD*we found a LOD score of 3.25 on chromosome 17 at 136 cM. To better characterize these results, we performed interaction modeling of all suggestive and significant LOD scores. For factor 1, the putative quantitative trait locus (QTL) on chromosome 15 was found to have a significant epistatic interaction with another locus on chromosome 1 at 24 cM giving a LOD score of 3.58. For factor 1 again, the putative QTL on chromosome 15 was found to exhibit significant QTL × age interaction ($p = 0.005$). Modeling QTL × age interaction for factor 1 increased the LOD score on chromosome 15 to 4.11, and revealed that the QTL variance decreased with increasing age. We conclude that this systems genetics approach is a powerful method for the detection of novel QTLs and of systems-level emergent properties.

## 914/W/Poster Board #572

**Transcriptomic epidemiology of human apolipoprotein E variation.** *E. Drigalenko, D.L. Rainwater, J.E. Curran, M.P. Johnson, J.C. Charlesworth, T.D. Dyer, M. Carless, S.A. Cole, M.C. Mahaney, E.K. Moses, J. Blangero, H.H.H. Göring.* Dept. of Genetics, Southwest Foundation of Biomedical Research, San Antonio, Texas.

Apolipoprotein E (apoE) plays an essential role in the metabolism of several different lipoproteins. Genetic variation in the APOE gene is known to be associated with atherosclerosis, Alzheimer's disease and other diseases. We sought to characterize the relationships between apoE plasma concentrations and gene expression levels in lymphocytes, in an effort to identify genes that act either upstream or downstream of this risk factor for multiple common diseases. Apolipoprotein E levels were measured by immunoassay in blood samples collected after an overnight fast in randomly ascertained participants in the San Antonio Family Heart Study, which seeks to shed light on the genetic etiology of cardiovascular disease risk factors in Mexican Americans. Expression profiles were generated on lymphocyte samples from 1,240 study participants. After adjustment for the effects of sex and age and normalization, we performed bivariate variance components-based quantitative genetic analysis on apoE level and 19,648 autosomal transcripts whose expression levels were significantly detected in the lymphocyte samples. A likelihood ratio test was used to examine whether apoE level and transcript level were significantly genetically correlated. At a 0.05 FDR, we identified 114 transcripts, corresponding to 93 unique genes with NCBI gene IDs, whose expression level is significantly genetically correlated with apoE blood levels. Bayesian model selection methods were employed to derive a subset of transcripts which optimally predict apoE protein levels. We also used Ingenuity Pathways Analysis v. 7.5 to examine the relationship among the identified genes. Compared to the total set of transcripts found to be expressed in lymphocytes, several functional annotation categories are overrepresented, including cell proliferation (16 genes; p=7.8x10-4) and immune response (11 genes; p=2.1x10-3). Based on the genetic correlations among transcripts, we connected 31 of the 93 genes into a single network. We are currently using association analysis, based on genotypes generated with Illumina's HumanHap 550 BeadChip, as well as the genotypes underlying the three main apoE isoforms, to disentangle which genes act upstream of apoE and which act obligately downstream of apoE. Our results show that transcriptional profiles represent a valuable adjunct to traditional epidemiological approaches utilized in studies of classical disease risk-related phenotypes.

## 915/W/Poster Board #573

**Metabolic syndrome candidate genes identified by linkage, genome-wide association, expression and protein interactions.** *A.T. Kraja[1], D.C. Rao[2], S.C. Hunt[3], R. Kume[4], U. Broeckel[5], R.H. Myers[6], D.K. Arnett[7], M.A. Province[1].* 1) Div. of Statistical Genomics, Washington U. MO; 2) Div. of Statistics, Washington U. MO; 3) Cardiovascular Genetics Div., U. of Utah, UT; 4) GEMS Training Program Washington U. MO; 5) HMGC Laboratories, Med. College of Wisconsin; 6) Dep. of Neurology, Boston U., MA; 7) Dep. of Epidemiology U. Alabama at Birmingham, AL.

Metabolic syndrome (MetS), a complex infirmity of obesity, dyslipidemia, glucose intolerance and insulin resistance, and hypertension, has become a debilitating disease in the US and developed countries. While a few candidate gene polymorphisms have been previously reported in relation to MetS, an integrated strategy involving genome-wide and candidate genes studies, in multiple studies for discovering a network of metabolic syndrome genes has not been reported. In this work we investigate the contributions of genes to 4 quantitative latent factors underlying MetS (obesity-insulin, lipids-insulin, blood pressure and central obesity factors). We start with 123 MetS candidate genes identified from the literature. These candidate genes were found to interact with 846 genes based on protein x protein databases. The polymorphisms available in MetS candidate genes and in the interacting genes were tested for their significant main effects in The Hypertension Genetic Epidemiology Network Study (Caucasians, n=1,316, which already were genotyped for about 0.5M SNPs and African Americans, n=1,263, genotyped for about 1M SNPs using the Affymetrix platforms). Linkage results have been published from these two ethnicities for the four MetS latent factors. GWAs are now presented for each latent factor from HyperGEN Caucasians and African Americans. For example, polymorphisms in 90 SNPs of 49 genes were identified in Caucasians to be significantly associated with MetS. A meta-analysis between Caucasians and African Americans for each of the four latent factors of MetS was performed to identify overlaps of significant results between two ethnicities. Three SNPs, among the most significant SNPs in the HyperGEN Caucasians, were genotyped in the Longitudinal Utah Pedigree Study (n=1,126) for further validation. A comparison of the significant genes with the ones differentially expressed in the STRRIDE study (GSE1295, n=10) (a gene expression of exercise intervention in individuals with MetS) is being performed. We use the MetS candidate genes in model building as well as in classifying individuals' MetS status. The main focus of this work is to present the methodology for an integrated approach to discovery of clusters of genes with relatively small effects that contribute to the MetS development.

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## 916/W/Poster Board #574

**The presence of cell-free male DNA in the plasma from non-pregnant women with scleroderma.** *A. Higashijima, K. Yamasaki, K. Miura, H. Masuzaki.* OB/GYN, Nagasaki University Hospital, Nagasaki, Nagasaki, Japan.

Object; Microchimerism, which has been suggested to occur by pregnancy, is the condition that low-level fetal (donor) cells maintain in mother (host). Recently, the condition of microcimerism is considered to play a role in the pathogenesis of scleroderma, suggesting that circulating fetal DNAs affect on immune reaction in mothers. However, how microchimerism affects on immune system remains unknown. In this study, to get further information between the microchimerism and the autoimmune diseases, we examined whether cell-free male DNA (donor) exist in the plasma from non-pregnant women (host) with scleroderma or not. Methods; Written informed consents were obtained from all non-pregnant women with autoimmune diseases, who were followed-up in Nagasaki university hospital. Peripheral blood was taken from non-pregnant women and cell-free DNA was extracted from 1.6 mL plasma. The SRY gene and the DYS 14 gene were selected as the markers of male DNAs of donor components, while the beta-globin gene was selected as the marker of total DNAs including both donor and host components. Quantitative real-time PCR analysis and regular PCR analysis were performed to detect the male sequences. When male DNA was detected in same non-pregnant woman two times separately, we decided that patient had a microchimerism. Results; Regular PCR could not detect the male sequences in all non-pregnant women examined. When the quantitative real-time PCR was performed, the DYS 14 sequence was detected in three out of 12 non-pregnant women with scleroderma, while not in women with autoimmune disease except for scleroderma. However, the quantitative real-time PCR using SRY probe could not detect the male sequences in same women with scleroderma. Conclusions; In this study, circulating male DNAs were detected in plasma from non-pregnant women with scleroderma, while not in other autoimmune diseases. The condition of microchimerism was detected by only the quantitative real-time PCR and the detection rate of it depends on the difference of TaqMan probes. Therefore, our results suggested that the level of microchimerism, which could be involved in the pathogenesis of scleroderma, is a very small amount.

## 917/W/Poster Board #575

**Gene set enrichment analysis of a genome-wide association study of fasting glucose in the Framingham Heart Study.** *A. Manning[1], L.A. Cupples[1], J. Dupuis[1], J.C. Florez[2,3,4], J.B. Meigs[4,5].* 1) School of Public Health, Boston University, Boston, MA; 2) Diabetes Research Center (Diabetes Unit) and Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 3) Program in Medical and Population Genetics, Broad Institute, Cambridge, MA; 4) Department of Medicine, Harvard Medical School, Boston, MA; 5) General Medicine Unit, Massachusetts General Hospital, Boston, MA.

**Introduction:** Gene Set Enrichment Analysis (GSEA) has been developed to detect multiple small changes in several genes belonging to a functional set where single-gene changes are too modest to achieve statistical significance. When applied to genetic association studies, the goal of GSEA is to find gene sets with a greater than expected number of low p-values for association between single nucleotide polymorphisms (SNPs) and a trait. We apply GSEA to a genome-wide association scan (GWAS) of glycemic traits in a family-based population cohort. **Methods:** Using genotypes from the Framingham Heart Study SNP Health Association Resource project, we selected imputed tag SNPs +/- 20 Kb of genes. Associations with fasting glucose were assessed using linear mixed effect models. Genes were annotated to gene sets using the Molecular Signatures Database. Kolmogorov-Smirnov Enrichment Scores and hypergeometric statistics were computed for each gene set to determine if it was enriched with low p-values. Because these test statistics assume independence between tests, we present the result of a simulation study which explores the effect of high linkage disequilibrium (LD) among the tagSNPs. To adjust for LD, the empirical enrichment of the top gene sets was assessed by comparing the proportion of tests in the gene set with p-values less than 0.001 to the null distribution of the proportion of p-values less than 0.001 obtained by performing association tests with 1000 unassociated traits. **Results:** For fasting glucose, the most significant associations from the GWAS were observed in the ABCB11 gene ($p<10^{-8}$), and thus every gene set containing this gene was observed to be enriched. The GSEA was repeated after removing ABCB11. Among the highest scoring gene sets (N Genes, Multinomial P, Enrichment Score, Empirical Enrichment P) are: chr11p11 (29, $8.44 \times 10^{-25}$, 0.71, 0.001), chr11q11 (43, 0.0005, 0.65, 0.03), RECEPTOR_ACTIVITY (352, $5.1 \times 10^{-9}$, 0.5, <0.001) and NAKAJIMA_MCSMBP_MAST (26, $2.1 \times 10^{-5}$, 0.65, 0.007). RECEPTOR_ACTIVITY describes molecular function defined as “Combining with an extracellular or intracellular messenger to initiate a change in cell activity.” NAKAJIMA_MCSMBP_MAST is a curated gene set of the “top 50 most-increased mast cell specific transcripts.” **Discussion:** A GSEA was performed with a continuous trait in a family sample. Several gene sets were enriched with low p-values, allowing plausible weak associations to be considered for replication.

## 918/W/Poster Board #576

**Influence of genetics, smoking, age and obesity on urine metabolomic profiles among healthy Korean adult men - the Healthy Twin Study.** *J. Lee[1], J. Sung[1], Y. Song[2], K. Lee[3], S. Cho[1], J. Lee[4], M. Lee[1].* 1) Department of Epidemiology, Seoul National University School of Public Health; 2) Department of Family Medicine, Samsung Medical Center, SungKyunKwan University School of Medicine; 3) Department of Family Medicine, Busan Paik Hospital, School of Medicine, Inje University; 4) Korea Institute of Science and Technology.

Providing personalized evidence for disease prevention/therapy is one of the important goals of the genome research. Findings from multidisciplinary rather than single “omics” research will be more informative to those purposes. Each metabolite level in human body is known to be determined by both environments and genes. However, studies exploring the overall associations between metabolomic and genetic profile, especially in general population, have been rare. We attempted to explore the associations between metabolomic profiles and alleged determinants such as genetic contributions, age, obesity and smoking. Among the participants in the Healthy Twin study(a total of 2,750 individuals), 104 adult men were selected, comprising 35 monozygotic twin(MZ) pairs, 7 dizygotic twin(DZ) pairs, and 6 families with MZ plus their brothers. Zygosity of twins was identified by forensic genotype markers. Collected 12-hour urine(7PM to 7AM) samples were analyzed by non-targeted, high-throughput metabolomic profiling methods (Gas Chromatography-Mass Spectrometry). 251 metabolites from 97 individuals were finally analyzed. Genetic influence was measured by the heritability(h2), and associations between metabolites determinants were estimated by linear regression. Associations with current smoking amount, age, body mass index(BMI), and waist-circumference were tested adjusting family relationship as random effects. Among 251 metabolites, 37 showed significant h2(>.3), and 12 indicated high genetic influence(h2>.5). Conversely, there were only 2 metabolites showing positive associations with smoking amount, while 8 were negatively associated. With age, however, 19 metabolites exhibited positive, and 4 showed negative associations. There were 4 and 3 metabolites with positive associations with BMI and waist-circumference, while 11 and 12 metabolites with negative associations.Although ours is non-targeted metabolomic profiling which cannot estimate the health significance of each metabolite, the patterns based on the proportion of metabolites associated with common risk factors revealed new findings. First, more metabolites were associated with genetics, aging, or obesity than with smoking. Second, more negative associations were observed with major determinants. Considering that most existing biomarkers have positive associations with exposure/effects, this abundance of negatively associated metabolites may require revision of conventional view on biomarkers of exposure.

## 919/W/Poster Board #577

**A new methodology for gene-mapping in complex disorders: pathway based analysis using logistic regression.** *G. Kalsi[1], M. Reimers[2], B.P. Riley[1], K.S. Kendler[1].* 1) Dept Psychiatry, Virginia Commonwealth Univ, Richmond, VA; 2) Dept Biostatistics, Virginia Commonwealth University, Richmond, VA.

For complex disorders such as alcohol dependence (AD), genetic factors contribute 40-60% of the variance in liability to AD. Although epidemiological and linkage findings do not support the existence of single genes of major effect in complex disorders, gene-finding efforts have largely focused on single gene studies. Consequently, gene-mapping studies have not replicated in other samples or have produced inconsistent findings. In contrast, functional studies in model organisms and humans provide considerable information about changes following alcohol exposure and about systems that may explain differences in response to alcohol and alcohol-related behavior. This information can be utilized in designing new tools for performing systems based investigations into complex disorders. In this study, we employed a logistic regression methodology to test the hypothesis that multiple alleles within a functional set have significantly higher than expected predictive value, even though none may have strong individual effects. We tested 1350 single nucleotide polymorphisms in 130 addiction candidate genes studied in a sample of 575 AD cases and 530 controls. The analysis produced empirical evidence indicating that AD is related to variation in genes participating in glutamate (P=0.05), GABA signaling (P=0.04) and in stress response pathways (NOR, P=0.02; CRH, P=0.01) but not with genes in several other systems implicated in other drugs of abuse. One concern about this study may be that the genes were a priori selected to be likely candidates for association with addictive behavior. We are currently following up our initial testing of the pathway based methodology with results from a genome-wide association study performed by the Genetics Association Information Network.

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## 920/W/Poster Board #578

**A Disequilibrium Model for Detecting Genes Associated with the Aneuploidy of Cancer Cells.** *Y. Li[1], Z. Wang[2], R. Wu[2].* 1) Department of Statistics, West Virginia University, Morgantown, WV; 2) Department of Public Health Sciences, PSU College of Medicine, Hershey, PA.

Because of the occurrence of one or more extra or missing chromosomes, aneuploidy is thought to affect the metabolic control of cells and then cause cancer. Thus, it is interesting to predict the incidence of aneuploid cancer through genetic data. Here, we present a statistical model for estimating and testing the non-random associations between diploid loci and aneuploid loci. The model formulates a series of disequilibrium coefficients to characterize genes in the normal tissue that are associated with aneuploidy. We provide a procedure for testing these disequilibria individually or jointly within the EM framework. Simulation studies are performed to investigate the statistical properties of the model. Our model can be useful for unraveling the genetic architecture of cancer.

## 921/W/Poster Board #579

**Predictive modeling for Parkinson Disease.** *R.-H. Chung[1], T.L. Edwards[1], W.K. Scott[1], C. Almonte[1], A. Burt[1], E.H. Powell[1], G.W. Beecham[1], I. Konidari[1], M.A. Pericak-Vance[1], J.L. Haines[2], S. Zuchner[1], G. Wang[1], L. Wang[1], J.M. Vance[1], E.R. Martin[1].* 1) Miami Institute for Human Genomics, and Dr. John T Macdonald Foundation Department of Human Genetics, Miller School of Medicine, University of Miami, Miami, FL, USA; 2) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN, USA.

Parkinson disease (PD) is a neurodegenerative disorder with complex etiology. One of the goals of identifying susceptibility genes for PD is to improve predictive models for PD risk. To explore this possibility at our current knowledge state, both candidate-gene and GWAS approaches were used to create predictive models. We used two GWAS datasets: our recent GWAS (605 cases and 621 controls) and the CIDR GWAS dataset (891 cases and 863 controls). We randomly selected 200 cases and 200 controls from the combined dataset as the test dataset, while the remaining 1296 cases and 1284 controls were used as the training dataset. In the candidate-gene approach, SNPs in the 24 most strongly associated genes reported in the PDGene database were selected. These SNPs were first tested with BIMBAM, a Bayesian modeling method, to identify associated SNPs [Bayes factor (BF) > 5]. Then the Random Forest method was used to create a predictive model based on these SNPs. In the GWAS approach, SNPs with BF > 10 and in gene regions were selected across autosomes. These SNPs were also used in Random Forest analysis. The candidate gene approach identified 46 significant SNPs in nine genes. The resulting model from Random Forests had 59.6% sensitivity and 52% specificity for the training dataset. For the test dataset, the predictive model had 58.5% sensitivity and 57.5% specificity. In the GWAS approach, 600 SNPs in 405 genes were identified. The predictive model reached 81.65% sensitivity and 77.7% specificity for the training dataset. For the test dataset, the predictive model had 66% sensitivity and 50% specificity. The results suggest that the predictive models can have > 60% sensitivity in both candidate gene and GWAS approaches, while specificity ranges between 50% and 78%. The results also show the difficulty with over-fitting in the GWAS approach. Although the models may not yet be ideal to predict diseases for clinical practice or to enrich for high risk groups for preclinical imaging, these results are encouraging. Further, known environmental exposures associated with PD were not used in this experiment, but their incorporation should improve the results further, as they have stronger associations with PD than the genetic risk factors identified thus far. With more candidate genes to be discovered for PD and more powerful datasets being assembled, we expect that the sensitivity and specificity for PD prediction using genetic data and other factors will be increased.

## 922/W/Poster Board #580

**Joint Genetic Influences on Height and Bone Mineral Density.** *L.M. Yerges-Armstrong[1], J.R. Shaffer[2], K.A. Ryan[1], J. Liu[1], J.M. Bruder[3], M.A. Carless[4], T.D. Dyer[4], J. Blangero[4], C.M. Kammerer[2], B.D. Mitchell[1].* 1) Division of Endocrinology Diabetes and Nutrition, University of Maryland, Baltimore, MD; 2) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 3) University of Texas Health Science Center, San Antonio, TX; 4) Southwest Foundation for Biomedical Research, San Antonio, TX.

Height and bone mineral density (BMD) are both under substantial genetic influence. Whether genes related to skeletal growth contribute jointly to variation in both of these traits is unknown. We evaluated whether common sets of genes may exert joint influence on both human stature and BMD in 685 Mexican Americans participating in the San Antonio Family Osteoporosis Study. Study participants were relatively healthy and comprised 41 distinct families. Study participants were ≥23 yrs old; BMD was measured by dual-energy x-ray absorptiometry.

We first estimated the overall degree of pleiotropy between height and BMD by using quantitative genetic methods to partition the phenotypic covariance between height and BMD into components attributable to genetic and environmental sources. Then, we estimated genetic correlation ($\rho_G$) which represents the proportion of covariance due to shared genetic factors with non-zero genetic correlations reflecting evidence for pleiotropy. Both height and BMD were highly heritable ($h^2$=0.55-0.85) in this population. Genetic correlations exhibiting evidence for pleiotropy were observed for height and whole body BMD ($\rho_G$=0.26) and height and hip BMD ($\rho_G$ =0.19-0.25), but not height and spine BMD. To identify possible pleiotropic loci, we genotyped 24 single nucleotide polymorphisms that have been reliably associated with variation in height, and tested their associations with BMD. In general, there was little evidence for association of any of these loci with height or BMD in this Mexican American population. SNP rs7560657 was nominally associated with height (p=0.03), but this SNP was not associated with whole body, hip or spine BMD. In fact, only one of the 24 loci showed a nominal association (p=0.01) with any BMD measure. Our data are consistent with joint genetic effects on height and BMD. However, none of the replicated height loci account for the shared genetic effects in our data. Given the very small effect sizes of these previously reported height loci, any effects on BMD may be small and undetectable with our sample size.

## 923/W/Poster Board #581

**Single Nucleotide Polymorphisms of Genes in Folate Metabolism Associate with Spina Bifida.** *H. Northrup[1,7], C.A. Martinez[1], M.R. O'Byrne[1], J.-I. Lin[2], A.C. Morrison[2], J.M. Fletcher[3], K.K. Ostermaier[4], G.H. Tyerman[5], S. Doebel[6], K.S. Au[1].* 1) Dept Peds/Div Med Gen, Univ Texas Med Sch-Houston, Houston, TX, USA; 2) Human Genetics Center, Sch Public Health, Univ Texas, Houston, TX, USA; 3) Dept Psychology, Univ Houston, Houston, TX, USA; 4) Texas Children Hospital, Houston, TX, USA; 5) Shriners Hospital for Children, Los Angeles, CA, USA; 6) The Hospital for Sick Children, Univ Toronto, Toronto, ON, Canada; 7) Shriners Hospital for Children, Houston, TX, USA.

**OBJECTIVE:** Neural tube defects (NTDs) are common birth defects with complex etiology involving both genetic and environmental factors on the developing neural tube. Spina bifida (SB) is one of the most common NTDs affecting 2 to 4 live births per 10,000 live births in the United States. Maternal deficiency in folate and diabetes are two major risk factors for NTDs. We tested single nucleotide polymorphisms (SNPs) in genes that regulate the folate/homocysteine metabolism to evaluate their contribution to SB susceptibility. **STUDY DESIGN:** A total of 608 unrelated simplex SB patient families were included in this study. We examined genotypes of 77 SNPs located in 13 folate metabolic pathway genes. Association was evaluated by the reconstruction-combined transmission disequilibrium test (RC-TDT). **RESULTS:** We found significant association in SNPs in the respective indicated genes: rs5742905 in cystathionine-beta-synthase (*CBS*), rs1643649 in dihydrofolate reductase (*DHFR*), rs2853533 in thymidylate synthetase (*TYMS*), rs327592 in 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*) and rs3737965 in methylene-tetrahydrofolate-reductase (*MTHFR*) with p-values of: 0.015, 0.041, 0.021, and 0.007 respectively. We also observed significant interactions between the SNPs with a demonstrated association. **CONCLUSION:** Transmission of specific SNPs alleles in the *CBS*, *DHFR*, *MTHFR*,*MTRR* and *TYMS* confer increased susceptibility to SB. The presence of risk alleles with interactions between risk allele of genes may contribute to dys-regulated folate/homocysteine metabolism subsequently increasing SB susceptibility.

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## 924/W/Poster Board #582

**Mutational screening and functional analyses in the zebrafish model of GIGYF2 as a candidate gene for Parkinson disease.** *I. Guella[1], A. Pistocchi[2], R. Asselta[1], V. Rimoldi[1], F. Sironi[3,4], L. Trotta[3,4], P. Primignani[3], M. Zini[4], A. Zecchinelli[4], D. Coviello[3], G. Pezzoli[4], L. Del Giacco[2], S. Duga[1], S. Goldwurm[4].* 1) Dipartimento di Biologia e Genetica per le Scienze Mediche, Università degli studi di Milano, Milan, Italy; 2) Dipartimento di Biologia, Università degli studi di Milano, Milan, Italy; 3) Laboratorio di Genetica Medica, Fondazione IRCCS "Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena", Milan, Italy; 4) Parkinson Institute, Istituti Clinici di Perfezionamento, Milan, Italy.

Parkinson disease (PD) is the second most common neurodegenerative disorder, with an age-associate prevalence of approximately 2% in the population above 65 years of age. Several loci (PARK1-15) for familial PD have been described, but for some of them the specific gene remains elusive. GIGYF2 (Grb10-Interacting GYF Protein-2) has been proposed as the PD susceptibility gene underlying the PARK11 locus after the identification of 7 different genetic variants in this gene in 123 Italian and 126 French familial PD cases, which were absent in 318 controls. Although GIGYF2 represents a good candidate gene for PD, being known to interact with the Grb10 protein and consequently to be potentially involved in insulin and insulin-like growth factor (IGF) signaling, several further studies did not confirm its association with the disease. However, the majority of these replication studies did not screen the whole gene and/or focused on sporadic PD patients, notwithstanding the fact that both the PARK 11 locus and the GIGYF2 gene were pointed out by studying familial PD. Moreover, a functional validation of GIGYF2 mutations is still lacking. In this frame, to elucidate the role of GIGYF2 in the pathogenesis of familial and sporadic PD, we performed a comprehensive mutational screening of the gene in a large Italian population of familial and sporadic PD patients and controls. Moreover, we analyzed the function of GIGYF2 in vivo, using the zebrafish model. Mutational screening of GIGYF2 was performed by a combination of high-resolution melting analysis and direct DNA sequencing. Exons containing previously reported mutations were analyzed in 552 cases (243 familial, 309 sporadic) and 552 controls. Thereafter, a subset of 184 familial PD cases and an equal number of controls were subjected to a full screening of all 27 coding exons. This analysis identified 8 different missense variations in a total of 9 individuals (4 PD patients and 5 controls). Abrogation of gigyf2 function in zebrafish embryos by morpholino oligonucleotide injection did not lead to a drastic loss of neurons in the diencephalic dopaminergic (DA) neuron clusters, neither caused a change in the expression levels of otp1 and prox1 genes, two of the major players in diencephalic DA neurons development, suggesting that gigyf2 is not required for proper DA neuron differentiation. These data, together with those recently reported by other groups, suggest that GIGYF2 is unlikely to be the PARK11 gene.

## 925/W/Poster Board #583

**Assessing the association between PAX6 polymorphism and high myopia.** *E. Hsi[1,2,3], C. Liang[4], Y. Pan[3], W. Chang[3], F. Lin[2], S. Juo[2,3,5].* 1) Dept Medicine, Kaohsiung Med Univ, Kaohsiung, Taiwan; 2) Dept Medical Research, Kaohsiung Med Univ Hospital, Kaohsiung, Taiwan; 3) Dept Med Genetics, Kaohsiung Med Univ, Kaohsiung, Taiwan; 4) Bright-Eyes Clinic, Kaohsiung, Taiwan; 5) Center of Excellence for Environmental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

**Purpose:** Pax-6 mutations are associated with a variety of human ocular diseases. In addition to the biological plausibility, a genome-wide linkage study revealed strong linkage of refractive error to the PAX6 locus. PAX6 has long been speculated as a potential gene involved in the development of myopia. Recently, studies on non-syndromic myopia have reported some significant susceptibility genes but most of the findings cannot be replicated. Therefore, replication of the genetic effect in large and independent samples is an important way to reduce the false positive finding. **Methods:** A total of 1083 cases and 1096 controls were included in the study. A case was defined as refraction ≤ -6 D and control ≥ -1.5 D. Four tagging single nucleotide polymorphisms (tSNPs) were genotyped. Statistical analyses included Hardy-Weinberg equilibrium (HWE) test, chi-squared test to evaluate the genotypic effect, linkage disequilibrium (LD) estimation, LD block construction and haplotype analysis. Subset analysis was also conducted for cases with highly myopic parents. The permutation was used for multiple test correction. **Results:** We only found a marginally significant result for SNP2 in the original definition for cases (≤ -6.0 D) and controls (≥ -1.5 D), the common genotype CC became significant when extreme myopia (≤ -11 D) was used to define a case. In addition, the frequency of the CC genotype increased with the severity of myopia in our population, which indicates a genetic dose effect. **Conclusions:** we conducted a large scale study to systematically evaluate the genetic effect of the PAX6 gene. We found a tagging polymorphism of PAX6 associated with extreme myopia but not high myopia among Chinese living in Taiwan.

## 926/W/Poster Board #584

**Assessing the association between *MAPK9* polymorphism and high myopia.** *C. Hu[1], E. Hsi[1,4], Y. Pan[2], S. Juo[2,3,4].* 1) Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Graduate Institute of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan; 3) Center of Excellence for Environmental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 4) Departments of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

Purpose: We investigate the association between high myopia and the MAPK9 gene. Methods: A total of 114 candidate genes were selected from our GWAS data. We conducted the pathway analysis to identify promising genes, and 114 candidate genes were entered into MetaCore software suite developed by GeneGo. We enrolled 967 high myopic individuals (≤-6 diopters) and 1087 controls (≥-1.5 diopters) . One SNP rs13190345 at MAPK9, associated in GWAS data, was genotyped initially. We further genotyped 12 tSNPs in a pilot sample of 170 cases and 200 controls. Hardy-Weinberg equilibrium was tested for each SNP, and genetic effects were evaluated by the chi-square test. Subset analyses were performed according to the family history and educational level. Results: Our pathway analysis showed that MAPK9 might relate to the development of myopia, which is involved in variety of cellular processes such as proliferation, differentiation, and transcriptional regulation. SNP rs13190345 at MAPK9 showed a borderline significance with myopia. Compared to the common homozygote CC, the rare allele carriers (TC and TT) appeared to have a dominant effect and was associated with myopia (OR = 0.72, p=0.057). Subset analysis on cases with a strong family history or higher educational level did not demonstrate significant results. We further genotyped 12 tSNPs to systematically assessed whether MAPK9 eliminate a susceptibility to myopia. No significant associations were seen with further tSNPs tests. Conclusions: From the borderline significance in rs13190345, it reveals that MAPK9 gene may play a moderate role in the development of myopia in the Taiwanese population. More subjects will be recruited in the near future to provide a more solid result.

## 927/W/Poster Board #585

**Analysis of 9p24 and 11p12-13 regions in Autism Spectrum Disorders: rs1340513 in the *JMJD2C* gene is associated with ASDs in the Finnish sample.** *K. Kantojärvi[1], P. Onkamo[2], R. Vanhala[3], R. Alen[4], M. Hedman[5], A. Sajantila[5], I. Järvelä[1,6].* 1) Dept Med Gen, Univ Helsinki, Helsinki, Finland; 2) Department of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland; 3) Hospital of Children and Adolescents, University of Helsinki, Helsinki, Finland; 4) Department of Child Neurology, Jyväskylä Central Hospital, Jyväskylä, Finland; 5) Department of Forensic Medicine, University of Helsinki, Finland; 6) Laboratory of Molecular Genetics, Helsinki University Central Hospital, Helsinki, Finland.

Autism spectrum disorders often show obsessive repetitive symptoms characteristic to obsessive compulsive disorder (OCD). Aberrant glutamate function has been suggested as a risk for both autism spectrum disorders (ASDs) and obsessive compulsive disorders (OCD). Two independent studies for OCD have shown suggestive evidence for linkage in the chromosomal region of 9p24. Furthermore, several studies for OCD have detected association at SNPs covering the glutamatergic gene *SLC1A1* region. Interestingly, comprehensive autism family material analyzed by the Autism Genome Project Consortium (2007) reported linkage peaks at 9p24 and 11p12-13 containing glutamatergic genes *SLC1A1* and *SLC1A2*. In order to answer the question whether there is common molecular background for obsessive repetitive symptoms in ASDs and OCD we have analyzed ten single nucleotide polymorphisms (SNPs) at 9p24 and 11p12-p13 covering glutamate transporter genes *SLC1A1* and *SLC1A2* and their neighboring genes in one hundred seventy-five patients with autism spectrum disorders of Finnish origin. 139 of them had infantile autism and 36 Asperger syndrome. The control material consisted of 216 Finnish anonymous blood donors. The SNPs were analyzed using real-time-PCR or direct sequencing. The results were calculated using Fischer's exact test, Plink and logistic regression.

Interestingly, the strongest association was detected with rs1340513 in the *JMJD2C* gene at 9p24.1 (p=0.007; permuted p=0.011) that is the same SNP that showed significant association with infantile autism (p-value= 0.0007) in the Autism Genome Project Consortium (2007). In a recent study by Shugart et al., (2009) the strongest association was found at rs301443 (p-value = 0.000067) residing 7.5kb outside at the 3'end of the *SLC1A1* gene in over 1000 OCD-patients. This location suggests that the true associated SNP for OCD will reside at the 3' end of the *SLC1A1* gene closer to the *JMJD2C* gene. Thus, these data is in agreement with ours and encourages further analysis on the 3'end of the *SLC1A1* gene and it's 3'UTR area with bigger sample set. No association was detected at 11p12-p13 with ASD. In summary, our results further suggest a common locus for OCD and ASDs at 9p24. We speculate that the region is a special candidate region for obsessive repetitive symptoms.

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## 928/W/Poster Board #586

**MC4R SNP is associated with BMI but not BMI response to resistance training in young females.** *F.E. Orkunoglu-Suer[1], H. Gordish-Dressman[1], P.M. Clarkson[2], P.D. Thompson[3], T.J. Angelopoulos[4], P.M. Gordon[5], N.M. Moyna[6], L.S. Pescatello[7], P.S. Visich[8], R.F. Zoeller[9], D.W. Moore[1], J.M. Devaney[1], E.P. Hoffman[1].* 1) Rsrch Cntr Genetics Med, Children's Natl Med Cntr, Washington, DC; 2) Dept of Kinesiology, U of Massachusetts, Amherst, MA; 3) Div of Cardiology, Henry Low Heart Cntr, Hartford Hospital, Hartford, CT, US; 4) Cntr for Lifestyle Med and Dept of Health Profes, U of Cntrl Florida, Orlando, FL; 5) Dept of Phys Med and Rehab, Sch of Med, U of Michigan, Ann Arbor, MI; 6) Sch of Health and Hum Perf, Dublin City University, Dublin 9, Ireland; 7) Dept of Kinesiology & Hum Perf Lab, University of Connecticut, Storrs, CT, US, 06269; 8) Hum Perf Lab, Cntrl Michigan University, Mount Pleasant, MI,; 9) Dept of Exer Sci and Health Promotion, Florida Atlantic University, Davie, FL.

**Background:** Recently, a genome wide association study (GWAS) that identified eight single nucleotide polymorphisms (SNPs) associated with body mass index (BMI) highlighted a possible neuronal influence on the development of obesity. We hypothesized that these SNPs will be validated with measures of BMI, and govern change in BMI in response to resistance training in young, college-aged individuals. **Methods:** We genotyped the following SNPs: NEGR1 (rs2815752), MTCH2 (rs10838738), TMEM18 (rs6548238), GNPDA2 (rs10938397), SH2B1 (rs7498665), MCR4 (rs17782313), FTO (rs9939609), and KCTD15 (rs11084753) in our cohort of young, healthy individuals, FAMUSS (Functional polymorphisms associated with human muscle size and strength) (n= 755). The individuals in our cohort underwent a 12-week resistance-training program on their non-dominant upper arm. Genotype/phenotype associations for BMI and subcutaneous fat levels before and after the exercise program were tested using an analysis of covariance model with age and weight as covariates. The percent variation in each phenotype attributable to genotype was determined using hierarchical models with a likelihood ratio test. **Results:** Females with a copy of the rare allele (C allele) for rs17782313 had significantly higher BMIs than TT females (CC/CT: n=174; 24.70 ± 0.33 kg/m2, TT; n=278; 23.41 ± 0.26 kg/m2, p=0.0025). This SNP accouted for 1.9% of overall variation in BMI in females and was statistically significant after correcting for multiple testing (p= 0.0071). Males with a copy of the rare allele (A allele) for rs9939609 lost a significant amount of subcutaneous fat with exercise (AT/AA; n=83; -798.35 ± 2624.30 mm3, TT; n=47; 9435.23 ± 3494.44 mm3, p=0.0190). This variant attributed 4.1% variation in phenotype but was not significant after correcting for multiple testing. We did not observe any effect of the other six loci on baseline subcutaneous fat levels, BMI, or change in BMI or subcutaneous fat following resistance training. **Conclusions:** This research supports and extends the original finding that there is an association between measures of obesity and variant near the MC4R gene in a healthy, young population. In addition seven other GWAS variants associated with BMI were not validated in our young population. There is no association between exercise and change in BMI with these GWAS SNPs.

## 929/W/Poster Board #587

**SCAN Database Promotes Gene Discovery and Elucidates Expression-level Replication Across Diabetes Studies: Pleiotropic Effects in Diabetes.** *J. Below[1], E. Gamazon[2], E. Kistner[3], D. Nicolae[2,4], N.J. Cox[1,2].* 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Dept Medicine, Univ Chicago, Chicago, IL; 3) Dept Health Studies, Univ Chicago, Chicago, IL; 4) Dept Statistics, Univ Chicago, Chicago, IL.

As in type 2 diabetes (T2D), the modest effect sizes of common susceptibility variants have impeded replication in many studies of common diseases. We demonstrate that the use of tools that incorporate information from expression quantitative trait loci (eQTLs) as an adjunct to physical linkage disequilibrium (LD) and known function of polymorphisms can identify relationships within phenotypes to prioritize SNPs and genes for additional study as well as novel relationships across phenotypes. The SCAN (SNP and Copy number variant ANnotation; http://www.scandb.org) database provides a novel approach to annotate genetic variation by aggregating large-scale published physical and functional information as well as multilocus LD and associations to gene expression level in the HapMap CEU and YRI lymphoblastoid cell lines at a user specified significance threshold. Using SCAN we have identified significant (pval < 0.001 by permutation) overlap in the list of genes whose expression is predicted (pval < 0.0001) by the most significant SNPs (pval < 0.001) implicated in the previously published independent T2D studies by the WTCCC, DGI, and FUSION, and the list similarly generated from SNPs associated with HbA1C level, a measure of diabetic control, in the GoKinD study of complications in type 1 diabetics (T1D). Among these genes showing significant pleiotropic effects are replicated T1D genes HLA-B, HLA-DRB1, and RNLS as well as genes implicated in other autoimmune diseases such as BST2, and genes associated with hypertension such as EDN1. In addition SCAN identified significant overlap (p <0.0001) between genes whose expression is predicted (as above) by SNPs associated with HbA1C level in the GoKinD study and SNPs associated with T1D in the curated NHGRI catalogue, including both novel and established T1D genes such as IL2RA. SCAN-generated gene lists provide novel information about replication and pleiotropy across diabetic phenotypes: the SNPs associated with the overlapping genes are not replicated between the studies and show no evidence of linkage disequilibrium ($r^2$<0.3).

## 930/W/Poster Board #588

**MAP2K3 Variation is Reproducibly Associated with Body Mass Index in Pima Indians.** *L. Bian, J. Mack, S. Kobes, W.C. Knowler, R.L. Hanson, C. Bogardus, L.J. Baier.* PECRB, NIDDK, NIH, Phoenix, AZ 85004.

To identify genetic variation that contributes to obesity in Pima Indians, a population with a high rate of obesity and type 2 diabetes, we completed a genome-wide association study using the Affymetrix 1million SNP chip. Several SNPs in the mitogen-activated protein kinase kinase 3 gene (MAP2K3) were associated with body mass index (BMI) in 1149 Pima Indians (P=2.7x10-7-0.003, adjusted for age, sex, and family membership). As an upstream kinase, MAP2K3 can induce activation of p38 MAPK, which may play a role in human adipogenesis through regulation of C/EBPβ and PPARγ. MAP2K3 was therefore directly analyzed as both a positional and biologic candidate gene for obesity in Pima Indians. Six tag SNPs (rs10468608, rs2363226, rs9901404, rs12602109, rs2230435, and rs8074866) selected from HapMap CEU and/or CHB populations by the criteria of minor allele frequency ≥0.05 and r2≥0.80, were genotyped in a population-based sample of 2789 full-heritage Pima Indians who had been longitudinally studied and had a BMI measurement from an exam when they were at non-diabetic status. The maximum BMI after the age of 15 years, from a non-diabetic exam, was used for association analyses. Five tag SNPs (all but rs8074866) were associated with BMI in the population-based study (P=0.0001-0.02, adjusted for age, sex, birth year, and family membership). Rs9901404 provided the strongest association, where the homozygous risk allele carriers (A, major allele) had a mean BMI of 36.7± 0.2 kg/m2 as compared with a BMI of 34.5± 0.5 kg/m2 for the homozygous non-risk allele carriers (G) (adjusted P=0.0001). To assess whether the association with BMI could be replicated in an independent group of subjects, rs9901404 was further genotyped in 2918 subjects from the same community, most of whom were of mixed heritage (on average ½ Pima Indian). The association with BMI reproduced in the mixed heritage replication sample in the same direction (adjusted P=0.01), and combining the full-heritage Pima Indian and mixed heritage subjects provided the strongest evidence for association (adjusted P=5.3x10-6). Preliminary functional studies using real time PCR of mRNA isolated from subcutaneous adipose tissue biopsies from 78 non-diabetic Pima Indians showed a positive correlation between MAP2K expression and BMI (adjusted P=0.0002). Taken together, our results suggest that common variation in MAP2K3 may contribute to obesity in Pima Indians by affecting MAP2K3 expression in human adipocytes.

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## 931/W/Poster Board #589

**Epidemiologic Architecture for Genes Linked to Environment (EAGLE): Characterizing genome-wide association study variations associated with type 2 diabetes in the National Health and Nutrition Examination Surveys.** *K. Brown-Gentry, L. Dumitrescu, K. Spencer, J.A. Canter, D. Murdock, J.L. Haines, M.D. Ritchie, D.C. Crawford.* Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Type 2 diabetes (T2D) is a common, complex disease affecting 8.0 % of the US population. SNPs associated with T2D status are being discovered in large genome-wide association (GWA) studies in samples of European-descent, and associations from diverse cohorts are just now emerging. To characterize the growing list of GWA-identified variants, we, as part of the Population Architecture using Genomics and Epidemiology (PAGE) Study, are genotyping samples collected for the National Health and Nutrition Examination Surveys (NHANES), a cross-sectional survey of Americans representing at least three major groups: non-Hispanic **w**hites (n=3,974), non-Hispanic **b**lacks (n=1,334), and **M**exican-**A**mericans (n=1,877). To date, six SNPs previously associated with T2D have been genotyped in NHANES 1999-2002. In preceding GWA studies, these SNPs explained a relatively small proportion of the risk, with odds ratios ranging from 1.1 to 1.6. T2D status can be defined four different ways in NHANES 1999-2002, three based on the self-reported status ("Doctor told you had diabetes," "Taking diabetic pills to lower blood sugar," "Taking insulin now,") and one based on laboratory values (fasting glucose levels ≥ 200 mg/dL). Using logistic regression, SNPs were tested for an association with each of the four T2D status definitions among adults assuming an additive genetic model and adjusting for age, sex, and body mass index. Despite being under-powered, we were able to detect several associations at the p=0.05 level. In **W**, *TCF7L2* rs7903146 was associated with T2D based on fasting glucose levels (n=15 cases, 2242 controls; p=0.036; OR=2.18; 95% CI: 1.05-4.50) and self-reported medication usage (n=51 cases, 34 controls; p=0.026; OR= 2.39; 95% CI: 1.11-5.16). In **B**, *IGF2BP2* rs4402960 (p=0.047; OR=1.48; 95% CI=1.01-2.18) and *TCF7L2* rs7903146 (p=0.021; OR=0.619; 95% CI= 0.41-0.93) were both associated with self-reported T2D status (n=57 cases, 716 controls). For **MA** (25 cases, 1170 controls), *FTO* rs8050136 was associated with fasting glucose-defined T2D (p=0.04; OR=0.54; 95% CI=0.30-0.96). No associations were observed for *CDKN2B* rs10811661, *HHEX* rs1111875, and *PPARG* rs1801282 for any T2D case definition in any group, and none of the observed associations were consistent across T2D definitions or groups. Limitations of sample size and refined case definition make characterization of T2D GWA-identified variations challenging in large epidemiologic cohorts such as NHANES.

## 932/W/Poster Board #590

**A moderate association of the *MTNR1B* and *G6PC2* gene variants with fasting plasma glucose levels in Asian Sikhs.** *J.L. Hatfield[1], L. Been[1], S.K. Ralhan[2], G.S. Wander[2], N.K. Mehra[3], J.R. Singh[4], D.K. Sanghera[1].* 1) Pediatrics, University of Oklahoma HSC, Oklahoma City, OK; 2) Hero DMC Heart Institute, Ludhiana, Punjab, India; 3) All India Institute of Medical Sciences, New Delhi, India; 4) Guru Nanak Dev University, Amritsar, Punjab, India.

Increased fasting plasma glucose (FPG) levels are a hallmark sign of type 2 diabetes (T2D). Recent genome-wide association scans (GWAS) and meta-analyses have identified common SNPs in the *MTNR1B* (encoding melatonin receptor 1-B), and *G6PC2* encoding an islet-specific protein (glucose-6-phosphatase catalytic unit 2) that contribute to individual variation in FPG levels and T2D risk in Europeans. This investigation examines the role of three SNPs with T2D and associated sub-phenotypes in a case-control sample from our Sikh Diabetes Study (SDS). We genotyped two variants from *MTNR1B* including rs1387153, located near the 5' untranslated region, and an intronic variant (rs108309630) located in *MTNR1B*. We also genotyped an intronic variant (rs560887) from *G6PC2*. These SNPs were screened on 2,194 SDS subjects comprising 1,379 T2D cases and 815 normoglycemic (NG) controls. We analyzed the association of these variants with T2D using logistic regression. We also examined the impact of these variants on quantitative sub-phenotypes of T2D [FPG, 2 hour plasma glucose, fasting insulin, and homeostatic model assessment of insulin resistance (HOMA-IR)] using multiple linear regression analysis. None of these variants were associated with T2D in this sample. However, our study confirmed prior evidence of association of these three variants with FPG levels in NG controls and these associations remained significant after adjusting for the effects of age, sex, and BMI. With the exception of FPG, no other quantitative trait revealed any association with these SNPs. A variant near the 5'untranslated region of *MTNR1B* (rs1387153) was significantly associated with FPG levels (p=0.02) under an additive model. A gender-specific significant association (p=0.022) was also observed in rs10830963 in *MTNR1B* with FPG in NG males. The *G6PC2* variant (rs560887) also revealed an 'A' allele associated significant decrease in FPG levels ($\beta$= -0.094; p=0.021) under the dominant model in controls. These associations would not remain significant in multiple testing corrections. From these results it appears that genetic variation in these genes could be a strong modulator of FPG levels in this population and may lead to the development of T2D in Asian Indians. As the overall contribution of each of these variants for controlling FPG levels in this sample is <2%, it is likely that causal variants in these loci with larger effects remain unidentified.

## 933/W/Poster Board #591

**Replication and joint effect analyses of genetic variants from type 2 diabetes susceptible loci in a Chinese population.** *C. Hu[1,2], R. Zhang[1], C. Wang[1], J. Wang[2], X. Ma[1], K. Xiang[1,2], W. Jia[1,2].* 1) Shanghai Diabetes Inst, Shanghai, China; 2) Dept Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People's Hospital.

**Objective**: Recent advance in genetic studies added the confirmed susceptible loci for type 2 diabetes to eighteen. In this study, we attempt to analyze the independent and joint effect of variants from these loci on type 2 diabetes and clinical phenotypes related to glucose metabolism. **Research Design and Methods**: Twenty-one single nucleotide polymorphisms (SNPs) from fourteen loci were successfully genotyped in 1,849 subjects with type 2 diabetes and 1,785 subjects with normal glucose regulation. We analyzed the allele and genotype distribution between the cases and controls of these SNPs as well as the joint effects of the susceptible loci on type 2 diabetes risk. **Results**: We confirmed the effects of SNPs from *PPARG*, *KCNJ11*, *CDKAL1*, *CDKN2A-CDKN2B*, *IDE-KIF11-HHEX*, *IGF2BP2* and *SLC30A8* on risk for type 2 diabetes, with odds ratios ranging from 1.114 to 1.406 (*P* value range from 0.0335 to 1.37E-12). But no significant association was detected between SNPs from *WFS1*, *FTO*, *JAZF1*, *TSPAN8-LGR5*, *THADA*, *ADAMTS9*, *NOTCH2-ADAM30* and type 2 diabetes. Analyses on the quantitative traits in the control subjects showed that *THADA* SNP rs7578597 was association with 2-h insulin during oral glucose tolerance tests (*P*=0.0005, empirical *P*=0.0090). The joint effect analysis of SNPs from eleven loci showed the individual carrying more risk alleles had a significantly higher risk for type 2 diabetes. And the type 2 diabetes patients with more risk allele tended to have earlier diagnostic ages (*P*=0.0006). **Conclusions**: Our findings suggested that genetic variants played important role in the susceptibility of type 2 diabetes in the Chinese.

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## 934/W/Poster Board #592

**ENPP1 Variants associate with quantitative metabolic traits and T2D in Afro-Caribbean men from Tobago.** *T.S. Leak[1], I. Miljkovic[1], A.L. Patrick[2], V.W. Wheeler[2], C.H. Bunker[1], C.S. Nestlerode[1], J.M. Zmuda[1].* 1) Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania; 2) Tobago Health Studies Office, Scarborough, Tobago, Trinidad and Tobago.

The ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*) gene, also referred to as plasma cell membrane glycoprotein (PC-1), has been shown to be associated with type 2 diabetes (T2D) and quantitative metabolic traits in multiple ethnic groups. Using HapMap data, we identified 41 tag single nucleotide polymorphisms (SNPs), based on a threshold linkage disequilibrium score $r^2 \geq 0.80$ and minor allele frequency ≥5%. These SNPs were genotyped in 281 unrelated Afro-Caribbean cases with T2D defined based on the criteria of fasting serum glucose levels ≥126 mg/dl and 934 unrelated Afro-Caribbean controls without a known diabetes diagnosis from Tobago. We performed age- and body mass index (BMI)adjusted linear regression on genotypic (additive,dominant, recessive) models to test for association with quantitative metabolic traits fasting plasma glucose (FPG), fasting insulin and insulin resistance by homeostasis model assessment (HOMA-IR), while logistic regression was used to test for association with T2D alone. We identified four noncoding SNPs (rs6916495, rs858338, rs943004 and rs7749493) associated with increased FPG, fasting insulin and insulin resistance by HOMA-IR (all P = 0.020-0.050). Additionally, rs9373000 (P = 0.025 dominant model; P = 0.011 additive) located in the downstream region, and an intron 1 SNP (rs6569759: P = 0.015 recessive) showed compelling evidence of association with an increased risk for T2D. We conclude that *ENPP1* gene is associated with quantitative metabolic traits and T2D in this population of Afro-Caribbean men from Tobago. However, the extensively studied K121Q variant (rs1044498) did not reveal evidence of association. The consistent evidence of association suggests pleiotropic effects for this gene.

## 935/W/Poster Board #593

**Contribution of TNFSF15 but not IL23R to ulcerative colitis susceptibility in Koreans.** *K. Song[1], J. You[1], M. Park[1], S. Yang[2].* 1) Dept Biochem & Molec Biol, Univ Ulsan Col Medicine, Seoul, Korea; 2) Dept Internal Medicine, Univ Ulsan Col Medicine, Seoul, Korea.

Inflammatory bowel diseases are chronic intractable diseases that consist of Crohn's disease and ulcerative colitis. Of the two subtypes of IBD, the genetic contribution to disease risk has been documented more clearly for CD than for UC. Linkage and genome-wide association studies in Caucasian populations identified over 30 susceptibility loci to Crohn's disease (CD). Some of the CD candidate genes were shown to be shared with UC. Previously, we have shown that TNFSF15 and IL23R genes are associated with susceptibility to CD in Koreans. As the IL23R gene was reported to be associated with UC as well as CD in Caucasian samples, we tested the two CD susceptibility genes, TNFSF15 and IL23R, in Korean UC samples. We analyzed four IL23R SNPs and four TNFSF15 SNPs in 380 patients with UC and 380 healthy controls. Two TNFSF15 gene variants, rs3810936 and rs7848647, showed marginal associations with UC; odds ratio (OR) for rs3810936 was 1.447 (95% CI = 1.024-2.044, P = 0.036) and OR for rs7848647 was 1.451 (95% CI = 1.059-1.990, P = 0.021). The contribution of TNFSF15 variants in Korean UC patients overall is much lower than in comparison with studies of CD (for rs3810936 aOR = 2.81, 95 % CI = 1.94-4.07, P = 4.4 x 10-8, for rs7848647 aOR = 3.49, 95 % CI = 2.42-5.03, P = 2.2 x 10-11 respectively). Our results of no association of IL23R with UC in Koreans do not support the previous Caucasian reports of shared association of the IL23R gene between CD and UC.

## 936/W/Poster Board #594

**Replication of markers from recent inflammatory bowel disease genome wide association scans in a Lithuanian cohort.** *J. Sventoraityte[1], A. Zvirbliene[1], L. Kupcinskas[1], G. Kiudelis[1], S. Schreiber[2], A. Franke[2].* 1) Dept. Gastroenterology, Kaunas University of Medicine, Kaunas, Lithuania; 2) Institute for Clinical Molecular Biology, Christian-Albrechts University, Kiel, Germany.

**INTRODUCTION.** Inflammatory bowel disease (IBD), a chronic inflammatory disorder affecting the intestinal mucosa, most commonly presents as either one of the two subtypes, ulcerative colitis (UC) or Crohn disease (CD). The genetic variations conferring susceptibility to IBD has increased substantially over the past few years after the introduction of genome-wide association scans (GWAS). Although several susceptibilty loci have been replicated, some of the associations have been reported to be unique for certain ethnicities. **AIM.** To perform a comprehensive association analysis of genetic markers reported by the two previous GWAS studies [1,2] to further characterize the CD and UC associations in a Lithuanian case-control sample set. To our knowledge, this is the first case-control study using an Eastern-European panel of IBD patients and healthy controls. **MATERIAL AND METHODS.** A set of 43 SNPs from three different categories were selected: (1) the 6 SNPs reported by Rioux *et al.*; (2) the 12 'non-converging' CD SNPs from Parkes *et al.* and (3) 25 SNPs - markers 'converging' between CD and nonautoimmune disease cases of the same study. They were genotyped in a cohort of 152 UC (mean age: 41.80±17.07) and 73 CD (mean age: 35.72±15.38) patients plus 249 unrelated healthy controls (mean age: 43.30±12.39) using ligation-based SNPlex genotyping (Applied Biosystems, USA) technology. The SNPs were quality-controlled for: genotyping success rate (>90%), allele frequency (>1% in healthy controls), deviation from Hardy-Weinberg equilibrium (pHWE>0.01 in the control sample). Assessment of all SNPs and single-marker association analyses were performed using the software program Haploview 4.0. **RESULTS.** Single-marker analyses revealed marginal associations between IBD and genetic variants from category (2) and (3): CD and rs17419032 (1q32.1; p=$4.96x10^{-2}$) and rs9993022 (4q13.1; p=$4.78x10^{-2}$); UC and rs10883365 (*NKX2-3*; p=$2.67x10^{-2}$), rs17419032 (1q32.1; p=$3.44x10^{-2}$), rs12529198 (*LYRM4*; p=$3.22x10^{-2}$, OR=0.37 (95%C.I.:0.15-0.93)) and rs9895062 (*STX8*; p=$9.64x10^{-3}$, OR=0.34 (95%C.I.:0.16-0.78)). **CONCLUSION.** We replicated genetic associations for CD with 4q13.1, UC with *NKX2-3*, *LYRM4*, *STX8*, both subtypes with 1q32.1. However, the lack of non-replication for the other loci is probably due to the small sample size and the lack of statistical power. **REFERENCES.** 1. Rioux, J.D. *et al.* Nat. Genet.39,596-604 (2007). 2. Parkes, M. *et al.* Nat. Genet.39,830-832 (2007).

## 937/W/Poster Board #595

**Positive association of genetic variants in the upstream region of *NKX2-3* with Crohn's disease in Japanese patients.** *K. Yamazaki[1], A. Takahashi[2], M. Takazoe[3], N. Kamatani[2], Y. Nakamura[4, 5], A. Hata[6], M. Kubo[1].* 1) Lab for Genotyping Development, RIKEN, Center for Genomic Medicine, Yokohama, Japan; 2) Laboratory for Statistical Analysis, RIKEN, Center for Genomic Medicine, Yokohama, Japan; 3) Department of Medicine, Division of Gastroenterology, Social Insurance Chuo General Hospital, Tokyo, Japan; 4) Laboratory of Molecular Medicine, Institute of Medical Science, The University of Tokyo, Japan; 5) Center for Genomic Medicine, Yokohama, Japan; 6) Department of Public Health, Chiba University Graduate School of Medicine, Chiba, Japan.

A number of genome-wide association (GWA) studies have been performed as a robust means of identifying susceptibility loci for Crohn's disease (CD). The loci detected after the completion of the HapMap project are quite concordant among these studies, suggesting that the results are reliable. Recently, the Wellcome Trust Case Control Consortium (WTCCC) reported the primary scanning of 17,000 individuals for seven diseases, including CD, and a subsequent study has validated these susceptible genetic variants in independent UK sample sets. To study the possible association of the variants reported by WTCCC with CD in a Japanese population, a total of 484 patients with Crohn's disease and 470 healthy controls were examined. Seventeen genetic variants at eight newly identified loci, including *IRGM*, *NKX2-3* and *PTPN2*, were genotyped using the TaqMan assay or the invader assay. We detected positive association signal presumably common to different ethnic groups for rs10883365 in the upstream region of *NKX2-3* (p = 0.019 under the genotypic model, p = 0.0065 under the allelic model, p = 0.019 under the recessive model, p = 0.036 under the dominant model). In addition to rs10883365 , marginal associations for two SNPs were detected in Japanese population; rs6887695 near *IL12B* and rs10761659 on 10q21. Further genotype-phenotype analysis found a significant association between rs6887695 and pure ileal CD patients. Our results indicate that the three loci are possible candidates for conferring susceptibility to Crohn's disease in people of different ethnicities.

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## 938/W/Poster Board #596

**Transferability and Fine-Mapping of Genome-wide Associated Loci for Adult Height across Human Populations.** *D. Shriner[1], A. Adeyemo[1], N. Gerry[2], A. Herbert[3], G. Chen[1], A. Doumatey[1], H. Huang[1], J. Zhou[1], M. Christman[2], C. Rotimi[1].* 1) National Institutes of Health, Bethesda, MD; 2) Coriell Institute for Medical Research, Camden, NJ; 3) Boston University School of Medicine, Boston, MA.

Human height is the prototypical polygenic quantitative trait. Recently, several genetic variants influencing adult height were identified, primarily in individuals of East Asian (Chinese Han or Korean) or European ancestry. Here, we examined 152 previously identified genetic variants influencing adult height for replication in a sample of 1,016 unrelated African Americans. In a well-powered replication analysis, nine genetic associations originally identified in individuals of East Asian or European ancestry replicated ($p$-values $\leq 0.05$ under an additive genetic model with directionally consistent effects) in our African American sample. Association $z$-scores, scaled for differences in sample sizes, were an average of 7.4-fold smaller in magnitude in our African American sample compared to the original East Asian or European samples. Power analysis indicated that our sample size was sufficient to overcome most differences in allele frequencies as well as most differences in linkage disequilibrium patterns. Effect size estimates were directionally consistent for 90% of associations with replication $p$-values $\leq 0.05$. In contrast, directional consistency was observed for 57% of associations with replication $p$-values $> 0.05$, consistent with the null hypothesis of sampling variability ($p = 0.23$). Our findings suggest that most previously reported associations for adult height are likely to be population-specific. Only one association transferred across all three groups, implying substantial heterogeneity across human populations among genetic variants explaining heritability for adult height. Using differences in linkage disequilibrium patterns between HapMap CEU or CHB reference data and our African American sample, we fine-mapped eight independent height loci, improving both the localization and the annotation of these transferable associations.

## 939/W/Poster Board #597

**Common variants in the *MEIS1*, *BTBD9*, and *MAP2K5/LBXCOR1* gene regions are major risk factors for Restless Legs Syndrome and Periodic Leg Movements during Sleep.** *L. Xiong[1], M.-P. Dubé[2], C. Gaspar[1], Y. Feroz Zada[2], G. Asselin[2], A. Levchenko[1], P. Thibodeau[1], J.-B. Rivière[1], J. St-Onge[1], G. Turecki[3], A. Desautels[1,4], J.Y. Montplaisir[4], G.A. Rouleau[1].* 1) Center of Excellence in Neuromics, CHUM Research Center, University of Montreal, Montréal, QC, Canada; 2) Research Centre, Montreal Heart Institute, University of Montreal, Montréal, QC, Canada; 3) Research Center, Douglas Hospital, McGill University, Montréal, QC, Canada; 4) Centre d'étude du sommeil, Hôpitall du Sacré-Cœur de Montréal and Centre de recherche en sciences neurologiques, Université de Montréal, QC, Canada.

Restless legs syndrome (RLS) and periodic leg movements during sleep (PLMS) are two largely overlapping common neurological phenotypes. Family and twin studies have indicated a significant genetic contribution to RLS. Recently, genome-wide association studies (GWAS) have identified three genomic regions associated with RLS or PLMS, i.e., the *MEIS1* gene, the *BTBD9* gene, and the overlapped regions between the *MAP2K5* and *LBXCOR1* genes on chromosomes 2p, 6p, and 15q, respectively. We carried out confirmation studies of 14 selected associated markers from these three regions in an enlarged French-Canadian (FC) case-control sample (281 cases and 803 controls). We also performed family-based association and linkage analyses in 116 pedigrees with RLS. The case-control samples showed significant association with all markers tested. Two markers (rs12469063 and rs2300478) from the *MEIS1* gene showed significant association, with P values of $4.97 \times 10^{-8}$ (allelic OR: 1.78; 95% CI: 1.45-2.19) and $3.24 \times 10^{-7}$ (allelic OR: 1.71; 95% CI: 1.40-2.09), respectively. The OR of the homozygous GG genotype for marker rs12469063 was 3.46 (95% CI: 2.12-5.60) when compared to the wild-type AA genotype. Among the five SNPs in the *BTBD9* region, only rs3923809 showed significant association with RLS (P = 0.0008; allelic OR: 1.46; 95% CI: 1.16-1.82), with the homozygous OR of AA versus GG being 2.28 (95% CI: 1.21-4.62). All seven markers from the *MAP2K5/LBXCOR1* region showed significant association with RLS, with ORs of 1.40-1.50, and with higher OR for the homozygous genotype of the risk allele for each marker (OR: 1.79-1.90), withe the exception of rs4489954. The concordant significant associations for the RLS, PLMS, and RLS/PLMS phenotypes in the FC case-control sample at these three loci respectively confirmed the highly correlated phenotypes and indicate shared pathways. Family-based analyses also showed excessive transmission of the associated alleles to the affected individuals and excessive allele-sharing among the affected siblings at the *MEIS1* and *MAP2K5/LBXCOR1* loci. The estimated population attributable fraction (PAF) of the risk alleles in the FC population was 18% at the *MEIS1* locus, 25% at the *BTBD9* locus, and 25% for the *MAKP2K5/LBXCOR1* locus. The joint PAF was estimated as 54%. Therefore, common variants in the *MEIS1*, *BTBD9*, and *MAP2K5/LBXCOR1* regions are major risk factors for both sporadic and familial RLS.

## 940/W/Poster Board #598

**Replication of association of *ZBTB38* variants with height and subsequent fine mapping in independent adult and adolescent Caucasian populations.** *A.E. Duncan[1], C.A. Olsson[2,3,4], G.C. Patton[2,3], S.B. Harrap[1], J.A. Ellis[1,2].* 1) Department of Physiology, University of Melbourne, Melbourne, Australia; 2) Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Australia; 3) Department of Paediatrics, University of Melbourne, Melbourne, Australia; 4) Department of Psychology, University of Melbourne, Melbourne, Australia.

Adult height is a polygenic trait with heritability estimated to be around 80%. Recent genome-wide association (GWA) studies of adult height have implicated numerous genes. SNPs in one such gene, *ZBTB38* (zinc finger and BTB domain containing 38) located on chromosome 3q23, were identified by three separate, simultaneously published, GWA studies. ZBTB38 appears to bind methylated DNA and repress transcription, although its exact function has yet to be determined. Here, we report on the replication and finer-scale mapping of the association of *ZBTB38* SNPs with height in two independent Australian Caucasian populations, the Victorian Family Heart Study (VFHS, 2876 family-based adult samples) and the Victorian Adolescent Health Cohort Study (VAHCS, 1001 unrelated samples with height measured at eight separate time points from age 15 years to adulthood). SNPs studied included those most strongly implicated by previous GWA studies (rs6440003, rs6763931 and rs724016), those in linkage disequilibrium ($r^2 \geq 0.5$) with these SNPs in the Caucasian population based on HapMap data, and those in close physical proximity based on dbSNP data. SNPs were genotyped using the Sequenom MassARRAY platform and stringent QC was applied to the data. Results for a total of 38 SNPs were analysed using PLINK or SOLAR, adjusting for age and sex. In the VFHS, SNP rs6763931 (chr3: 142585523, intron 4) was the most significantly associated with height ($p = 4.5 \times 10^{-6}$) when testing for total evidence of association. In the VAHCS, rs6440003 (chr3: 142576899, intron 3) was the most significantly associated ($p = 2.4 \times 10^{-7}$), although this SNP is in strong LD with rs6763931 ($r^2 = 0.99$ in the VFHS). The association with height was consistent across the adolescent time points of the VAHCS, suggesting that this gene has an effect on growth that occurs prior to age 15 years. The functionality of the associated SNPs is unknown. However there was some evidence that haplotypes of SNPs were more strongly associated with height than individual SNPs, suggesting that these haplotypes could be better tagging other functional SNPs in the region than single SNPs alone. In conclusion, we have replicated the GWA studies' findings of association of SNPs around *ZBTB38* in two independent populations. The effect of this gene on growth appears to occur at an early age. The functional variant(s) are yet to be identified.

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## 941/W/Poster Board #599

**_ITGAM_ is more strongly associated to discoid lupus erythematosus than systemic disease in Finnish patients.** *T.M. Järvinen[1,2,3,4], A. Hellquist[4], S. Koskenmies[5], J. Panelius[5], T. Hasan[6], H. Julkunen[7], J. Kere[2,3,4], U. Saarialho-Kere[1,8].* 1) Department of Dermatology, University of Helsinki, Helsinki, Finland; 2) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 3) Folkhälsan Institute of Genetics, Helsinki, Finland; 4) Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden; 5) Department of Dermatology, Helsinki University Central Hospital, Helsinki, Finland; 6) Department of Dermatology, University of Tampere and Tampere University Hospital, Tampere, Finland; 7) Peijas Hospital, Helsinki University Central Hospital, Vantaa, Finland; 8) Section of Dermatology and Department of of Clinical Science and Education, Karolinska Institutet at Stockholm Söder Hospital, Stockholm, Sweden.

Lupus erythematosus (LE) is a heterogeneous autoimmune disease with abnormal immune responses including production of autoantibodies and immune complexes. We have recruited a population-based cohort representing different subphenotypes of LE: 177 discoid (DLE), 42 subacute cutaneous (SCLE), 275 systemic (SLE) patients and 356 control individuals from Finland. To test whether these different subphenotypes possess similar genetic predisposition, 47 SNPs in 16 genes with strongest evidence of susceptibility for SLE implicated in previous genome-wide association studies were investigated. The samples were genotyped using the Sequenom iPLEX Gold chemistry and association analyses were performed with HaploView 4.0. As a novel finding, *ITGAM* marker rs1143679 showed strongly increased risk for DLE ($p=4.7 \times 10^{-11}$, OR=3.2 [95% CI=2.2-4.6]) compared to SLE ($p=8.3 \times 10^{-6}$, OR=2.1 [95% CI=1.5-3.0]). Also other markers in *ITGAM* were significantly associated to DLE (p-value range from $10^{-11}$ to $10^{-4}$, OR-range from 1.6 to 3.1). Interestingly, a modest association between *ITGAM* and SLE patients with malar and/or discoid lesions was observed (p=0.05). In addition, *BLK/C8orf13* gene region on chromosome 8p was associated more strongly to DLE ($p=2.0 \times 10^{-4}$, OR=1.7 [95% CI=1.3-2.3]) than SLE ($p=1.5 \times 10^{-3}$, OR=1.5 [95% CI=1.2-2.0]). A known SLE susceptibility gene *STAT4* showed significant association to SLE ($p=1.1 \times 10^{-6}$, OR=1.9 [95% CI=1.5-2.5]), whereas only moderate association was observed in DLE ($p= 4.4 \times 10^{-2}$, OR=1.4 [95% CI=1.0-1.9]). We conclude that different subphenotypes of LE share much the same genetic predisposition. The observed association between skin manifestations and *ITGAM* may implicate that abnormal function of this protein is a hallmark of more severe disease progression towards SLE in patients with cutaneous LE. Furthermore, *ITGAM* is involved in inflammation and complement system, both showing defects in LE patients. We are currently replicating these results in Swedish patients with LE or primary Sjögren Syndrome and positive for Ro/SSA antibody.

## 942/W/Poster Board #600

**A common variant in BMP2 modulates severity of the iron burden in HFE p.C282Y homozygous patients: a follow-up study.** *G. LeGac[1], J. Milet[2], V. Scotet[1], I. Gourlaouen[1], C. Thèze[1], J. Mosser[3], C. Bourgain[4], Y. Deugnier[5].* 1) Inserm U613, EFS-Bretagne, Centre Hospitalier Universitaire; Brest, France; 2) IRD - UR010; Paris, France; 3) Medical Genomics Unit, Pontchaillou Hospital and CNRS, UMPR6061, IFR 140, Université Rennes, France; 4) Inserm U535 and Univ. Paris-SUD; Villejuif, France; 5) Center of Clinal Investigation (CIC INSERM 0203) and Service des Maladies du Foie; Rennes, France.

It is now generally admitted that penetrance of the common HFE p.C282Y/p.C282Y genotype is incomplete, and identification of modifier genes is the concern of a growing number of research projects. We recently identified a significant association between pretherapeutic serum ferritin level and the common rs235756 single nucleotide polymorphism (SNP) of the BMP2 gene region. Our results further suggested an interactive effect between the BMP2 rs235756 SNP and the rs16827043 SNP in HJV, with a small additive effect of the rs4901474 SNP in BMP4. The present study has been designed as a replication study in an independent cohort of 450 HFE p.C282Y homozygous patients from a nearby French region (Brittany). Information on individual alcohol consumption and amount of iron removed by phlebotomy being available for a substantial part of this cohort, additional analyses were conducted. Although we failed to replicate results of the initial study, we argue that, altogether, our results help to consider genes involved in regulation of hepcidin synthesis as potential modifiers of the p.C282Y/pC282Y genotype expression and especially BMP2.

## 943/W/Poster Board #601

**Fine-mapping the _MAPT_ H1 haplotype clade in Parkinson's disease.** *I. Mata[1], A. Samii[2], S. Factor[3], D. Higgins[4], A. Griffith[5], J. Roberts[6], H. Kim[2], P. Agarwal[5], D. Yearout[1], C. Hutter[7], K. Edwards[8], D. Kay[9], H. Payami[9], C. Zabetian[1,2].* 1) Dept Neuroscience, VA Puget Sound Healthcare Sys, Seattle, WA; 2) Dept of Neurology,University of Washington, Seattle, WA; 3) Dept. of Neurology, Emory University School of Medicine, Atlanta, GA; 4) Parkinson's Disease and Movement Disorder Clinic, Albany Medical Center, Albany, NY; 5) Booth Gardner Parkinson's Care Center, Evergreen Hospital Medical Center, Kirkland, WA; 6) VA Puget Sound Health Care System, Virginia Mason Medical Center , Seattle, WA; 7) Fred Hutchinson Cancer Research Center, Seattle, WA; 8) Dept. of Epidemiology, University of Washington, Seattle, WA; 9) Division of Genetic Disorders, Wadsworth Center, New York State Department of Health, Albany, NY.

Background: An inversion polymorphism of ~900kb on chromosome 17q21, which includes the microtubule associated protein tau (*MAPT*) gene defines two haplotype clades, H1 and H2. The H1 clade has consistently been demonstrated to associate with Parkinson's disease (PD) and the tauopathies. While putative functional risk variants within H1 have been nominated for the tauopathies, none have been identified for PD. Methods: We fine-mapped the H1 clade in a large case-control sample of white individuals from the NeuroGenetics Research Consortium using a two-tiered approach. In Tier 1 (n=692 cases and 692 controls), we genotyped a comprehensive set of 87 tagging single nucleotide polymorphisms (tagSNPs) selected from the International HapMap Project CEU panel. SNPs associated with PD under an additive model ($\alpha=0.05$) in Tier 1 were then validated in Tier 2 (n=1,277 cases and 1,422 controls). For comparison with the literature, we genotyped three additional SNPs reported to associate with the tauopathies in both Tiers 1 and 2. To account for the effect of the H1 versus H2 clade, analyses were restricted to individuals of the H1/H1 diplotype. Results: As expected, there was a significant excess of the H1/H1 diplotype within the Tier 1 (p=2.2 x 10-3), Tier 2 (p=8.9 x 10-5), and combined PD groups (p=7.2 x 10-7). Among H1/H1 individuals in Tier 1, nine tagSNPs (six within *MAPT*) were marginally associated with PD, with p values ranging between 0.01-0.05. However, none of these nine tagSNPs was replicated in Tier 2 after adjustment for age and sex. Finally, none of three tauopathy-related SNPs was associated with PD in Tier 1 or Tier 2. Conclusions: Our data suggest that the MAPT variants that convey disease risk are distinct between PD and the tauopathies. However, the identification of the true risk allele(s) for PD remains to be determined. We are now seeking to address the problem using deep resequencing of the *MAPT* region as an alternate strategy to discovery potential risk variants of low frequency.

## 944/W/Poster Board #602

**A population specific susceptibility and genotype risk to Crohn's disease and ulcerative colitis.** *S. Nakagome[1], Y. Takeyama[2], S. Mano[3], S. Sakisaka[2], T. Matsui[4], S. Kawamura[1], H. Oota[1].* 1) Department of Integrated Biosciences, University of Tokyo, Kashiwa, Chiba, Japan; 2) Department of Gastroenterology and Medicine, Fukuoka University Faculty of Medicine, Fukuoka, Japan; 3) Graduate School of Natural Sciences, Nagoya City University, Nagoya, Japan; 4) Department of Gastroenterology, Fukuoka University Chikushi Hospital, Fukuoka, Japan.

Inflammatory bowel disease (IBD) is a chronic inflammation in the digestive tract. The Crohn's disease (CD) and the ulcerative colitis (UC) are named generically as IBD. IBD is common in Europe, but not in East Asia. However, the prevalence rate of CD is increasing in East Asian in the last 20 years. Recent genome-wide association studies (GWAS) have identified more than 30 CD-associated genomic loci in Europeans. Risk alleles in some loci (*IL23R*, *NKX2-3*, and *MST1*) are reported to be associated also with UC in Europeans. Regarding these loci, however, no clear association has been shown in East Asians, except *TNFSF15*. Furthermore, little is known whether the IBD-causative alleles identified in Europeans work dominantly or recessively in any population. To clarify whether there is any population-specific susceptibility and genotype risk, we conducted genotyping for 212 IBD patients and 168 controls from Kyushu, Japan, of the seven loci (*NOD2*, *IL23R*, *ATG16L1*, *TNFSF15*, 5q31, *IRGM*, 10q21) that are shown associated with CD in Europeans by multiple studies. We confirmed that *TNFSF15* is associated with CD also in Japanese. We found that this gene is also associated with UC, which has not been reported for Europeans. By the modified genotype relative risk (mGRR) test we showed that the risk variant of TNFSF15 works dominantly in CD whereas it works recessively in UC. These results suggest that susceptibility and genotype risk to multifactorial diseases, such as IBD, differs among populations.

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## 945/W/Poster Board #603

**Association between a functional polymorphism of *ITPKC* gene and Kawasaki disease: a replication study.** *Y. Onouchi[1], K. Ozaki[1], H. Suzuki[2], M. Terai[3], Y. Suzuki[4], A. Hata[4], Y. Nakamura[5,6], T. Tanaka[1].* 1) Lab. Cardiovascular Diseases, Center for Genomic Medicine RIKEN, Yokohama, Japan; 2) Dept. Pediatrics, Wakayama Medical Univ., Wakayama, Japan; 3) Dept. Pediatrics, Tokyo Women's Medical Univ. Yachiyo Medical Center, Yachiyo, Japan; 4) Dept. Public Health, Graduate School of Medicine, Chiba Univ., Chiba, Japan; 5) Lab. Molecular Medicine, Human Genome Center, Institute of Medical Science, the Univ. of Tokyo, Tokyo, Japan; 6) Director, Center for Genomic Medicine RIKEN, Yokohama, Japan.

Kawasaki disease (KD) is an acute systemic vasculitis syndrome of infants and young children. Recently we reported that a single nucleotide polymorphism (SNP) located in intron 1 of inositol 1,4,5-trisphosphate 3-kinase C (*ITPKC*) gene was associated with KD and the risk for formation of coronary artery lesions (CALs) both in Japanese and Caucasian populations. The SNP (rs28493229) was also associated with the risk for unresponsiveness to intravenous immunoglobulin (IVIG) therapy in Caucasian KD patients. However, the findings have not been replicated to date and the association between the SNP and unresponsiveness to IVIG therapy has not been investigated in the Japanese KD patients yet. We recruited 282 Japanese KD patients and 373 ethnically matched controls independent from those in the original report and performed an association study. In this new case-control panel, the C allele of rs28493229 was significantly associated with KD again (OR = 1.60, 95%CI 1.15 - 2.21, $P$ = 0.0047 in dominant model). When combined with the data in the original report (637 KD patients vs. 1034 controls; OR = 1.89, 95%CI 1.53 - 2.33, $P = 2.2 \times 10^{-9}$ in dominant model) using the Mantel-Haenszel method, the pooled OR was 1.80 (1.51 - 2.14) and the $P$ value was $5.5 \times 10^{-11}$. In addition, among the KD patients who received a single dose of 2g/kg or 1g/kg daily for 2 days of IVIG in the acute phase (n = 122), the C allele was significantly overrepresented in patients subgroup who responded poorly to the therapy (n = 34, OR = 2.76, 95%CI 1.23 - 6.23, $P$ = 0.013 in dominant model) and was marginally associated with CALs formation (n = 14, OR = 2.94, 95%CI 0.92 - 9.39, $P$ = 0.059 in dominant model). These data indicated that the C allele of rs28493229 truly confers susceptibility to KD and has the potential to be a tool for personalized medicine predicting patients who will experience severe clinical course and treating them with alternative or additional therapy before they are destined to develop CALs.

## 946/W/Poster Board #604

**Association analyses between 9 loci and metabolic traits in Chinese Han population.** *Y. Liu[1,3], DZ. Zhou[2], D. Zhang[2], Z. Zhang[2], L. He[1,2,3].* 1) Institutes Of Biomedical Scien, Fudan university, Shanghai, China; 2) Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China; 3) Bio-X Center, Shanghai Jiao Tong University, Shanghai, China.

Aims/hypothesis: Recently, genome-wide association studies had been performed in the genetics studies of plasma lipid level, and identified several common variants associated with LDL-C, HDL-C and triglycerides, including some new polymorphisms. The aim of this study was to replicate these associations in Han Chinese individuals. Methods: In the present study, nine SNPs in nine loci were genotyped in a Chinese Han population (n= 3,953), the associations of these SNPs with metabolic traits were assessed by linear regression adjusted for age, gender, diabetes status and body mass index. Results: Four variants (rs780094, p=4.43×10-11 ;rs17145738, p=4.28×10-7; rs439401, p=3.47×10-5;, rs326, p=1.51×10-6;) show strong evidence for association with TG after multiple testing, four variants (rs3764261, p=4.67×10-14; rs1800588, p=1.93×10-7; rs17145738, p= 1.90×10-4; rs326, p=1.14×10-3;) show strong evidence for association with HDL-C after multiple testing, two variants (rs3764261, p=6.24×10-7; rs12654264, p=1.57×10-6;) show strong evidence for association with total cholesterol after multiple testing, one variants (rs12654264 p=1.92×10-5) show strong evidence for association with LDL-C after multiple testing. Conclusions: Our findings indicate that these variants associated with metabolic traits in Europeans may also play a role in Chinese Han population.

## 947/W/Poster Board #605

**Variants in the TNFAIP3 locus form Three Risk Haplotypes Associated with SLE in Koreans.** *J.S. Bates[1], C.J. Lessard[1,2], S.C. Bae[3], Y.W. Song[4], B.P. Tsao[5], L.J. Battiest[1], T. Nguyen[1], J. Rodgers[1], A. Adler[1], K.M. Kaufman[1,2], J.A. Kelly[1], J.B. Harley[1,2], C. Gray-McGuire[1], K.L. Moser[1], P.M. Gaffney[1].* 1) Oklahoma Medical Research Foundation, Oklahoma City, OK, OK; 2) University of Oklahoma Health Sciences Center, Oklahoma City, OK; 3) Hanyang University, Seoul, Republic of Korea; 4) Seoul National University, Seoul, Republic of Korea; 5) University of California- Los Angeles, Los Angeles, CA.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by acute and chronic inflammation in multiple tissues and often leads to organ malfunction and failure. Variants in tumor necrosis factor alpha interacting protein 3 (TNFAIP3) have recently been associated with SLE and rheumatoid arthritis risk in Europeans. TNFAIP3 encodes A20, a zinc-finger protein with dual ubiquitin and de-ubiquitin properties required for efficient termination of the NF-κB signaling axis. Variants in TNFAIP3 (n = 22) were assessed for association with SLE in Koreans using 821 cases and 1008 controls in PLINK. Genotyping was performed using the Illumina GoldenGate chemistry. Association of SLE risk with the TNFAIP3 locus in Koreans was observed in 13 variants ($OR$ = 1.78-2.32, $P < 1 \times 10^{-4}$). Eight haplotypes (MHF > 0.5%) in the TNFAIP3 locus were formed with greater than 95% confidence per individual using DECIPHER in SAGE from 20 observed variants and evaluated for SLE risk using the LOGISTIC procedure in SAS. Three risk haplotypes explained 98.8% of SLE risk in the TNFAIP3 locus and each contained a primary risk block marked by minor alleles from rs5029937, rs5029939, rs2230926, rs610604, rs7752903, rs9494894, rs7749323, and rs6932056. The most strongly associated risk haplotype was further marked by the minor allele at rs9494883 ($OR$ = 3.35, $P = 3.4 \times 10^{-7}$). The second most strongly associated haplotype was further marked by the major allele at rs10499194 and the minor allele at rs10499197 ($OR$ = 2.27, $P = 8.9 \times 10^{-3}$). The third risk haplotype, further marked by the minor allele at rs10499194, was independent from the major frequency non-risk haplotype ($OR$ = 1.80, P = 0.005) and the most strongly associated risk haplotype ($OR$ = 0.54, $P$ = 0.04). Our results suggest variants in TNFAIP3 form three risk haplotypes associated with SLE risk in Koreans and that two risk haplotypes are independent of one another, suggesting multiple causal variants in the TNFAIP3 locus may exist.

## 948/W/Poster Board #606

**The Protein Kinase C Alpha (PRKCA) gene is associated with multiple sclerosis in the Italian population.** *R. Asselta[1], E.M. Paraboschi[1], G. Soldà[1], C. Dall'Osso[1], D. Gemmati[2], P. Zamboni[3], M.D. Benedetti[4], S. D'Alfonso[5], S. Duga[1].* 1) Dipartimento di Biologia e Genetica per le Scienze Mediche, Università degli Studi di Milano, Milano, Italia; 2) Center Hemostasis and Thrombosis, University of Ferrara, Ferrara, Italy; 3) Vascular Diseases Center, University of Ferrara, Ferrara, Italy; 4) Department of Neurological and Vision Sciences, Section of Clinical Neurology, University of Verona, Verona, Italy; 5) Department of Medical Sciences and IRCAD, Eastern Piedmont University, Novara, Italy.

Multiple sclerosis (MS) is a chronic neurological disorder characterized by multicentric inflammation, demyelination, and axonal damage. The Protein Kinase C Alpha (PRKCA) gene was found to be associated with MS in Finnish, Canadian, and UK populations, in which specific risk haplotypes were identified. Moreover, PRKCA transcript levels were shown to be higher in CD4- mononuclear cells of MS patients carrying the risk haplotypes, suggesting a contribution of PRKCA regulatory mechanisms in the pathogenesis of MS. In this study, we analyzed the role of PRKCA in MS susceptibility in a cohort of 358 cases and 662 controls from continental Italy. An association analysis was performed genotyping 3 microsatellites and 20 single nucleotide polymorphisms (SNPs) covering the whole gene. A significant association with 2 microsatellites, mapping respectively in the promoter region and in intron 2, was found (P=0.032 and P=0.027). The 5' regulatory region of the PRKCA gene was hence further investigated by sequencing the first 424 bp of the promoter and the entire exon 1 in all MS cases and controls. However, no genetic variants specific for MS cases were identified. Analysis of individual SNPs did not show any evidence for association; however, a haplotype of 7 SNPs, spanning a genomic region of 43 kb of PRKCA intron 3, resulted strongly associated with the disease status (P= 7.4*10-4; OR=1.54, 95% CI=1.22-1.95). This haplotype, which partially overlaps the risk haplotypes observed both in Finns and Canadians, includes an alternative exon characterizing a shorter PRKCA protein isoform. Since no information were available on the tissue distribution of this isoform, its expression pattern was analyzed and compared to the expression profile of PRKCA in a panel of 20 human tissues by RT-PCR: the shorter isoform showed a more marked tissue-specific expression. The possible differential expression of the 2 transcripts between MS patients and controls is currently underway by qRT-PCR on RNA extracted from peripheral blood mononuclear cells. In conclusion, our study provides a strong replication evidence that haplotypes characterizing PRKCA increase the risk of MS; a metanalysis including all studies investigating the role of PRKCA as a MS susceptibility gene reported so far showed the considerable OR of 1.52 (95% CI=1.36-1.69; P=2.2*10-14).

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## 949/W/Poster Board #607

**Influence of the HLA-DRB1*15 allele on Multiple Sclerosis in a Colombian population.** *M.C. Lattig[1], C. Perea[1], J.M. Gonzalez[2], H. Groot[1], D. Vela-Duarte[3], J. Toro[2,3].* 1) Laboratorio de Genetica Humana, Universidad de los Andes, Bogota - Colombia; 2) Facultad de Medicina, Universidad de los Andes, Bogota - Colombia; 3) Grupo Esclerosis Multiple Fundacion Santa Fe Bogota - Colombia.

Multiple Sclerosis (MS [MIM 126200]), a demyelinating disease of the central nervous system, varies in prevalence depending on the geographic region where the disease is encountered. A low MS prevalence has been reported in tropical regions of the world. In Bogota - Colombia, an area of defined low risk for the disease, an MS prevalence rate of 4,41/100,000 was previously reported by our group. Genetic factors including (HLA) class II genes such as (HLA)-DRB1*15 play a role in the susceptibility to multiple sclerosis, especially in Northern European populations. To determine the contribution of the (HLA)-DRB1*15 allele in the Colombian population we performed a case-control study involving 53 MS patients collected following the McDonald criteria and 51 matched controls observing that the allele was present in similar frequencies among patients and controls [odds ratio (OR) = 1.47; 95% CI = 0.59 - 3.70; p = 0.49]. The clinical course and symptoms of MS vary considerably between patients. Among the affected individuals we analyzed, the HLA DR15 contribution to various clinical phenotypes showed no evidence that the allele has an influence on the clinical course of MS. In summary, the contribution of the (HLA)-DRB1*15 allele appears to be less significant in the low risk zone known to have a high admixture component such as Bogota - Colombia.

## 950/W/Poster Board #608

**Association between insulin-like growth factor 2 gene polymorphism and normal tension glaucoma in the Japanese population.** *F. Mabuchi[1], Y. Sakurada[1], K. Kashiwagi[1], Z. Yamagata[2], H. Iijima[1], S. Tsukahara[1].* 1) Dept Ophthalmology, Univ Yamanashi, Chuo, Yamanashi, Japan; 2) Dept Health Sciences, Univ Yamanashi, Chuo, Yamanashi, Japan.

Purpose: Previous study reported that the insulin-like growth factor 2 (*IGF2*) gene polymorphism (rs680) was associated with primary open angle glaucoma (POAG) in the Chinese population. However, there have been no studies in the other ethnic populations. POAG is clinically classified into high tension glaucoma, in which elevated intraocular pressure is a major feature, and normal tension glaucoma (NTG), in which the intraocular pressures are consistently within the statistically normal population range. POAG is a complex and genetically heterogeneous disease characterized by the progressive apoptotic death of retinal ganglion cell, and apoptosis-related genes like *IGF2* seem to possibly be associated with POAG, especially NTG. We thus assessed whether the *IGF2* gene polymorphism was associated with NTG in the Japanese population. Methods: One hundred Japanese patients with NTG and 100 control subjects were analyzed for the *IGF2* gene polymorphism (rs680) using an allele specific primer PCR technique, and the genotype and allele frequencies were compared between the NTG patients and control subjects. The mean age at the time of blood sampling was 63.7 ± 13.5 years (mean ± SD) in patients with NTG and 65.5 ± 11.5 years in the control subjects. Results: No significant difference was observed (P = 0.98, Chi-square test) regarding the *IGF2* genotype between the NTG patients (GG: 36%, GA: 42%, AA: 22%) and the control subjects (GG: 37%, GA: 42%, AA: 21%). Additionally, there was no significant difference (P = 0.89, Fisher's exact test) in the frequencies of the *IGF2* alleles between the NTG patients (G allele: 57%, A allele: 43%) and the control subjects (G allele: 58%, A allele: 42%). Conclusion: The *IGF2* gene polymorphism was not found to be associated with NTG in the Japanese population. Further studies in the different ethnic populations are desirable to elucidate the relationship between the *IGF2* gene and POAG, especially NTG.

## 951/W/Poster Board #609

**The role of genetically determined metabotypes in the genetics of complex traits and polygenic disorders.** *C. Gieger[1], T. Illig[1], G. Zhai[2], W. Römisch-Margl[3], R. Wang-Sattler[1], C. Prehn[4], E. Altmaier[3,5], G. Kastenmüller[3], B.S. Kato[2], N. Soranzo[2,6], H.-W. Mewes[3,7], T. Meitinger[8,9], M. Hrabé de Angelis[4,10], F. Kronenberg[11], H.E. Wichmann[1,12], T.D. Spector[2], J. Adamski[4,9], K. Suhre[3,5].* 1) Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany; 2) Department of Twin Research & Genetic Epidemiology, King's College London, UK; 3) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Neuherberg, Germany; 4) Institute of Experimental Genetics, Genome Analysis Center, Helmholtz Zentrum München, Neuherberg, Germany; 5) Faculty of Biology, Ludwig-Maximilians-Universität, Germany; 6) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton UK; 7) Department of Genome-oriented Bioinformatics, Life and Food Science Center Weihenstephan, Technische Universität München, Germany; 8) Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany; 9) Institute of Human Genetics, Klinikum rechts der Isar, Technische Universität München, Germany; 10) Institute of Experimental Genetics, Life and Food Science Center Weihenstephan, Technische Universität München, Germany; 11) Division of Genetic Epidemiology, Department of Medical Genetics, Molecular and Clinical Pharmacology, Innsbruck Medical University, 6020 Innsbruck, Austria; 12) Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-Universität, Munich, Germany.

Serum metabolite concentrations provide a direct readout of biological processes in the human body, and are associated with disorders such as cardiovascular and metabolic diseases. We have previously identified frequent genetic polymorphisms with large effects sizes that alter an individual's metabolic capacities (Gieger et al., PLoS Genetics, 2008). We argue that knowledge of these "genetically determined metabotypes" in the human population is key to identifying the contributions and interaction of genetic and environmental factors in the etiology of complex diseases. Here we present a genome-wide association study with 163 metabolic traits, covering a biologically relevant panel of amino acids, sugars, acylcarnitines, and phospholipids, using 1809 participants from the KORA population, which we replicated in the TwinsUK cohort with 422 participants. Most often, the genetic variant is located in or near enzyme or solute carrier coding genes, where the associating metabolic traits match the proteins' function, many of these polymorphisms being located in rate limiting steps of important enzymatic reactions. For several loci, an association with clinical endpoints has previously been reported, such as *SLC22A4* with Crohn's disease, *FADS1* with hyperactivity and cholesterol/triglyceride levels, and *ACADS* with susceptibility to ethylmalonic aciduria. Here we report association of these loci with different metabolic phenotypes and show that these allow deriving new functional information about the underlying pathophysiology. In summary, this study allowed us to draw a systemic perspective of the genetic variation that is found in human metabolism. In contrast to most GWAS with clinical endpoints, it appears that for metabolic traits most of the associations are linked to genetic variants in genes with a matching metabolic function. Our study shows the exciting potential of Metabolomics to unravel the genetics of complex traits and polygenic disorders.

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## 952/W/Poster Board #610

**Genome-wide transcriptomic and genomic analyses of Ashkenazi Parkinson's disease patients.** *A. Orr-Urtreger[1,3], A. Bar-Shira[1], E. Kenny[4], I. Pe'er[4], N. Giladi[2,3], M. Kedmi[1].* 1) Genetic Institute, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel; 2) Movement Disorders Unit, Parkinson Center, Department of Neurology, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel; 3) Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel; 4) Department of Computer Sciences Columbia University, New-York, USA.

Parkinson's disease (PD) is a complex disorder. Aging, environmental variables and multiple genetic factors are involved in its pathogenesis. However, a surprisingly high proportion of disease-associated mutations were detected in about a third of Ashkenazi patients in the *LRRK2* and *GBA* genes, rendering this population valuable for genetic studies aimed to understand PD pathogenesis in the world population-at-large. In order to define novel genes, genomic regions and cellular pathways associated with PD, combined genome-wide analyses of transcription, SNPs and CNVs were performed. RNA (n=118) and DNA (n=428) samples from peripheral blood leukocyte (PBL) of patients and controls were tested. Four study groups were analyzed: Ashkenazi patients with either *LRRK2* G2019S or *GBA* mutations, patients without these mutations and age- and sex-matched controls. Analysis of Affymetrix Exon 1.0 Array "core data" (21,980 genes and 232,448 exons) revealed 173 differentially expressed exons between all PD patient and controls ($P<0.01$ and Fold Change>1.5). These exons were incorporated into 80 genes; some of which showed alternative splicing. When comparing only two experimental groups, non-carrier PD-patients and controls, 206 differentially expressed exons ($P<0.01$ and Fold Change>1.5) were detected, that incorporated into 114 genes. Analysis of the genome-wide Affymetrix SNP6.0 Arrays demonstrated high quality data, with call rates above 97.8% and Identity-by-Descent of up to 4% sharing in the study population. This analysis confirmed *LRRK2* and *GBA* as two major loci associated with PD in Ashkenazi Jews, and further identified novel candidate PD-associated SNPs on chromosomes 14 and 20. The CNV analysis demonstrated 4715 gain and loss events in patients and 1880 in controls, revealing an increased CNV burden in PD patients, as measured by the number of CNVs in each individual ($P$=0.023). Additionally, 33 novel CNVs were shared by two or more individuals, 13 of them by patients only. Together, our results demonstrate the importance of the Ashkenazi PD-patients population for studying the complex genetic basis of PD. Results from the expression studies support PBL as a relevant surrogate tissue to identify transcriptional changes involved in PD pathogenesis, and the SNP and CNV data will hopefully help identify genes and genetic pathways associated with PD.

## 953/W/Poster Board #611

**GWAS of systemic lupus erythematosus on a Chinese population in Hong Kong.** *W. Yang, Y.L. Lau, Chinese Consortium on SLE Genetics.* Paediatrics & Adolescent Med, Univ Hong Kong, Hong Kong, Hong Kong.

SLE is a prototype autoimmune disease with strong genetic involvement and population differences in both clinical manifestations and genetic susceptibilities. In this study, we have genotyped 600 SLE patients of Chinese ethnicity residing in Hong Kong using Illumina 550K Beadchip, and analyzed the data against 900 controls matched with ethnicity and geography. We have analyzed the data using Plink and Eigenstrat to correct for potential subpopulation structures. About 50 SNPs were selected for replication based on their P value, gene ontology, relationship to expression of nearby genes, multiple independent alleles in a region, etc, using the remaining samples from Hong Kong as well as two other Chinese cohorts collected in Shanghai and Hefei, China, together with a cohort from Bangkok, Thailand. Our data confirmed association of some known susceptibility genes identified in the previous studies on populations of European ancestry, but also pointed out some differences in terms of effect sizes, allele frequency differences, and independently contributing alleles in genes such as STAT4, ITGAM, Bank1, BLK, TNFAIP3, and TNFSF4. We also report some novel findings that are different from previous Caucasian studies. The newly-discovered susceptibility genes for our Asian populations may reflect genes and alleles that are either population-specific in terms of disease association, or different effect sizes between different populations, explaining differences in the study power between our study and the previous ones. Our data concludes that due to the extreme heterogeneity of the disease, the high prevalence and more severe clinical manifestations of the disease in Asians, many more new susceptibility genes are going to be discovered despite previous efforts focusing on the Caucasian populations.

## 954/W/Poster Board #612

**Discovery of Genes Controlling Mono- and Polygenic Diseases in Mice: Genome-Wide Association Analysis of Serum-transfer Induced Arthritis.** *V.A. Adarichev[1,2], E. Kudryavtseva[1], T.S. Forde[1].* 1) Dept Medicine, Division of Rheumatology, Albert Einstein College Med, Bronx, NY; 2) Dept Microbiology & Immunology, Albert Einstein College Med, Bronx, NY.

Genome-wide association (GWA) analysis was proven to be an effective tool in human genetics, but GWA in mice is not developed yet hindered by the lack of efficient algorithm for SNP association analysis and by arbitrary small number of inbred murine strains characterized for susceptibility to complex diseases.

Using information about several million SNPs in more than 70 inbred murine strains, we propose a two-phase GWA analysis. First, 100k SNP set covering the entire mouse genome was used. On a second phase, all available SNPs within the regions that were found positive after the first GWA phase were tested. Computations were based on a single gene model, hence, independent statistical test for every polymorphic genome position was performed using linear regression model for genotype-phenotype association. Raw $p$-values were corrected for multiple comparisons generating genome-wide $q$-values.

To address the efficiency of GWA gene discovery in inbred murine strains, we performed statistical modeling using coat color trait. Tyrosinase encoding gene was identified as a single major genome-wide peak when more than 45 strains were used. Two SNPs within tyrosinase gene were strongly associated with coat color ($p < 4.4\times10^{-16}$, genome-wide $q < 1.1\times10^{-10}$). Signal-to-noise ratio for the major peak was $10^9$ to 1; locus size was smaller than 1,500 bp.

The two-phase GWA analysis was applied for murine serum-transfer induced arthritis. Analysis successfully confirmed two human rheumatoid arthritis loci previously found near hemolytic complement component *C5* and phosphatidylinositol-phosphate kinase *Pip4k2c* encoding genes. Both genes showed highly significant association with clinical score of murine arthritis: $p < 4\times10^{-10}$ and $p < 8.2\times10^{-11}$, respectively. Novel genetic loci containing *Serpine type 2* ($p < 10^{-9}$), fibrillin *Fbn2* ($p < 10^{-8}$), nuclear factor of activated T cells *Nfatc4* ($p < 10^{-7}$), and *Nfkb1* ($p < 10^{-7}$) were also found to be significantly associated with the effector inflammatory phase of murine arthritis.

The novel approach opens an avenue for discovery genes associated with common pathologies in a short period of time avoiding long and expensive genetic linkage studies.

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## 955/W/Poster Board #613

**Genome-wide association study of degenerative disc disease (DDD).** *P.Y.P. Kao[1], D. Chan[1], K.S.E. Cheah[1], K.M.C. Cheung[2], D.W.H. Ho[1], J. Karppinen[5], J.C.Y. Leong[2], K.D.K. Luk[2], S.P. Yip[6], Y.Q. Song[1, 2], P.C. Sham[3, 4].* 1) Dept. of Biochemistry, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong, Hong Kong, China; 2) Dept. of Orthopaedics & Traumatology, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong, China; 3) Dept. of Psychiatry, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong, China; 4) Genome Research Centre, University of Hong Kong, Hong Kong, China; 5) Dept. of Rehabilitation, University of Oulu, Oulu, Finland; 6) Dept. of Health Technology and Informatics, Hong Kong Polytechnic University, Hong Kong, China.

Low back pain is a common problem, where 70-85% of people experienced back pain in their life. There are a number of causes for LBP and the most important one is degenerative disc disease (DDD). The etiology of DDD is complex. Both environmental and genetic factors are possible contributing factors. Familial aggregation and similar degeneration patterns observed in twins suggested that genetic factors play an important role in DDD. Genetic variants related to DDD were already suggested in several candidate gene studies. To decipher the genetic component of DDD, we have initiated a whole-genome case-control association study for DDD using Affymetrix Mapping 500K microarray. Volunteers of Southern Chinese origin, aged from 18 - 65 years, were recruited and underwent blood sampling and magnetic resonance imaging (MRI) of the spine with consent. DDD status was graded according to Schneiderman's classification to give a DDD score. MRIs were analyzed by two experienced physicians (K.M.C.C., J.K.) blinded to the results of genetic analysis and clinical history. Scores of each of the five lumbar intervertebral discs were summed. An age-adjustment procedure for the DDD score was carried out using a standardization process to give age-adjusted DDD score (AADS). In this procedure, each sample's DDD score was standardized with the mean and standard deviation calculated from individuals having ages below and above a specified bandwidth. Cases and controls were selected from samples having the highest and lowest, respectively, AADSs. From these criteria, 200 cases and controls, respectively, were selected. DNA was extracted from whole blood and quality was checked to be conforming to the quality threshold for genotyping with 500K array set. Genotypes were called with BRLMM algorithm. Analysis was carried out using Plink, and Haploview was used for visualization of the data. Quality control was performed with Plink. Samples and markers with bad qualities were excluded. Relationships between individuals were checked by means and variances of the identity-by-state (IBS) to ensure there are no close relatives. The filtered samples and markers were corrected for hidden population structure with Eigenstrat. 318,747 SNPs remained after per-SNP QC filtering. After a further correction of hidden population substructure by Eigenstrat, markers with p-values of order 10-5 could be detected.

## 956/W/Poster Board #614

**Bone Density GWAS in Premenopausal White Women and Replication in Other Cohorts.** *D.L. Koller, S. Ichikawa, D. Lai, L. Curry, S.L. Hui, M. Peacock, T. Foroud, M.J. Econs.* Departments of Medical & Molecular Genetics and Medicine, Indiana Univ Sch Medicine, Indianapolis, IN.

Several genomewide association studies (GWAS) have been performed to identify genes contributing to bone mineral density (BMD), typically in samples of elderly women and men. In order identify genes contributing to peak BMD we completed a GWAS in a sample of 1,552 premenopausal white women aged 20-45 years from 762 sibships. BMD was measured at the lumbar spine and femoral neck by DXA. Genotyping was performed by the Center for Inherited Disease Research using the Illumina 610Quad. SNPs and samples with call rates below 95%, SNPs with deviation from Hardy-Weinberg equilibrium (p<0.00001), or minor allele frequency below 1% were removed, as were samples with evidence of population stratification. The final dataset consisted of 547,971 SNPs and 1,524 women. Age and weight-adjusted BMD values were tested for association with each SNP using PLINK, with p-values determined empirically via permutation. The top 50 SNPs for both peak femoral neck and lumbar spine BMD were genotyped in a sample of 669 black premenopausal women and 1,092 white men. Associations with $p<1.5 \times 10^{-5}$ for femoral neck or lumbar spine BMD were selected for replication genotyping. A SNP located 180 kb 3' of SOX6 was associated with lumbar spine BMD (rs10832519, $p=1.5 \times 10^{-5}$) and replicated in the sample of white men (p=0.01). This gene has been demonstrated to have a role in bone formation. SNPs in CATSPERB on chromosome 14 provided evidence of association with femoral neck BMD (rs1298989, $p= 2.7 \times 10^{-5}$; rs1285635, $p=3.0 \times 10^{-5}$) which was replicated in the black women (rs1298989, p=0.006; rs1285635, p=0.003). We also detected association with rs1826601 on chromosome 16 ($p=1.1 \times 10^{-5}$) near the 5' terminus of ADAMTS18. Association with SNPs in this gene has been previously reported in a GWAS and replicated by Xiong et al (AJHG 84:388-398, 2009). We conclude that a GWAS for peak BMD in a sample of premenopausal women replicates several genes previously identified, and that several of these findings replicate in black women or white men.

## 957/W/Poster Board #615

**Genome-wide association study of migraine with aura in a large international consortium sample identifies first SNP and CNV associations affecting migraine susceptibility.** *V. Anttila[1,2], K. Alakurtti[1], M. Kallela[3], G. Terwindt[4], U. Todt[5], T. Freilinger[6], V. Artto[3], M. Inouye[1], K. Kristiansson[1], S. Calafato[1], A. van den Maagdenberg[4], L. Peltonen[1,2,7,8], M. Wessman[2,9], J.-A. Zwart[10], M. Daly[7], D. Nyholt[11], M. Dichgans[6], C. Kubisch[5], R. Frants[4], A. Palotie[1,2,7], International Headache Genetics Consortium.* 1) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, UK; 2) Institute for Molecular Medicine Finland and Center of Excellence for Complex Disease Genetics, University of Helsinki, Helsinki, Finland; 3) Dept. of Neurology, Helsinki University Central Hospital, Helsinki, Finland; 4) Leiden University Medical Center, University of Leiden, Leiden, the Netherlands; 5) Institute of Human Genetics, University Hospital Cologne, Cologne, Germany; 6) Klinikum Groβhadern, Ludwig-Maximilians-Universität, Munich, Germany; 7) Broad Institute at Harvard and MIT, Cambridge, MA, USA; 8) National Institute for Health and Welfare, Helsinki, Finland; 9) Folkhälsan Research Center, Helsinki, Finland; 10) Norwegian National Headache Centre, Department of Neurology and Clinical Neurophysiology, St Olavs Hospital, Trondheim, Norway; 11) Queensland Institute for Medical Research, Brisbane, Australia.

Here we present the results of the first genome-wide association (GWA) study in migraine. Despite a well-established genetic component for migraine and numerous studies, no variants or genes influencing migraine susceptibility have been convincingly identified. Given the recent successes by large-scale multinational collaborations in tackling complex diseases, we formed the International Headache Genetics Consortium comprised of seven top-tier migraine centres. In this study, we have performed a genome-wide analysis of 2,900 cases and 11,000 controls, roughly equally distributed between Finland, Germany and the Netherlands. All cases have extensive phenotype information available and diagnosis has been verified by a migraine specialist. For genotyping, we used the Illumina 610k and 550k GWA chip platforms, and obtained population-matched controls from other studies (Health2000 and the Helsinki Birth Cohort from Finland [www.nationalbiobanks.fi], the ERGO study from the Netherlands [www.epib.nl/ergo.htm], and KORA [www.helmholtz-muenchen.de/kora/], PopGen [www.popgen.de] and HNR [www.recall-studie.uni-essen.de] studies from Germany) genotyped on the same chips. Association analyses were performed using PLINK and Haploview for SNPs and PennCNV, QuantiSNP and CNVtools for CNV discovery and copy number assignment. We report the results of genome-wide SNP analyses, including both population-specific and population-agnostic genes, and several associating copy number variants. Specifically, we have identified several SNP associations (on chromosomes 4q, 10q and 17p), with a high degree of overlap with previous linkage findings, as well as CNV associations with similar overlap. Furthermore, we demonstrate that the previously published trait component approach has greater power to resolve the association signal at these loci. In this presentation we detail the first statistically robust and consistent genetic associations in migraine genetics, and the first step to quantify the contributions of CNVs to the genetic background of migraine.

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## 958/W/Poster Board #616

**Meta-analysis of GWAS for Handedness: results from the ENGAGE consortium.** *S.E Medland[1,2], C.M Lindgren[3,4], R. Magi[3,4,5], B.M Neale[6,7], E. Albrecht[8], T. Esko[5,9,10], D.M Evans[11], J.J Hottenga[12], M.A Ikram[13], M. Mangino[14], S. Ripatti[15], F. van Rooij[13], D.I Boomsma[12], G. Davey Smith[11], I.M Rückert[8], T.D Spector[14], C.D van Duijn[13], N.G Martin[1], L. Peltonen[15], M.I McCarthy[3,4], the ENGAGE Handedness Consortium.* 1) Genetic Epidemology, Queensland Institute of Medical Research , Brisbane, Queensland, Australia; 2) Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, USA; 3) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 4) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK; 5) Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia; 6) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA USA; 7) Program in Medical and Population Genetics, The Broad Institute, Boston, MA USA; 8) Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; 9) Estonian Biocentre, Genotyping Core Facility, Tartu, Estonia; 10) Estonian Genome Project, University of Tartu, Tartu, Estonia; 11) MRC Centre for Causal Analyses in Translational Epidemiology, Department of Social Medicine, University of Bristol, Bristol, UK; 12) Department of Biological Psychology, VU University Amsterdam, Amsterdam, The Netherlands; 13) Dept of Epidemiology, Erasmus MC, Rotterdam, The Netherlands; 14) Dep of Twin Research & Genetic Epidemiology, King's College, London, UK; 15) National Institute of Health and Welfare, Public Health Genomics Unit and FIMM, Institute for Molecular Medicine Finland, Helsinki, Finland.

**Background**: Handedness, a consistent asymmetry in skill or preferential use between the hands, is a moderately heritable trait which is related to the lateralization of language functions within the brain. The prevalence of left-handedness (~10% in the general population) is increased in a number of psychiatric, neurologic, and learning disorders suggesting that cerebral asymmetry may play a role in these disorders. Previous linkage and association studies have had only limited success. However these studies have used unselected samples resulting in low numbers of cases and low power. Here we present the results of a meta-analysis of 12 genome wide association studies, comprising 23,443 samples (2350 cases, 21093 controls). **Methods**: Writing hand was used to classify participants as left- or right-handers, and sex and year of birth were used as covariates. All samples were derived from European descent populations. We tested for trait associations under an additive model for a total of ~2.5 million common HapMap SNPs (directly genotyped and imputed). Weighted Z-score based meta-analysis was conducted using METAL with weights defined by effective sample size. **Results**: We observed a number of signals approaching genome-wide significance ($5x10^{-8}$) all of which were novel. Promising results were found on chromosome 5 ($p = 2.455e^{-07}$) and 13 ($p = 6.149e^{-07}$), in regions that encompasses *SLIT3* (which plays a role in the axon-guidance-pathways which guide motor neuronal development during fetal development), *MAB21L1* (involved in cerebellum development) and *NBEA* (which encodes a neuron-specific multidomain protein implicated in membrane trafficking that is predominantly expressed in the brain and during development). **Conclusions**: No evidence of association was found for any the traditional asymmetry candidate genes (*NODAL*, *LEFTY*). Large-scale replication efforts are currently underway. These results vastly extend the number of loci implicated in the development of handedness and lateralization in general.

## 959/W/Poster Board #617

**Heterogeneity in autism spectrum disorders (ASD): linkage analysis of four ASD subsets defined by ADI-R clustering.** *Z. Talebizadeh[1], E. Moore[1], V.W. Hu[2].* 1) Children's Mercy Hospital and University of Missouri-Kansas City MO; 2) Biochemistry and Molecular Biology, The George Washington University Medical Center, Washington, DC.

Autism is a genetically heterogeneous neurodevelopmental disorder which belongs to a group of conditions known as autism spectrum disorders (ASD). Recently a new ASD phenotypic sub-classification was reported by Hu et al. (2009) using ADI-R data, a gold standard autism diagnostic assessment tool. The objective of our current study was to determine the impact of this new ASD subject stratification on genome-wide linkage analysis. According to this classification, four reasonably distinct phenotypic subgroups were identified: (1) severe language impairment, (2) milder symptoms across all domains, (3) notable savant skills, and (4) intermediate phenotype. The genotyping data derived from the Affymetrix 10K SNP array for 426 families was downloaded from the Autism Genetic Research Exchange (AGRE) website. The AGRE SNP data files were sorted into separate files for each of the four ADI-R phenotypic sub-categories. Four group-related main lists were prepared by selecting families having at least one autistic sibling belonging to group 1, 2, 3, or 4. In multiplex families, affected members of each family may belong to different sub-categories reflecting intra-family heterogeneity. Therefore, once families were sorted based on these initial subject sub-categories, for increasing specificity the intra-family heterogeneity was further adjusted by removing affected siblings that were not in the same phenotypic subgroup. To assess gender affect, further analysis was done based on the affected individual's gender. Non parametric linkage (NPL) was calculated using MERLIN linkage analysis software. When the combined (non-subtyped) samples were analyzed, a suggestive linkage was found for 426 AGRE families for the following chromosomal bands: 3q25 (LOD=1.6, P=0.003), 4q22 (LOD=2.02, p=0.001), 5p13 (LOD=2.04, p= 0.001), 5q35 (LOD=2.7, P=0.0002), and 10q23 (LOD=2.84, P=0.0002). However, when analysis was performed based on the ADI-R subtypes, new group-specific suggestive linked regions were detected while the family sample size was smaller in each group compared with no grouping (e.g., n=496 no grouping; n=253 group 1; n=203 group 2; n=173 group 3; and n= 138 group 4). Our linkage analysis results indicate that application of such a phenotypic sub-classification method will improve the detection power in genome-wide linkage study by reducing heterogeneity in ASD study subjects.

## 960/W/Poster Board #618

**The nonsynonymous ATBF1 variant S72A is associated with Atrial Fibrillation.** *M. Müller[1], M.F. Sinner[2], S. Lindhof[3], S. Perz[4], H.E. Wichmann[1,5], T. Meitinger[3,6], A. Pfeufer[3,6].* 1) Institute of Epidemiology, Helmholtz Center Munich, Munich, Germany; 2) Department of Medicine I, Klinikum Grosshadern, Munich, Germany; 3) Institute of Human Genetics, Helmholtz Center Munich, Germany; 4) Institute of Medical Informatics, Helmholtz Center Munich, Germany; 5) Institute of Informatics, Biometry and Epidemiology, Ludwig-Maximilians-Universität, Munich, Germany; 6) Institute of Human Genetics, Technical University Munich, Germany.

Atrial fibrillation (AF) is the most common sustained arrhythmia in man with a population prevalence of about 1 percent. A recent genome-wide association study has identified association with AF in the 16q22 locus near the ATBF1 (ZFHX3) gene, encoding a zinc finger transcription factor. In order to further characterize this association we intended to investigate the presence of independent association signals in the locus. We analyzed 2,145 AF cases from the German Kompetenznetzwerk Vorhoflimmern (AFNET) and 4,073 controls from the population based KORA S4 study in a LD-based SNP-tagging replication approach. We confirmed association at the AFBF1 gene. The strongest association was with rs9302644 (C>G, MAF= 0.19) in intron 1 of the gene with the rare G allele predisposing to AF under an additive model. (OR= 1.48, (1.35 - 1.62), p= 1.1e-16). In a multivariable regression model we found independent association with rs9302644 (OR = 1.28, (1.10-1.50), p= 1.5E-03), rs2106261 OR = (1.20, (1.02-1.41), p= 2.4E-02) and rs7193297 (OR = 1.23, (1.12-1.35), p= 2.1E-05). While rs9302644 and rs2106261 are significantly correlated (r2 = 0.416) neither of them is correlated to rs7193297 (r2 < 0.01). rs7193297 is the nonsynonymous coding variant ATBF1 p.S72A (MAF= 0.23). Overall we could identify two novel common variants in ATBF1 that were independently associated with AF. One of them is a nonsynonymous coding variant which may greatly facilitate the funcitonal investigation of how ATBF1 influences predisposition to AF.

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## 961/W/Poster Board #619

**Genetic variation in insulin-like growth factor 1 receptor (IGF1R) and metabolic syndrome-related phenotypes: Maximizing data from Genome-wide association studies.** *C. Pirola, T. Fernandez Gianotti, C. Gemma, A. Burgueño, S. Sookoian.* Department of Molecular Genetics and Biology of Complex Diseases. Institute of Medical Research A. Lanari-IDIM, University of Buenos Aires-National Council of Scientific and Technological Research (CONICET). Ciudad Autónoma de Buenos Aires, Argentina.

Background: The application of stringent statistical thresholds in genome-wide association studies (GWAS) may dismiss SNPs in genes that are biologically meaningful in the pathogenesis of a disease. A first stage of in silico exploratory assessment of GWAS data may be considered for the identification either of new or candidate loci in the genetic susceptibility of metabolic syndrome (MS)-related phenotypes, and then for further replication stages. Aims and Research design: We perform a two-stage study to explore the role of gene variants and derived haplotypes in MS-related phenotypes. The selection of putative variants was performed by a first stage of in silico analysis of the open data of the GWAS on genes involved in common human diseases, granted by the Diabetes Genetics Initiative-DGI and the Welcome Trust Case Control Consortium-WTCCC, available at public websites. The complete dataset encompass a total of 3000 individuals in the DGI study (1464 patients with T2D and 1467 controls) and 7000 individuals in the WTCCC (T2D and hypertension, 2000 patients for each disease, and 3000 control samples). Among the more promising variants, IGF1R ones with modest significant P values for association with MS-related phenotypes in both datasets (close to $10^{-3}$-$10^{-4}$) were selected for further replication in our population. Altogether, 1094 men of self-reported European ancestry were included in a population-based study. Then, in the second stage of the analysis, a total of six SNPs in the IGF1R were genotyped in our sample: rs11247362 C/T, rs10902606 G/C, rs1317459 C/G, rs11854132 A/G, rs2684761 A/G and rs2715416 G/C. Association was tested by PLINK software.Results: Genotypes of rs2684761 showed significant association with insulin resistance (as measured by HOMA-IR, OR per G allele: 1.13, 95%CI: 1.02-1.25, p<0.026) and HOMA-IR as a continuous trait p<0.011, and fasting serum insulin p<0.011. The association with HOMA-IR was even stronger with the haplotype TCGGGG (P<0.003).Interestingly, a significant association of the rs2684761 with arterial hypertension was observed: OR 1.14 per G allele, 95% CI 1.01-1.28, P<0.034, after adjusting by age and HOMA-IR. As expected, by ordinal multinomial analysis, the cumulative number of metabolic syndrome components was associated with the rs2684761 G variant ($chi^2$: 7.4, p<0.026). Conclusions: Our study suggests a putative role of the IGF1R variants in the individual susceptibility to the MS-related phenotypes.

## 962/W/Poster Board #620

**Genomewide association meta-analysis of >120,000 individuals reveals 39 loci that influence body weight.** *C.J. Willer[1], S.I. Berndt[2], C.M. Lindgren[3,4], J.C. Randall[3], G. Þorleifsson[5], K.L. Monda[6], K.E. North[6,7], J. Luan[8], S. Vedantam[9], I.M. Heid[10], H.M. Stringham[1], M. Boehnke[1], U. Thorsteinsdottir[5], I. Barroso[11], G. Abecasis[1], J.N. Hirschhorn[9,12,13,14], M.I. McCarthy[3,4], E. Speliotes[12,15], R.J.F Loos[8], E. Ingelsson[16], the GIANT Consortium.* 1) Dept Biostatistics and Center for Statistical Genetics, Univ Michigan, Ann Arbor, MI, 48109. USA; 2) Division of Cancer Epidemiology and Genetics, National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, Maryland, USA; 3) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, UK; 4) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford OX3 7LJ, UK; 5) deCODE Genetics, Reykjavik, Iceland; 6) Department of Epidemiology University of North Carolina at Chapel Hill Chapel Hill,NC 27516-3997, USA; 7) Carolina Center for Genome Sciences University of North Carolina at Chapel Hill Chapel Hill, NC 27516, USA; 8) MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK; 9) Division of Genetics, Children's Hospital, Boston, MA 02115, USA; 10) Institute of Epidemiology,University of Regensburg, Germany; Institute of Epidemiology, Helmholtz Zentrum München, Germany; 11) Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK; 12) Broad Institute at MIT and Harvard, Boston MA 02115, USA; 13) Departments of Genetics and Pediatrics, Harvard Medical School, Boston, MA 02115, USA; 14) Division of Endocrinology, Children's Hospital, Boston, MA 02115, USA; 15) Department of Gastroenterology, Massachusetts General Hospital and Harvard Medical School, Boston MA. 02114, USA; 16) Dept. of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden.

Body shape, size, and composition are influenced by multiple genes and environmental factors, including secular trends. Furthermore, different measures of body size and shape may have different sensitivity to the influences of individual genetic loci, and may therefore facilitate the discovery of novel genetic associations. Body weight, a commonly measured phenotype, integrates both height and body shape. We conducted a genomewide association scan meta-analysis for body weight of >120,000 individuals from 48 separate cohorts, examining ~2.8 million directly genotyped or imputed SNPs. Overall, 39 unique loci reached genomewide significance ($p < 5x10^{-8}$), highlighting the polygenic nature of body weight regulation.

The three strongest signals were observed near *FTO* ($p \approx 10^{-47}$), *MC4R* ($p \approx 10^{-25}$), and *TMEM18* ($p \approx 10^{-21}$), three loci previously associated with body mass index (BMI) and other measures of obesity. Other prominent loci in the list include *ZBTB38* ($p \approx 10^{-18}$), *HMGA2* ($p \approx 10^{-16}$), and *GDF5* ($p \approx 10^{-10}$), all loci with common variants strongly associated with human height. Still, not all the loci previously associated with height and/or BMI showed association with body weight (e.g. *MTCH2* $p \approx 10^{-3}$ for weight and $10^{-10}$ for BMI), and conversely, 7 loci that did not reach genomewide significance in parallel analyses of BMI, waist circumference, waist-hip ratio and height, did reach genomewide significance for body weight (e.g. *MTIF1* $p \approx 10^{-10}$, *CADM2* $p \approx 10^{-9}$ , and *HNF4G* $p \approx 10^{-8}$). Although not genomewide significant, an additional 46 loci had p-values < $1x10^{-6}$, and efforts to replicate these may reveal many new associated loci.

By using body weight as a phenotype, we have augmented the catalog of loci implicated in the regulation of body shape and size. The loci include genes that are likely to act in the brain to regulate appetite and energy balance, and genes that are likely to influence overall growth -- consistent with the observed overlap of many of our findings with previous genetic studies of height and BMI. We expect that, over the next few years, continued progress will be made in the identification of loci and variants that influence body shape and size. Furthermore, we expect this progress will be enhanced by the examination of multiple quantitative summaries of body size, shape and composition.

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## 963/W/Poster Board #621

**Genome wide search for genes controlling risk to non-syndromic oral clefts in a consortium case-parent trio study.** *T.H. Beaty[1], J.B. Hetmanski[1], K.Y. Liang[1], I. Ruczinski[1], R.A. Redett[2], A.F. Scott[2], M.L. Marazita[3], J.C. Murray[4], R.G. Munger[5], A. Wilcox[6], R.T. Lie[7], Y.H. Wu-Chou[8], H. Wang[9], X. Ye[10], V. Yeow[11], S.S. Chong[12], S.H. Jee[13], B. Shi[14], K. Christensen[15], GENEVA. Consortium[16].* 1) School of Public Health, Johns Hopkins Univ, Baltimore, MD; 2) School of Medicine, Johns Hopkins Univ., Baltimore MD; 3) School of Dental Medicine, University of Pttsburgh, Pittsburgh PA; 4) Children's Hospital, School of Medicine, University of Iowa, Iowa City, IA; 5) Dept. of Nutrition and Food Sciences, Utah State Univ. Logan UT; 6) Epidemiology Branch, NIEHS/NIH, Durham NC; 7) University of Bergen, Bergen Norway; 8) Chang Gung Memorial Hospital, Taoyuan, Taiwan; 9) Peking University Health Sciences Center, Beijing, China; 10) School of Stomatology, Wuhan University, Wuhan, China; 11) KK Women's and Children's Hospital, Singapore, Singapore; 12) School of Medicine, National University of Singapore, Singapore; 13) Yonsei University, Seoul, Korea; 14) West China School of Stomatology, Sichuan University, Chengdu, China; 15) University of Southern Denmark, Odense, Denmark; 16) NIDCR/NHGRI.

As part of an international consortium, we assembled a collection of 2463 case-parent trios from 9 countries in the first genome wide association study (GWAS) of oral clefts utilizing a family-based study design. These trios included both major cleft phenotypes [cleft lip with/without cleft palate: CL/P; and cleft palate: CP], and incorporated information on recognized environmental risk factors. Oral clefts are thought to be influenced by several different genes and by different environmental risk factors (including maternal smoking, alcohol consumption and vitamin supplementation). Thus, this consortium was designed to conduct a genome-wide search for genes important in the etiology of oral clefts where both environmental risk factors and parent-of-origin effects could be considered. Probands were drawn from 13 recruitment sites in 9 countries and were all diagnosed as having either an isolated, non-syndromic CL/P (n=1920) or CP (n=563). Review and approval for this study was obtained from IRBs at each participating institution, and informed consent was obtained from all participants (including parental consent for minor children). Forty-five percent of these trios are of European ancestry and 51.9% are of Asian ancestry. DNA was obtained from the case child and both parents and genotyped at the Center of Inherited Disease Research (CIDR) for 589,945 single nucleotide polymorphic (SNP) markers on the Illumina 610Q chip. Using 580,307 autosomal SNPs in a transmission disequilibrium test (TDT), we scanned the genome for evidence of linkage and association in CL/P trios and CP trios separately. We first focused on chromosome 8 to confirm evidence that a region of 8q24 influences risk to CL/P as reported by a case-control GWAS from Germany (Birnbaum et al. 2009 NAT GENET 41:473). Our preliminary analysis confirmed strong evidence of linkage and association in a 270 kb region of 8q24 among CL/P trios, but not CP trios. Testing for heterogeneity between European and Asian derived trios showed much stronger evidence of linkage and association in the European derived families. This region of chromosome 8q24 appears to be a "gene desert", approximately 300 kb from a region reported to be associated with prostate and other cancers.

## 964/W/Poster Board #622

**A genome-wide survey of SNPs and CNVs for association to body mass index in 1,931 individuals from African-derived populations.** *C.W.K. Chiang[1,2,3], S.J. Kang[4], B. Tayo[5], C. Palmer[2,3], G. Lettre[6], J.L. Butler[2,3], R. Hackett[3], C. Guiducci[3], T. Nguyen[2,3], A. Adeyemo[7], C. Rotimi[7], A. Luke[5], H.N. Lyon[2,3], R. Cooper[5], J.N. Hirschhorn[1,2,3], X. Zhu[4].* 1) Department of Genetics, Harvard Med School, Boston, MA; 2) Children's Hospital Boston, Boston, MA; 3) Broad Institute, Cambridge, MA; 4) Case Western Reserve University, Cleveland, OH; 5) Stritch School of Medicine, Loyola University Chicago, Chicago, IL; 6) University of Montreal, Montreal, Canada; 7) Howard University, Washington, District of Columbia.

Genome-wide association studies (GWAS) in large cohorts have identified numerous loci associated with central and overall obesity. However, most studies were conducted in European-derived populations, and it is not known whether the identified loci generally replicate in populations with recent African ancestry. Lack of replication could reflect genetic, environmental or phenotypic heterogeneity, or the different recombination history in African-derived populations. To begin to identify genetic variants associated with obesity in populations with recent African ancestry, we conducted a comprehensive GWAS in 743 African Americans and 1188 Nigerians using the Affymetrix 6.0 platform and surveyed SNPs as well as common and rare copy number variants (CNVs). Additionally, we devised a method using a mixed reference panel to impute genotypes at over 2 million HapMap SNPs in our African-American cohort, and showed that this method achieved > 95% accuracy in imputation calls, comparable to imputation of non-admixed population using a single reference panel. After applying quality controls and correcting for genetic ancestry, we observed no associations that reached genome-wide significance for any SNPs or common CNVs. There was a nominal association of the lean individuals with heavier burden of longer ($P = 0.004$) and total ($P = 0.001$) rare CNVs (population frequency < 5%). We also attempted to replicate in additional African-American cohorts the top independent SNPs from this GWAS and the best SNPs at known loci. There were no strong consistent associations, with the best evidence of replication coming from SNPs near a previously known gene, MC4R. It does not appear that there are common variants with large effects on BMI that are unique to African ancestry population. Replication of further SNP associations with suggestive evidence will be done in additional well-powered cohorts, and meta-analysis with other GWA in African-derived populations, should detect valid associations in African-derived populations.

## 965/W/Poster Board #623

**Genome-wide profile of copy number variants for Hirschsprung disease.** *C.S. Tang[1], M.M. Garcia-Barceló[2], S.S. Cherny[1], P.C. Sham[1], P.K.H. Tam[2].* 1) Department of Psychiatry, Li Ka Shing Faculty of Medicine, The University of Hong Kong, HKSAR; 2) Department of Surgery, Li Ka Shing Faculty of Medicine, The University of Hong Kong, HKSAR.

Hirschsprung disease (HSCR, aganglionic megacolon) is a developmental disorder characterised by the absence of the enteric ganglia along the intestinal tract. In addition to the major implicating gene *RET*, common polymorphisms, rare mutations and chromosomal abnormalities have been found to be associated with HSCR. The most common associated chromosomal abnormality is trisomy 21 (Down syndrome). Other chromosomal aberrations including 13q chromosomal deletions were also reported, which pinpointed the region for the discovery of disease susceptibility loci, such as *EDNRB*. To characterize the copy number variants for HSCR, we analyzed 139 HSCR patients and 333 controls previously genotyped by Affymetrix 500k SNP arrays using Birdsuite. For HSCR patients, we identified 92 duplications and 60 deletions of at least 100kb. The total burden for rare and singleton CNVs increased for cases when compared to controls, both for the number of CNVs per individual ($p = 0.0008$) and the number of overlapping genes ($p < 0.0004$). In particular, long singleton duplications and deletions were identified for HSCR patients. Focusing on highly confident structural variations revealed three duplications ranging from 1.8 to 10Mb, spanning 8p21, 15q11-13 and 16p12. Two nonoverlapping deletions (14.9 & 29.7Mb) with both breakpoints residing in 11q23 were also shown. As previously reported, a 16Mb deletion overlapping with *EDNRB* was found on one HSCR patient. Together with other shorter variants, this finding could account for a sustainable proportion of susceptibilty to HSCR.

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## 966/W/Poster Board #624

**Meta-analysis of six large type 1 diabetes cohorts identifies multiple loci.** *J.P. Bradfield[1], H.Q. Qu[2], K. Wang[1], H. Zhang[1], P.M. Sleiman[1], C.E. Kim[1], K. Annaiah[1], J.T. Glessner[1], K. Thomas[1], E.C. Frackelton[1], R. Chiavacci[1], M. Imielinski[1], D.S. Monos[3,4], S.F.A. Grant[1,3,5], C. Polychronakos[2], H. Hakonarson[1,3,5].* 1) Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; 2) Departments of Pediatrics and Human Genetics, McGill University, Montreal, Quebec, Canada; 3) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; 4) Department of Pathology and Laboratory Medicine, Abramson Research Center, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; 5) Division of Human Genetics, Abramson Research Center, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania.

Type 1 diabetes is an autoimmune disease leading to the destruction of the pancreatic islets. The prevalence of T1D in the United States in individuals below the age of 20 is 1.7 per 1000. Genome wide association studies (GWAs) have been particularly valuable in identifying the genetic susceptibility loci underlying type 1 diabetes with over 40 associations described in the last two years. One inherent limitation of GWAs is the large sample sizes required to detect loci of modest effect. Meta-analyses are the most efficient and economical way to assemble such large cohorts. In an effort to find more loci, we analyzed the largest combined T1D cohort to date, totaling 10,000 cases and 17,500 controls, comprising ~14,000 samples (~3,500 cases, ~10,500 controls) genotyped on Affymetrix 500K chips (Gokind, WTCCC) and ~13,500 samples (~6,500 cases, ~7,000 controls) genotyped on Illumina 550K chips (McGill, CHOP, DCCT-EDIC, T1DGC). Power calculations based on these sample sizes indicate we have greater than 80% power to detect a variant with relative risk of 1.13 and above assuming a minor allele frequency of 20%. Methodologically, we initially imputed all datasets up to the 2 million CEU Hapmap SNPs using Mach 1.0 so that platform independent comparisons could be made. To control for population stratification, we used SNPs common to both platforms as input into a Multi-Dimensional Scaling(MDS) algorithm. The results of the MDS analysis were then used as covariates in a logistic regression. Summary stats were then computed on the combined cohort at all 2 million SNPs. The results of this, the largest T1D meta-analysis to date, will be presented at the meeting.

## 967/W/Poster Board #625

**Predictive model for type 2 diabetes mellitus using Korean community based on chort.** *J. Lee[1], B. Keam[2], M. Park[3], J. Heo[3], J. Lee[1].* 1) Division ofStructural and Genomics, Korea Centers for Disease Control and Prevention, Seoul, Korea; 2) Division of BioBank for Health Sciences, Korea Centers for Disease Control and Prevention, Seoul, Korea; 3) Division of B-medical Informatics, Korea Centers for Disease Control and Prevention, Seoul, Korea.

Recent genetic association studies have provided convincing evidence that several novel loci and single nucleotide polymorphisms (SNPs) are associated with the risk of type 2 diabetes mellitus (T2DM). The aims of this study were to compare misclassification rates of different models. We surveyed and had second follow-up 10,038 individuals from the Korean Genome and Epidemiology Study (KoGES). And then we also genotyped 500,568 SNPs using SNP array(Affymetrix). We selected 1,745 individuals with T2DM and 1,857 controls from the Korean Genome and Epidemiology Study (KoGES). We also considered their past-history of Type 2 diabetes. There were 1,745 cases of diabetes during 8 years of follow-up. For selection of significant SNPs, it was tested their associations of SNPs(additive model) using PLINK. SNPs were analyzed for significant association with T2DM using statistical tree QUEST algorithm. We tested these models using the complete KoGES cohort and computed the T2DM misclassification rates for each models. The misclassification rates of the statistical tree QUEST algorithms were estimated in 512 cases(with T2DM) and 568 controls. The dataset was then divided randomly into the training set (70% of the observations) and the test set (30% of the observations). In model, we analyzed misclassification rate of QUEST and optimized the model using 10-folds cross validation pruning method. The rate of misclassification was 0.41 in test data using 10-fold CV pruning method. We identified CDKAL1(rs7767391), ADAM12(rs11244960) and PEX19(rs2820421) SNPs that were significantly associated with Type 2 diabetes. Their SNPs are major risk factors related T2DM. If CDKAL1(rs7767391) and PEX19(rs2820421) had minor genotype, they were predicted diabetes. Alternative, if CDKAL1(rs7767391) was not minor genotype and then ADAM12(rs11244960) was hetero genotype, they were predicted diabetes. We have developed predictive models for T2DM using genotype data. However, the misclassification rates are relatively high. To improve the performance of these models, further studies using large sample sizes are warranted, and a better strategy to integrate the genotype data classifiers for the quantification of SNPs needs to be developed.

## 968/W/Poster Board #626

**A Genome-Wide Association Study of Diabetic Nephropathy in African Americans.** *C.W. McDonough[1,2], N.D. Palmer[2], M.A. Bostrom[2], P.J. Hicks[2], L. Lu[3], J. Divers[3], C.D. Langefeld[3], B.I. Freedman[4], D.W. Bowden[1,2,4].* 1) Program in Molecular Medicine and Translation Science, Wake Forest University School of Medicine, Winston-Salem, NC, USA; 2) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC, USA; 3) Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC, USA; 4) Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, USA.

African Americans (AA) have a 3.6-fold increased risk of developing end-stage renal disease (ESRD) compared to Caucasian Americans. Diabetes-associated nephropathy is the most common cause of ESRD, accounting for approximately 45% of cases in the U.S. The incidence of ESRD continues to increase. Multiple studies have concluded that there is a genetic component to ESRD in the general population and in AAs. In order to identify genes associated with type 2 diabetic nephropathy (DN), we performed a genome-wide association study (GWAS) in 966 AA type 2 diabetic (T2DM)-ESRD cases and 1,031 AA non-diabetic, non-nephropathy controls using the Affymetrix Genome-Wide Human SNP Array v6.0. The top 577 SNPs were genotyped in a replication set of 709 AA T2DM-ESRD cases and 693 AA controls. Those SNPs that replicated were then genotyped in 1,246 AA T2DM non-nephropathy DNAs in order to determine association with diabetes or diabetic nephropathy/ESRD. Admixture adjusted analysis has identified four candidate genes for T2DM-ESRD to date. SNP rs3775043 in *UNC5C*, unc-5 homolog (*C. elegans*), was associated in the GWAS under a dominant model (P=1.82E-05). This SNP replicated (dominant P=0.028), and had a combined GWAS-replication dominant p-value of 2.89E-06. SNP rs3775043 was not associated with T2DM alone. One SNP upstream of ribosomal protein S12 (*RPS12*), rs9493454, was associated in the GWAS, replication and combined set (additive P= 0.0009, 0.009, and 1.53E-05 respectively). rs9493454 was not associated with T2DM alone. SNP rs773506, upstream of the *AUH* gene, AU RNA binding protein/enoyl-Coenzyme A hydratase, had dominant p-values in the GWAS, replication set and combined set of P=0.0004, 0.01, and 1.73E-05 respectively, and was not associated with T2DM. Lastly, *LIMK2*, LIM domain kinase 2, SNPs rs2106294 and rs4820043 showed nominal association in the GWAS (additive P=0.027 and 0.024 respectively). These SNPs remained associated in the replication and combined analyses (rs2106294 dominant P=0.003 and 6.63E-06 respectively, rs4820043 dominant P=0.003 and 6.59E-06 respectively). Again, these SNPs were not associated in the T2DM non-nephropathy analysis. Genotype frequencies for each SNP were also significantly different when T2DM alone and DN samples were compared, suggesting they are DN genes. While these results need further replication to achieve genome wide significance, these four genes are strong candidates to play a role in the development of DN in AAs.

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## 969/W/Poster Board #627

**A Genome-wide Association Study in 1997 African Americans Reveals Candidate Susceptibility Loci for Type 2 Diabetes.** *N.D. Palmer[1,2], C.W. McDonough[2,3], S.C. Smith[3], C.D. Langefeld[4], J. Divers[4], L. Liu[4], B.I. Freedman[5], D.W. Bowden[1,2].* 1) Biochemistry, Wake Forest University, Winston-Salem, N.C; 2) Center for Human Genomics, Wake Forest University, Winston-Salem, N.C; 3) Molecular Medicine and Translation Science, Wake Forest University, Winston-Salem, N.C; 4) Public Health Sciences, Wake Forest University, Winston-Salem, N.C; 5) Nephrology, Wake Forest University, Winston-Salem, N.C.

The prevalence of diabetes in the U.S. is on the rise with African Americans disproportionately affected by the disease. To identify type 2 diabetes (T2D) susceptibility variants, we analyzed genome wide association data (Affy6.0) from 966 African American T2D cases and 1031 controls. All subjects were recruited from the southeastern U.S. and case subjects were concordant for diabetes and diabetic nephropathy. Principal components analysis was used to correct for population stratification. PC1, which explained 22% of the variance, was highly correlated with African ancestry as determined from ancestry informative markers (AIMs) data, PC2 accounted for 3% and the remaining ≤2% each. Genotype analysis of the autosomal SNPs (>832K), adjusted for age at recruitment, gender, PC1 and PC2, resulted in a genomic inflation factor value of <1.046 and revealed several signals of association. Within the GWAS, we examined the impact of known T2D-susceptibility loci (the majority of which were identified from European-derived populations). With the exception of *TCF7L2*, these T2D-susceptibility loci do not appear to explain individual variation in T2D risk in African Americans. Replication of the top 577 SNPs from the GWAS was completed in an independent collection of African American T2D cases (*n*=709) and controls (*n*=693) recruited under identical ascertainment criteria. To distinguish between loci for diabetes and diabetic nephropathy, loci nominally associated in the replication were genotyped on a sample of 1246 T2D-only cases for validation. Among the top associations seen after admixture adjustment in the overall analysis (GWAS+Rep+Val) were SNPs in *TCF7L2* ($P_{ADD}$=6.11x$10^{-9}$) and intergenic regions on chr10 and chr14 ($P_{ADD}$=8.81x$10^{-5}$ and $P_{DOM}$=7.88x$10^{-5}$, respectively). In addition, these SNPs have been typed on an additional 640 controls with comprehensive metabolic phenotypes derived from the Frequently Sampled Intravenous Glucose Tolerance Test (FSIGT) with MINMOD analysis. SNPs which have been validated for association with T2D are now the focus of fine-mapping efforts to identify putative causal variants. In parallel, top hits from imputation, which include a hit on chr19 in a zinc finger gene ($P_{ADD}$=1.55x$10^{-16}$), are being tested. This study highlights the complexity of T2D and likely implicates ethnic-specific variants in the etiology of the disease.

## 970/W/Poster Board #628

**Analysis of gene-environment interactions with smoking on a genome-wide basis reveals a genetic network and a new pathway in the development of rheumatoid arthritis.** *L. Padyukov[1], B. Ding[2], H. Källberg[2], M. Seielstad[3], L. Klareskog[1], L. Alfredsson[2].* 1) Rheumatology Unit, Karolinska Inst, Stockholm, Sweden; 2) Department for Environmental Medicine, Karolinska Inst, Stockholm, Sweden; 3) The Genome Institute of Singapore, Singapore.

The search for the etiology of complex diseases is hampered by the involvement of multiple genetic and environmental factors. By examining the effect of a known environmental risk factor (smoking) in a genome-wide scan of 3000 Swedish rheumatoid arthritis patients and matched controls, we uncover multiple potential gene-environment interactions. Importantly, the genetic risk factors identified in this way are not apparent in the conventional genome-wide scan of main-effects, due to their modest risk ratios. This genome-wide interaction analysis with smoking and conservative correction for multiple comparisons revealed a set of genetic variants with an unexpected feature. While protective in the absence of smoking, these variants in combination with smoking increased the risk of RA dramatically. In order to enhance the validity for detection of potential mechanisms we used the corresponding genes in a pathway analysis by IPA software. The network of genes is pointing towards involvement of the Wnt/beta-catenin signaling pathway in the development of rheumatoid arthritis. In this study we introduce the notion of genome-wide interaction study (GWIS) that could be employed in investigating any complex disease for which at least one environmental or genetic risk factor is known. Genome-wide interaction analysis is an efficient way for the detection of new candidate genes and we successfully applied this strategy to rheumatoid arthritis.

## 971/W/Poster Board #629

**The Gene, Environment Association Studies (GENEVA) Consortium.** *M.C. Cornelis[1], A. Agrawal[2], J.W. Cole[3], N.N Hansel[4], T. Beaty[4], S. Bennett[5], K. Doheny[4], E.L. Harris[6], J.H. Kang[1], C.C. Laurie[5], H. Ling[4], T.A. Manolio[7], D.B. Mirel[8], E. Pugh[4], J. Udren[5], X.J. Wang[9], K. Williams[5] for the GENEVA Consortium.* 1) Harvard School of Public Health, Boston, MA; 2) Washington University School of Medicine, Saint Louis, MO; 3) University of Maryland School of Medicine, Baltimore, MD; 4) Johns Hopkins University, Baltimore, MD; 5) University of Washington, Seattle, WA; 6) National Institute of Dental and Craniofacial Research, Bethesda, MD; 7) National Human Genome Research Institute, Bethesda, MD; 8) Broad Institute of Massachusetts Institute of Technology and Harvard; 9) University of Pittsburgh, Pittsburgh, PA.

Genome-wide association studies (GWAS) have emerged as a powerful tool for identifying genetic loci related to complex disease and traits. The role of environment and its potential to interact with genes, however, has not been adequately addressed at a genome-wide level. Formation of networks of collaborative GWAS involving different study samples and multiple phenotypes is a powerful approach for addressing the many challenges in analysis and interpretation common across different GWAS. The Gene, Environment Association Studies (GENEVA) consortium was established in 2007 by the NHGRI to identify genetic variants related to common, complex disease or traits; to identify variations in gene-trait associations related to environmental exposures; and to ensure the rapid sharing of data to the general scientific community. This consortium consists of several NIH-based organizations and extramural participants including a coordinating center, 2 genotyping centers and a growing list of study investigators. GWAS completed or in progress target type 2 diabetes, oral clefts, alcohol addiction, lung cancer, chronic obstructive pulmonary disease, birth weight/maternal glycemia, premature birth, dental caries, blood pressure, prostate cancer, venous thrombosis, ischemic stroke, and glaucoma. A key feature which distinguishes GENEVA from other existing consortia is the availability of common and detailed environmental exposure information from multiple studies which allow analysis of additional traits (smoking behavior, anthropometric measures, substance use, lifestyle and dietary intake behavior) common to many studies and explicit tests for gene-environment interactions. Facilitated by its unique infrastructure to promote collaboration among groups, GENEVA has established a unified framework for sample submission and genotyping, data quality control and assurance, as well as independent and cross-study analysis and interpretation. Data from each study are shared with the scientific community through NIH's dbGaP. By maximizing knowledge obtained through collaborative GWAS which incorporate environmental exposure information, GENEVA will enhance our understanding of both disease etiology and identify opportunities for prevention.

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## 972/W/Poster Board #630

**Meta-analysis of Genome-Wide Association Scans Identifies CARD11 as a Determinant of Bone Strength in Caucasian Women: The Genetic Factors For Osteoporosis (GEFOS) Consortium.**
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GWA, an approach with hypothesis-free association testing of genetic determinants, has proved successful identifying at least 20 BMD loci. Hip geometry has been shown an important predictor of fracture. We performed meta-analysis of ~2.5 million imputed SNPs from three large GWA studies (including 9,021 Caucasian men and women) in relation to proximal femoral geometry phenotypes. We analyzed femoral neck length (FNL), neck-shaft angle (NSA), neck width (Wid), and section modulus (Z) estimated by hip structure analysis (HSA) algorithms from DXA scans. In each cohort sex-specific and sex- combined association analyses were performed under an additive model adjusted for age, age$^2$, height, BMI (and estrogen history in FOS women). The genomic inflation factor $\lambda_{GC}$ ranged from 0.977 to 1.025 across different traits and cohorts. A z statistic-based fixed-effect meta-analysis weighted by effective sample sizes across studies (METAL) was run on GWA results from the Rotterdam Study (RS, n=4,102; Illumina550), Framingham Osteoporosis Study (FOS, n=3,409; Affymetrix 550), TwinsUK Study (TUK, n=1,510 women; Illumina317) with 2,543,686 SNPs imputed from the HapMap CEU reference panel. The genome-wide significance (GWS) threshold was set at $p<5x10^{-8}$. Despite some GWS signals in isolated studies, the sex-combined meta-analysis identified no GWS loci. In contrast, several sex-specific associations were found with diverse traits for SNPs mapping to chromosome 1p13.3 (Wid, women), 5q13.1 (NSA, men), and 7p22 (Z, women), 12q21.2 (NSA, women), of which the notable association was between Z and variants in a novel gene CARD11 in women ($p=4.80x10^{-8}$); CARD11 was not previously associated with BMD. Other well-known candidate genes for bone strength were also identified (p-values: $3.8x10^{-6}$ to $2.0x10^{-10}$) including EXT1, PTH2R, TNFRSF11B, and TSHB. In conclusion, we showed at GWS level that genetic variation in CARD11 influences bone strength in Caucasian women. CARD11, which plays a role in NF-kappaB activation, may be a potential factor acting through the OPG-RANK-RANKL signaling pathway which is already known to be influential on bone strength. Further replication in additional independent samples is underway to maximize power as well as to determine the relation with hip fractures.

## 973/W/Poster Board #631

**Population-based cohorts can help validate genetic associations with asthma.**
*H.N. Lyon[1,2], J.L. Butler[1], C. Palmer[1,3], J. Reibman[4], M. Liu[4], G. O'Connor[5], J.N. Hirschhorn[1,3,6].* 1) Div Gen, Boston Children's Hosp, Boston, MA; 2) Dept of Pediatrics, Harvard Med School, Boston, MA; 3) Broad Institute, Cambridge, MA; 4) Depts of Med and Environmental Med, NYU School of Med, NY; 5) Div Pulmonary Critical Care Med, Boston Univ School of Med, Boston, MA; 6) Dept Genetics, Harvard Med School, Boston, MA.

Asthma is a common disease with strong hereditary influences. Genome-wide association (GWA) studies in cohorts of people with asthma and controls highlight genetic variants that are predictors of disease. Large consortia will soon be available for studies of asthma, but GWA data exist for much larger sets of population-based cohorts with asthma and related phenotypes. We hypothesized that population-based cohorts could provide valuable GWA data to replicate or discover new genetic associations with asthma. Methods: We used data from the SHARe database which consists of SNP (single nucleotide polymorphism) genotypes across the genome in 3 generations of individuals from the Framingham Heart Study (FHS) and information on physician diagnosed asthma. We imputed genotypes at 2.2 million SNPs using the HapMap CEU reference panel. These SNPs were tested for association with asthma by comparing allele frequencies for 975 people with asthma and 3576 control individuals; smokers and people with lung diseases excluded. We used logistic regression and applied genomic control to correct for inflation of test statistics from relatedness within the 3 generation cohort. We validated previously reported variants for association with asthma and also found novel asthma SNPs for replication in further cohorts. To examine the utility of population- based data on related phenotypes, we also tested SNPs reported to influence eosinophil counts for association with atopy (allergen-specific IgE) within an asthma cohort from NY City. Results: We found that of 9 loci previously reported as associated with asthma, 8 SNP associations were validated in the FHS cohorts, and 6 had P-values below 0.05. We also examined the top novel signals for association. Although none reached genome-wide significance, the top ten loci highlighted several interesting candidate genes, including variants near TRPV1/TRPV3 (thermosensitive airway nocireceptor), RNASE3 (eosinophil cationic protein) and ALOX5AP (regulator in the leukotriene biosynthetic pathway). Genomic controlled P-values were $1.3x10^{-5}$, $1.3x10^{-6}$, and $2x10^{-5}$ respectively. Replication in asthma case-control cohorts is ongoing. Finally, we found a strong association between a SH2B3 eosinophil associated variant and atopy in an asthma case-control cohort from NY City (P<0.0006). Conclusion: Asthma and related phenotypes available in population-based cohorts are useful complements to case-control asthma association studies.

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## 974/W/Poster Board #632

**A Genome-wide Association Study of Advanced Age-Related Macular Degeneration Identifies a New Susceptibility Locus in the Lipid Metabolism Pathway and Comprehensively Evaluates Copy Number Variations.** *J.A. Fagerness[1], B.M. Neale[1,2], R. Reynolds[3], L. Sobrin[4], S. Raychaudhuri[1], M. Parker[3], P. Tan[6], E.C. Oh[6], J.E. Merriam[7], E. Souied[8], P.S. Bernstein[9], K. Zhang[9,10], D.J. Zack[6], B. Campochiaro[6], P. Campochiaro[6], N. Katsanis[6], R. Allikmets[7,11], A.M.D. GWAS Consortium[1-11], M.J. Daly[1], J.M. Seddon[3,5].* 1) Center for Human Genetic Research, Massachusetts General Hospital, Program in Medical and Population Genetics, Boston and Broad Institute of Harvard and MIT, Cambridge, Massachusetts 02142, USA; 2) Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, London SE5 8AP, UK; 3) Ophthalmic Epidemiology and Genetics Service, New England Eye Center, Tufts Medical Center, Boston, MA 02111, USA; 4) Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston, Harvard Medical School, Boston, Massachusetts 02114, USA; 5) Department of Ophthalmology, Tufts University School of Medicine, Boston, Massachusetts 02111, USA; 6) McKusick-Nathans Institute of Genetic Medicine, Department of Ophthalmology, Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; 7) Department of Ophthalmology, Columbia University, New York, NY, 10032, USA; 8) Department of Ophthalmology, University Paris 12, Hopital Intercommunal de Creteil, Creteil, 94000, France; 9) Department of Ophthalmology and Visual Sciences, Moran Eye Center, University of Utah School of Medicine, Salt Lake City, UT 84132, USA; 10) Institute for Genomic Medicine and Shiley Eye Center, University of California San Diego, La Jolla, CA 92093, USA; 11) Department of Pathology and Cell Biology, Columbia University, New York, NY 10032, USA.

Age-related macular degeneration (AMD) is the leading cause of late onset blindness and arises from retinal damage associated with accumulation of drusen and subsequent atrophy or neovascularization that leads to loss of central vision. Prior studies have identified several loci explaining roughly half of the overall heritability of AMD, most of which are in the alternative complement pathway. We performed a genome-wide association study (GWAS) of 1000 cases of advanced AMD and 1709 controls, who were all Caucasian and unrelated, using the Affymetrix 6.0 platform (906,000 genotyped single nucleotide polymorphisms(SNPs)and 946,000 genotyped copy number variations(CNVs), to identify additional genetic variants and other pathways contributing to the development of this complex disease. We also conducted several stages of replication of our more significant findings (P < 10-4) using 4013 cases and 1915 controls from five additional independent Caucasian samples with similar phenotypes. Previously associated loci were confirmed to be strongly related to AMD including CFH rs1061170 - 3.84E-56; CFH rs1410996 - 3.52E-48; ARMS2/HTRA1 rs10490924 - 1.32E-60; CFB/C2 rs641153 - 1.07E-07; CFI rs10033900 - 1.26E-05; and C3 rs2230199 - 8.48E-04. Our most significant novel finding is an association with a common functional variant of a gene involved in the lipid metabolism pathway (P = 2.6 x 10-8), and we found this gene to be expressed in the retina. The association shows a consistent effect for both advanced dry and wet AMD and implicates a novel biologic pathway for this disease. We also conducted the first comprehensive analysis of CNVs in AMD. No new CNVs were shown to have genome-wide association to AMD. A previously reported common deletion variant at CFHR1 was associated with advanced AMD in our discovery cohort (P=1 x 10-19), but this observation was due to strong linkage disequilibrium to the two previously reported CFH locus SNPs. The deletion variant showed no association when conditioning on the CFH SNPs, yet both CFH SNPs remained strongly associated with AMD conditional on the deletion variant and the other SNP. The novel association to a gene in the lipid metabolism pathway adds to our previously published prediction algorithm for AMD which includes multiple genetic loci and environmental factors. This result further provides new information about the mechanisms associated with AMD and may provide new avenues for prevention and treatment.

## 975/W/Poster Board #633

**Quantitative trait loci for CD4:CD8 lymphocyte ratio in the general population associate with risk of type 1 diabetes.** *M.A.R. Ferreira[1], M. Mangino[2], M.J. Wright[1], D.R. Nyholt[1], S. Gordon[1], M. Campbell[1], B.P. McEvoy[1], A. Henders[1], D.M. Evans[1,3], J.S. Lanchbury[2], N. Soranzo[2], T.D. Spector[2], I.H. Frazer[4], G. Montgomery[1], N.G. Martin[1].* 1) Queensland Institute of Medical Research, Brisbane, Australia; 2) Twin Research and Genetic Epidemiology Department, King's College London St Thomas' Hospital, London SE1 7EH, UK; 3) Current address: MRC Centre for Causal Analyses in Translational Epidemiology, University of Bristol, Bristol BS8 2BN, UK; 4) The University of Queensland Diamantina Institute for Cancer, Immunology and Metabolic Medicine, Princess Alexandra Hospital, Brisbane, QLD 4102, Australia.

Through a whole-genome association study of lymphocyte levels in the general population, we identified variants in the major histocompatibility complex explaining 7% of the variation in CD4:CD8 ratio, with both class I and II genes independently regulating T cell numbers. Class II variants also associated with type-1 diabetes, suggesting that genetically determined variation in T cell numbers may influence immune-related diseases.

## 976/W/Poster Board #634

**Admixture mapping to identify risk genes for preterm premature rupture of membranes in African-American women.** *L.N. Pearson[1], J.P. Kusanovic[2], R. Romero[2], M.D. Shriver[1], J.F. Strauss 3rd[3].* 1) Department of Anthropology, Pennsylvania State University, University Park, PA; 2) Perinatology Research Branch of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health in Bethesda, Maryland and Wayne State University, Detroit MI; 3) School of Medicine, Virginia Commonwealth University, Richmond, VA.

Preterm delivery is a complex disorder that is a leading contributor to infant mortality in the United States, particularly among African-American women, who are at increased risk. It is evident that risk of preterm delivery is not limited to social and environmental factors, suggesting a role for genetic influences. We are using genome-wide admixture mapping to identify gene regions that affect the risk of preterm delivery, specifically preterm premature rupture of membranes (PPROM), in African-American women using an admixture mapping approach. PPROM is characterized by spontaneous rupture of fetal membranes prior to 37 weeks gestation and constitutes 30% of preterm deliveries. This methodology has revealed novel candidate genes for future studies of PPROM in African-American women.

Self-reported African-American women were recruited at Hutzel Hospital (Detroit), the Hospital of the University of Pennsylvania (Philadelphia) and MCV Hospitals (Richmond) (n = 536). DNA from 275 women with confirmed PPROM and 261 women with normal pregnancy outcomes were genotyped on a genome-wide panel of 1,509 ancestry informative markers (AIMs), single nucleotide polymorphisms (SNPs) with large allele frequency differences between European and West African parental populations. This panel is specifically designed for admixture mapping, a method that exploits linkage disequilibrium created by recent admixture to identify risk regions associated with genetic ancestry, in African-American populations. We used ADMIXMAP, an admixture mapping statistical package, to control for admixture stratification and to test for associations between PPROM and ancestry across the genome. Ancestry estimates and phenotype-ancestry associations were generated using prior allele frequencies from three parental populations, West African, European, and East Asian. The average African contribution to genetic ancestry in the combined sample is 80%. Regions on eight chromosomes were identified as significantly contributing to risk of PPROM in the African-American sample, five associated with West African ancestry (Chromosomes 2, 3, 5, 8 and 11) and three with European ancestry (chromosomes 3, 13 and 21). Several of these loci overlie genes involved in matrix metabolism that are candidate PPROM genes.

## 977/W/Poster Board #635

**Combining Genome Wide linkage and association reveals new candidate gene loci for Celiac Disease.** *A. Torinsson Naluai[1], M. Östensson[2], A.H. Gudjonsdottir[3], H. Ascher[3,4], S. Nilsson[2], J. Wahlström[1].* 1) Medical and Clinical Genetics, Inst. of Biomedicine, Gothenburg, Sweden; 2) Inst. of Mathematical sciences, Chalmers university of Technology, Gothenburg, Sweden; 3) Sahlgrenska University hospital, Gothenburg, Sweden; 4) The Nordic School of Public Health, Gothenburg, Sweden.

The GWASes published so far, including the one on Celiac Disease (CD) are based on a comparison between affected cases and healthy controls. The results provide disease gene variants, which are relatively common in the population and with a small effect on disease risk. If there are gene variants influencing these diseases with large effects they are most likely to be rare in the population. Rare variants are often located on different haplotype backgrounds due to separate founder mutations both within but most certainly between populations. Apart from massive parallell sequencing, there are two ways to detect associations due to rare variants; one way is to stratify the material in some way which selects for those specific individuals that carry the rare variant, e.g. by subphenotype or by an isolated ethnic population. Also, one can use families and combine both genetic linkage and association. The first GWAS in CD, like other diseases, revealed only a small percentage of the genetic variance responsible for CD. Rare variants with a larger effect could not be detected due to limitations of the case-control design. Family-based study designs from the earlier microsatellite linkage scans (including our own) do not have the same limitations. Linkage scans, on the other hand, can not generally pick up common variation underlying the disease. So why then, were the linkage scans not successful? The answer is partly that they were unable to extract all the genetic information from the families. It has been suggested that all these scans should be re-run with today's technology. As far as we know, we are the first to perform a GWAS in families taking advantage of a test using both family linkage information as well as a population based association test. Doing this, we will be able to extract all available information under our linkage peak on chromosome 5. Since we have the strongest linkage of the populations tested so far we also have the best chance of identifying the disease risk variants in this region. Second, we will get complete linkage information across all chromosomes. Third, we will be able to detect new disease variants by combining the linkage and association signals. A selection of 50 CD families have been genotyped using the Illumina 660w-quad chip. The results from the analysis will be presented.

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## 978/W/Poster Board #636

**Testing association with a Reference Control Population: evaluation of case-control matching strategies.** *M.-C. Babron[1,2], H. Perdry[3,4], R. Kazma[1,3], S. Heath[5,6], G.M. Lathrop[5,6], E. Genin[1,2].* 1) INSERM UMR-S946, Paris, France; 2) Univ Paris-Diderot, Paris, France; 3) Univ Paris-Sud, Le Kremlin-Bicêtre, France; 4) INSERM UMR-S535, Villejuif, France; 5) Centre National de Génotypage, CEA, Evry, France; 6) Fondation Jean Dausset/CEPH, Paris, France.

Genome-wide association studies, involving the genotyping of hundreds of thousands of markers in thousands of subjects, are increasingly used to evidence genetic risk factors involved in multifactorial diseases. An attractive idea to minimise cost and work load is to gather a common Reference Control Panel (RCP). Each centre only needs to collect and genotype its own sample of patients, and compare them to the RCP. However, controls must be carefully selected to avoid false positives due to population stratification.

Here, we focus on local studies where cases originate from one country and compare two control matching designs: global matching where the set of cases is compared to a set of controls, and individual matching where each case is compared to one or two genetically matched controls. We investigated three selection schemes: selection at-random, selection based on the IBS, and selection based on the Principal Components Analysis.

From the RCP set up by the Centre National de Génotypage, that includes 6,000 controls from 13 European countries, genotyped on an Illumina 317K chip, we simulated samples of 600 French cases, based on their genotypes for chosen disease susceptibility SNPs, under an additive model (GRR= 1.5). We then selected up to 1,200 controls among the remaining individuals. The chosen disease susceptibility SNPs represent the different stratification characteristics observed across the European populations (no stratification, North-South and East-West clines, ...).

Selection at-random or based on IBS does not perform well. Overall, selecting controls on the basis of the results of Principal Components Analysis is better, both in terms of Type I error and power to detect association. However, even that approach does not correct well when the SNP under study exhibits important stratification.

## 979/W/Poster Board #637

**Multiple genome-wide association studies reveal variants with large effects on biochemical traits measured in blood serum.** *B. Benyamin, A. McRae, M. Ferreira, S. Gordon, R. Middelberg, B. McEvoy, G. Zhu, A. Henders, M. Campbell, L. Wallace, D. Nyholt, N. Martin, G. Montgomery, J. Whitfield, P. Visscher.* Queensland Institute of Medical Research, 300 Herston Road, Brisbane 4029, Australia.

Hundreds of genetic variants associated with complex traits have been identified via genome-wide association studies (GWAS). The individual effects of these variants are typically small and together only explains a small proportion of the heritability. One exception to this general finding appears to be quantitative biochemical traits measured in blood serum. Using GWAS in multiple cohorts, we found genetic variant(s) with large effect influencing variation in serum transferrin levels (a marker for iron overload- and deficiency-related diseases) and serum bilirubin levels (a marker for cardiovascular diseases). Three variants in TF plus the known C282Y mutation in HFE explained ~40% of genetic variation in serum transferrin. Similarly, we also confirm variants within UGT1A1 explaining ~30% of genetic variation in serum bilirubin levels. These results strongly support the hypothesis that genetic architecture of some traits may be simpler than that of disease.

## 980/W/Poster Board #638

**Genome-wide association study of lipid related phenotypes in the Avon Longitudinal Study of Parents and Children (ALSPAC).** *C.R. Boustred[1], N. Timpson[1], B. Glaser[1], D.M Evans[1], P. Deloukas[2], A. Hingorani[5], N. Sattar[3], L. Peltonen[2], J.E. Deanfield[4], G. Davey-Smith[1].* 1) Medical Research Council Centre for Causal Analysis in Translational Epidemiology and Department of Social Medicine, University of Bristol, Bristol, UK; 2) Human Genetics Group, The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK; 3) British Heart Foundation Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, UK; 4) Great Ormond Street Hospital, 30 Guilford St, London, UK; 5) Department of Epidemiology and Public Health, University College London, London, UK.

Genome-wide association studies (GWAS) in population based birth cohorts enable detailed investigation of common genetic variation that is associated with complex human disease. Applying the GWAS strategy to population-based cohorts avoids problems of bias associated with case-control GWAS as well as the traditional problems associated with candidate gene studies. We report GWAS results for three lipid fractions, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides measured in the ALSPAC study. We have used direct genotyping (Illumina 300K chip) and indirect genotype imputation (MACH) to replicate previously reported associations for the first time in children (n = 1400, average age 9.55yrs) as well as to identify previously unreported associations. Replicated associations include the CEPT gene region on chromosome 16 (rs3764261, p = 2.41E-23) and its association with circulating HDL levels, and the CELSR2, PSRC1, SORT1 region on chromosome 1 and its association with circulating LDL levels (rs646776, p = 1.69E-06). Replicated results through genotype imputation include LIPC (15q21, rs4775041, p = 3.58E-06) associated with HDL, APOE (19q13, rs445925, p=2.71x10-19) and the PCSK9 gene region associated with LDL and ZNF259 (11q23, rs964184, p=1.86x10-06) associated with triglycerides. This work provided replication for the first time in a child cohort and substantiates their association with lipid concentrations from an early age. We also report 11 genetic loci that show novel associations with altered circulating lipid levels in children (p < 1 x 10-5). Of the eleven newly identified loci, two SNPs rs2039973 and rs1538833 (10q23 within ~140kb of each other near PPP1R3C and TNKS2) were associated with HDL cholesterol, three with LDL cholesterol, rs1569247 (7p12 near FLJ45974), rs8601 (12p2 in PLEKHA5) and rs5923437 (Xq21 near CHM and DACH2). Novel SNPs associated with triglycerides include, rs2293851 (8p24.1 gene desert), rs9291033 (3q28 in C3ORF59), rs1129055 (3q13.3 in CD86), rs614394 (11q13 near CCND1), rs7553212 (1q41 in ESRRG) and rs2387308 (10p15 in ADARB2). Abnormal variations in HDL, LDL and triglyceride levels are known to alter risk for coronary vascular disease (CVD) and stroke. Evidence suggests that the CVD process starts in childhood. Therefore the genetic variants identified here may be influencing blood lipid concentrations at an early age, resulting in an altered CVD risk later in life.

## 981/W/Poster Board #639

**Genome-Wide Association Study of Type 2 Diabetes and population-based quantitative traits in Asian populations.** *Y.S. Cho, M.J. Go, Y.J. Kim, J.H. Oh, J.Y. Heo, M.H. Lee, H. Min, J.-Y. Lee, B.-G. Han, H.-L. Kim.* Center for Genome Science, Korea National Institute of Health, Seoul, Korea.

Recent advances in genotyping technologies and analytic methods greatly contribute to investigating the entire genome in a large population in order to search for genetic factors controlling complex diseases and traits via genome-wide association study (GWAS). Many GWASs, however, have been accomplished primarily in samples of West European descent to date. Thus, it is not clear if the same or different loci play a role in Asian populations. To conduct GWAS aiming for exploring the genetic basis of lifestyle-related complex diseases such as T2D prevalent in Korea and complex traits with significant public health impact, we initiated the Korea Association Resource (KARE) project in 2007. In this project, almost all of the participants (n= 10,038) aged 40 to 69 in Ansung (n=5,018) and Ansan (n=5,020) cohorts, established as a part of the Korean Genome Epidemiology Study (KoGES) since 2001, were genotyped using the Affymetrix SNP Array 5.0. Using genotyping information and epidemiological data, we analyzed associations between common genetic variants and T2D as well as population-based quantitative traits. So far 11 SNPs showing strong evidence of association with given traits (SBP, pulse rate, WHR, BMI, height, and bone density) were identified and replicated in the independent population. Our results revealed both similarities and intriguing differences in the genes contributing to trait variation in Asian and European populations. In addition to carrying out GWAS, we were able to construct the genomic database for Korean genetic variations. This whole genome-wide SNP information from 10,038 participants in Korean population-based cohorts will facilitate research on the area of genomic science.

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## 982/W/Poster Board #640

**Genome-wide association study of four anthropometric traits in a cohort of 1,798 Filipino women from the Cebu Longitudinal Health and Nutrition Survey.** *D.C. Croteau-Chonka[1], A.F. Marvelle[1], E.M. Lange[1,2], N. Lee[3], L.S. Adair[4], L.A. Lange[1], K.L. Mohlke[1].* 1) Department of Genetics, University of North Carolina, Chapel Hill, NC; 2) Department of Biostatistics, University of North Carolina, Chapel Hill, NC; 3) Office of Population Studies, University of San Carlos, Cebu City, Philippines; 4) Department of Nutrition, University of North Carolina, Chapel Hill, NC.

Genome-wide association (GWA) studies in a variety of population contexts enhance our understanding of the contribution of common genetic variants to human phenotypes. We performed a GWA study for four anthropometric traits ($log_{10}$-transformed body mass index [BMI], weight, waist circumference [WC], and average height) in a cohort of 1,798 unrelated adult Filipino women from the Cebu Longitudinal Health and Nutrition Survey (CLHNS). The associations were adjusted for age, $age^2$, assets, $log_{10}$-transformed income, number of pregnancies, menopausal status, and five principal components of population substructure. We replicated 10 of 84 genome-wide significant SNP-trait associations previously reported in European populations ($p < 5 \times 10^{-8}$) for three of our traits (CLHNS $p < 0.05$, same direction of additive effect): both BMI and weight with *BDNF*, *MC4R*, and *FTO*; BMI alone with *KCTD15*; and height with *EFEMP1*, *ZBTB38*, and *TBX2*. We also replicated three loci with suggestive evidence of association ($p < 10^{-4}$) with either height (*HIST1H1PS2*, *C14orf145*) or BMI (*OTOL1*) in a Korean population. An inter-population difference in local linkage disequilibrium narrowed one of the putative association regions. The CLHNS association signal at *BDNF* is 61% smaller in genomic size (115 kb vs. 294 kb) than the signal described in European populations, and still includes a proposed functional non-synonymous amino acid substitution variant. The strongest SNP not previously reported with our four traits was located nearby the gene *KCNE4*, and was highly associated with both BMI and WC ($p < 5 \times 10^{-6}$). *KCNE4* codes for the potassium voltage-gated channel, Isk-related family, member 4 protein, which acts as an inhibitory subunit to *KCNQ1* (potassium voltage-gated channel, subfamily Q, member 1). *KCNQ1* is expressed in adipose tissue and has been associated with type 2 diabetes in European and Asian populations. Our study also identified two regions (near *RPS24-POLR3A* and *USP13*) containing SNPs that significantly interact with total daily caloric intake to influence WC (tail area-based genome-wide false discovery rate of $q < 0.1$). Replication of these findings and the biological relevance of the nearby genes to their associated traits remain to be determined. Our results are further suggestive evidence for the utility of both non-European populations and differing environmental backgrounds in determining the genetic underpinnings of complex human traits.

## 983/W/Poster Board #641

**The Tobacco and Genetics Consortium: Genome-wide meta-analyses for smoking behavior among 74,035 individuals.** *J. Dackor, H. Furberg, Tobacco and Genetics Consortium.* Department of Genetics, University of North Carolina, Chapel Hill, NC.

Substantial evidence supports that cigarette smoking behavior has genetic determinants. Genome-wide association studies (GWAS) entail an unbiased search of the human genome and have yielded numerous, unsuspected and robust associations for a variety of complex traits. We conducted meta-analyses for smoking behavior using genotype and smoking data from existing GWAS of other traits (e.g., diabetes, cancer, heart disease). Our sample is comprised of 74,035 individuals from 16 different studies in the U.S. and Europe. We examined associations between ~2.5 million imputed markers and four smoking phenotypes, including smoking initiation (ever vs. never smokers), age at onset of smoking, average number of cigarettes smoked per day (CPD) and smoking cessation (current vs. former smokers). We partnered with two other smoking GWAS consortia to provide independent validation for our top 15 loci, resulting in a total sample size of >150,000 individuals. We confirmed the genome-wide significant association between the nicotinic receptor cluster on chromosome 15q25.1 and average CPD (rs1051730 p=5.19 x 10-73), In addition, we identified other genome-wide significant loci associated with average CPD, Ever vs. Never smoking, and Current vs. Former smoking status. No SNPs met genome-wide significance for age at onset of smoking. Findings from this meta-analysis shed light on novel biological pathways involved in multiple smoking phenotypes, potentially identifying novel susceptibility genes and new targets for smoking cessation treatment development.

## 984/W/Poster Board #642

**A Genome-wide Approach For The Identification of Shared Susceptibility Loci.** *D. Ellinghaus[1], M. Nothnagel[2], S. Schreiber[1,3], A. Franke[1].* 1) DFG, Excellence Cluster Inflammation at Interfaces, Institute of Clinical Molecular Biology, Christian-Albrechts-University, Kiel, Germany; 2) PopGen Biobank, Christian-Albrechts-University, Kiel, Germany; 3) First Medical Department, University Clinic S.-H., Campus Kiel, Germany.

Historically, medical indications have been defined anatomic along organ structures. However, recent genetic discoveries indicate that an organ-based definition of disease may not be appropriate as discovered disease genes are often causative for several diseases. Hence, a novel definition of diseases along etiologically relevant pathways may improve our future ability to develop effective therapies. Genome-wide association studies (GWAS) and subsequent meta-analysis projects have been successful in identifying novel genetic susceptibility factors for several autoimmune and inflammatory diseases. Although many examples exist that different disorders share common genetic risk loci (e.g. IL23R in Crohn's disease, ulcerative colitis and psoriasis; NOD2 in Crohn's disease and sarcoidosis), systematic and genome-wide approaches are scarce. We therefore started a joint genome-wide analysis of various immune-related diseases to detect and characterize new disease susceptibility alleles that are relevant for more than one of these diseases. We have recently completed GWAS for various complex diseases. More than 5000 controls and 7000 cases for 11 distinct diseases were genotyped on Affymetrix 6.0 [1000k] or Illumina 550k arrays. Standard and uniform quality thresholds were applied to all data sets before and after imputation with MACH leaving >1.7 Mio. SNPs for inclusion in the analyses. Subsequent case-control analyses were carried out using MACH2DAT. Replication genotyping will be carried out using TaqMan/SNPlex genotyping technology. Different and complementary analysis strategies are currently developed to identify common disease loci and to prioritize associated loci for follow-up studies. Selected SNPs are then subjected to a replication phase using large and independent case-control collections. The project takes a systematic, genome-wide approach by studying an overlay of GWAS data sets from different diseases in clinically relevant combinations. A large-scale replication of the identified loci as well as subsequent resequencing of replicated disease loci will identify the broad spectrum of variation and will yield lists of potentially causative variants. Association analysis results for both the individual analyses and the combined analyses will be made publicly available through a UCSC custom track. The current status of the project will be presented at the conference.

## 985/W/Poster Board #643

**Genomewide association study of age of onset of asthma in the Childhood Asthma Management Program.** *E. Forno[1,2,5], J. Lasky-Su[1], C. Ramsey[4], J. Brehm[1,3,5], B. Klanderman[1], J. Ziniti[1], S.T. Weiss[1,5], J.C. Celedon[1,3,5].* 1) Channing Laboratory, Boston, MA; 2) Division of Pediatric Pulmonology, University of Miami, Miami, FL; 3) Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Boston, MA; 4) Division of Pulmonary and Critical Care Medicine, University of Manitoba, Canada; 5) Harvard Medical School, Boston, MA.

Asthma, a chronic obstructive airway disease, is a major public health problem with known heritability. An earlier age of onset of asthma is associated with increased disease severity in childhood. We performed a genome-wide association study of the age of onset of asthma in 573 affected children in the Childhood Asthma Management Program (CAMP) trial. Participants were 5-12 years of age at enrollment in the trial, and their reported age of onset of asthma ranged from 6 months to 12 years. Genome-wide SNP genotyping was performed by Illumina, Inc. (San Diego, CA) on the HumanHap550v3 BeadChip for CAMP subjects and their parents. After stringent quality-control filters, 512,296 SNPs remained for analysis. In order to control for population stratification, Eigenstrat was used to identify the main eigenvectors describing the population substructure, and these were used as covariates in the analysis. Testing for association between the SNP panel and age of onset of asthma in index children was performed by survival analysis in R via PLINK, using an additive model adjusted for age at enrollment, gender, environmental tobacco exposure during infancy, and the eigenvectors. Two SNPs, one on chromosome (chr.) 11q24 and one on chr. 17p12, were associated with age of onset of asthma after a Bonferroni correction for genomewide significance ($P < 9.8 \times 10^{-8}$). In addition, 12 SNPs among those with the lowest P values had evidence for a biologically plausible role on asthma from prior studies in animal models and/or human studies. Replication studies in independent cohorts are ongoing.

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## 986/W/Poster Board #644

**Interim analysis from a genome-wide association study of childhood obesity identifies *FTO* as strongly associated with the trait.** *S.F.A. Grant[1,2,3], J.P. Bradfield[1], J. Zhao[2], H. Zhang[1], K. Annaiah[1], C.E. Kim[1], J.T. Glessner[1], E.C. Frackelton[1], F.G. Otieno[1], K.A. Thomas[1], M. Garris[1], R.M. Chiavacci[1], K. Wang[1], M. Li[4], R.I. Berkowitz[5,6], H. Hakonarson[1,2,3].* 1) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 3) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA; 4) Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA; 5) Behavioral Health Center and Department of Child and Adolescent Psychiatry, The Children's Hospital of Philadelphia, Philadelphia, PA; 6) Center for Weight and Eating Disorders, Department of Psychiatry, University of Pennsylvania, Philadelphia, PA.

The prevalence of obesity in children and adults in the United States has increased dramatically over the past decade. A number of genetic determinants of adult obesity have already been established through genome wide association (GWA) studies. Although we are still in the midst of recruiting cases and controls for a GWA study of childhood obesity, we elected to carry out an interim analysis of the subjects that have already been genome wide genotyped to date. As such, we generated a data freeze of genome wide SNP genotyping data from 1,285 Caucasian obese children (BMI≥95th percentile) and 6,169 Caucasian controls (BMI<95th percentile) produced on the Illumina Infinium II HumanHap550 or 610S BeadChip platform. All subjects were consecutively recruited from the Greater Philadelphia area from 2006 to 2009 at the Children's Hospital of Philadelphia. BMI≥95th percentile was defined using the Center for Disease control (CDC) z-score= 1.645. All subjects were biologically unrelated and were aged between 2 and 18 years old. All subjects were between -3 and +3 standard deviations of CDC corrected BMI i.e. outliers were excluded to avoid the consequences of potential measurement error or Mendelian causes of extreme obesity. Six common variants (most notably rs9930333) in strong linkage disequilibrium in the *FTO* gene on chromosome 16q12 attained genome-wide significance for childhood obesity association (*P*-value range $6.81 \times 10^{-9}$ to $5.84 \times 10^{-11}$; odds ratio range 1.297-1.338). As such, variants in the *FTO* gene confer a similar magnitude of risk for obesity in children as to what has already been described in their adult counterparts. We did not observe association to any other chromosomal regions at the genome wide significance level, including to other loci previously described from adult obesity studies. Once our GWA study is complete, we will have the opportunity to search for novel variants in the genome that are associated specifically with childhood obesity.

## 987/W/Poster Board #645

**Evidence for significant allele frequency variation as a function of age within and across GWAS control datasets detected using PLINK and MAP.** *T.H. Hamza, D.M. Kay, H. Payami.* Wadsworth Center, New York State Department of Health, Albany, NY.

Background: Hidden age effects in association studies can lead to both false positive signals and missing true associations, therefore, identifying SNPs whose frequencies vary by age could help investigators guard against spurious results. We tested (1) age related allele frequency variation in reference and control populations from three GWAS datasets, and (2) compared allele frequencies over age between datasets. Methods: We used three publicly available GWAS: Intragen population genetics database (Intragen) (914 control subjects, 317,503 SNPs), GWAS in Familial Parkinson Disease (CIDR) (895 control subjects, 344,301 SNPs), and Population Reference Sample (POPRES) (3189 control subjects, 457,297 SNPs), genotyped on Illumina HapMap 300v1.1, Illumina Human CNV370-DUO and Affymetrix 500k respectively. SNPs with MAF <5%, HWE p≤0.00001, and call rates <95% were excluded. We used PLINK to test age-SNP association, treating age as a quantitative trait, and adjusting for population clusters. QQ-plot was used to examine the extent to which the observed distribution of the test statistic followed the expected distribution. We then used frequency moving average plots (MAP) to visualize allele frequency distribution across the age spectrum within each dataset, as well as to test differences between datasets, and to measure the degree of differences by a moving average odds ratios (the MAP method is in press in Genetic Epidemiology). Results: 198 SNPs in Intragen, 5 in CIDR and 1 in POPRES reached $p \leq 10^{-5}$ for association with age. None of these SNPs achieved a $p \leq 10^{-5}$ in all three studies. Comparing intragen and CIDR, both of which used Illumina, 3 SNPs achieved $p \leq 10^{-3}$ in both data sets. Visual inspection by MAPs showed that the pattern of the allele frequency change for all three SNPs was in the same direction in both data sets. We then compared the frequency distribution of these 3 SNPs between pairs of datasets (intragen vs. CIDR vs. POPRES), and found significant differences (p<0.001) that generated odds ratios as large as 2.0 (95% CI: 1.5-2.5). Conclusions: (1) There can be significant allele frequency variation as a function of age within a dataset which if undetected could skew the association results. (2) Control datasets can differ considerably; therefore caution should be taken when borrowing controls. Visual inspection of data using MAP can help detect and control for these anomalies.

## 988/W/Poster Board #646

**Combination of Case-Control and Family-based Asthma Genome-wide Association Results Using Probands from a Single Population.** *B.E. Himes[1,2,3,4], J. Lasky-Su[2], A.J. Murphy[2], G.M. Hunninghake[2], B. Klanderman[2], R. Lazarus[2], J.C. Celedón[2], B.A. Raby[2], E.K. Silverman[2], S.T. Weiss[2,4].* 1) Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA; 2) Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 3) Children's Hospital Informatics Program, Boston, MA; 4) Partners Center for Personalized Genetic Medicine, Boston, MA.

Asthma is a chronic inflammatory airway disease with well-established heritability that affects over 300 million people around the world. The Childhood Asthma Management Program (CAMP) was a clinical trial of asthma treatment that gathered genome-wide genotype data for family-based genomic studies of a subset of 422 Caucasian CAMP probands and their parents. In addition to using a family-based approach for the study of asthma, we increased our ability to detect associations by comparing CAMP probands to Caucasian controls that are publicly available through the Illumina ICONdb resource. After stringent quality-control filters were applied and genomic ancestry matching (GEM) was used to control for population stratification, data for 516,617 single nucleotide polymorphisms (SNPs) in 359 CAMP cases and 846 Illumina controls was used for case-control association analysis. The 5604 SNPs with case-control Cochran-Armitage trend test association p-values less than 0.01 were compared to those SNPs that had family-based FBAT additive model association p-values less than 0.05 in families. Both of these criteria were met by 1726 SNPs. A subset of 1377 of the 1726 SNPs was successfully genotyped in an Illumina 1536 GoldenGate assay in an independent population, the Genetics of Asthma in Costa Rica. Of the 1377 SNPs, 78 had 1-sided p-values less than 0.05 with effects in the same direction as CAMP. Replication of these results is ongoing in another independent population of Partners HealthCare patients recruited through the Informatics for Integrating Biology & the Bedside (i2b2) National Center for Biomedical Computing and the Crimson Project. Replication results in this third population will provide further evidence for the efficacy of screening SNPs based on both case-control and family-based analyses and of the validity of association of the 78 SNPs to asthma.

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## 989/W/Poster Board #647

**Genome-wide association study of serum lipoprotein(a) concentration identifies multiple associated SNPs in addition to the kringle repeat.**
*S.M. Hosseini[1,2], E. Shen[3], D. Waggott[3], A.P. Boright[4], L. Sun[1,5], S.B. Bull[3,5], S.M. Marcovina[6], J.D. Brunzell[6,7], A.D. Paterson[1,2,5], the DCCT/EDIC Research Group.* 1) Genetics & Genome Biology Program, The Hospital for Sick Children, Toronto, ON, Canada; 2) Institute of Medical Science, University of Toronto, Toronto, ON, Canada; 3) Samuel Lunenfeld Research Institute of Mount Sinai Hospital, Prosserman Centre for Health Research, Toronto, ON, Canada; 4) Department of Medicine and University Health Network, University of Toronto, Toronto, ON, Canada; 5) Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada; 6) Northwest Research Laboratory, Seattle, WA, USA; 7) Division of Metabolism, Endocrinology and Nutrition, Department of Medicine, University of Washington School of Medicine, Seattle, WA, USA.

**Background:** Lipoprotein (a) (Lp(a)) is an established independent risk factor for cardiovascular and cerebrovascular diseases. Serum level of Lp(a) is highly heritable. Based on linkage studies, most of the genetic effect is due to the *LPA* locus encoding for apolipoprotein(a), the unique structural protein of Lp(a). About half of the genetic effect is thought to be attributable to a VNTR, coding for kringle IV-2 (KIV-2) domain of apolipoprotein(a). The remainder of the effect at *LPA* locus is attributed to additional sequence variations, which are not fully understood. Genome-wide linkage studies have been inconsistent in detecting loci other than the main *LPA* locus.

**Methods:** A genome-wide association study was performed for serum Lp(a) level in 1236 white individuals with type 1 diabetes from the Diabetes Control and Complications Trial (DCCT) who were genotyped using the Illumina 1M chip. 841,342 SNPs with a minor allele frequency >1% were subjected to analysis. The number of KIV-2 repeats was determined in a multiplex qPCR reaction using two TaqMan® probes matching different regions of the repeat element. A series of analyses were performed: we tested for association between each SNP and serum Lp(a) level; SNPs in the *LPA* region (*LPA* gene and 0.5Mb on each side) were then evaluated for association with KIV-2 copy number; we finally fine-mapped the effect of SNPs on Lp(a), after adjusting for KIV-2, using forward selection in a linear regression framework.

**Results:** Significant evidence for association with serum Lp(a) levels was observed for a group of SNPs in the *LPA* region (57 SNPs with a $p<5\times10^{-8}$). KIV-2 copy number variation alone explained about 20% of variation in serum Lp(a) level ($p=4\times10^{-64}$). After adjusting for the effect of KIV-2 copy number variation, 54 SNPs from *LPA* region retained genome-wide significant ($p<5\times10^{-8}$) evidence of association with serum Lp(a) level. Using forward selection, 13 SNPs at the *LPA* locus showed evidence of association with Lp(a) in multivariate analysis (five of which had p-values $<5\times10^{-8}$).

**Conclusion:** We identify multiple SNPs in the *LPA* region that contribute to serum Lp(a) level independent of kringle copy number in the DCCT study population. Conditional genome-wide analysis after adjusting for the main effects at the *LPA* locus is underway to discover potential trans genetic effects.

## 990/W/Poster Board #648

**Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations.**
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Elevated serum uric acid levels cause gout and are a risk factor for cardiovascular disease and diabetes. To investigate the polygenetic basis of serum uric acid levels, we conducted a meta-analysis of genome-wide association scans from 14 studies totalling 28,141 participants of European descent, resulting in identification of 954 SNPs distributed across nine loci that exceeded the threshold of genome-wide significance, five of which are novel. Overall, the common variants associated with serum uric acid levels fall in the following nine regions: *SLC2A9* ($p=5.2 \times 10^{-201}$), *ABCG2* ($p=3.1 \times 10^{-26}$), *SLC17A1* ($p=3.0 \times 10^{-14}$), *SLC22A11* ($p=6.7 \times 10^{-14}$), *SLC22A12* ($p=2.0 \times 10^{-9}$), *SLC16A9* ($p=1.1 \times 10^{-8}$), *GCKR* ($p=1.4 \times 10^{-9}$), *LRRC16A* ($p=8.5 \times 10^{-9}$) and near *PDZK1* ($p=2.68 \times 10^{-9}$). Identified variants were analyzed for gender differences. We found that the minor allele for rs734553 in *SLC2A9* has greater influence in lowering uric acid levels in women and the minor allele of rs2231142 in *ABCG2* elevates uric acid levels more strongly in men compared to women. To further characterize the identified variants, we have analyzed their association with a panel of metabolites. rs12356193 within *SLC16A9*, was associated with DL-carnitine ($p=4.0 \times 10^{-26}$) and propionyl-L-carnitine ($p=5.0 \times 10^{-8}$) concentrations, which in turn were associated with serum UA levels ($p=1.4 \times 10^{-57}$ and $p=8.1 \times 10^{-54}$, respectively), forming a triangle between SNP, metabolites and UA levels. Taken together, these associations highlight additional pathways that are important in the regulation of serum uric acid levels and point towards novel potential targets for pharmacological intervention to prevent or treat hyperuricemia. In addition, these findings strongly support the hypothesis that transport proteins are key in regulating serum uric acid levels.

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## 991/W/Poster Board #649

**Fine scale analysis and gene dicovery for age related macular degeneration.** *L. Kopplin[1], R.P. Igo[2], Y. Wang[2], T.A. Sivakumaran[2], S.A. Hagstrom[4], N.S. Peachey[4, 5], P.J. Francis[6], M.L. Klein[6], J.P. SanGiovanni[7], E.Y. Chew[7], D.V. Leontiev[2], G. Jun[2], L. Tian[2], T. Joshi[2], Q. Xi[2], A.K. Henning[8], K.E. Lee[9], R. Klein[9], B.E.K. Klein[9], S.K. Iyengar[1,2,3].* 1) Department of Genetics, Case Western Reserve University, Cleveland, OH; 2) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH; 3) Department of Ophthalmology, Case Western Reserve University, Cleveland, OH; 4) Cole Eye Institute, Cleveland Clinic Foundation, Cleveland, OH; 5) Louis Stokes Veteran Affairs Medical Center, Cleveland, OH; 6) Casey Eye Institute, Oregon Health & Science University, Portland, OR; 7) Division of Epidemiology and Clinical Applications, National Eye Institute, Bethesda, Maryland; 8) The EMMES Corporation, Rockville, Maryland; 9) Department of Ophthalmology and Visual Science, University of Wisconsin School of Medicine and Public Health, Madison, WI.

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly in the developed world. To identify novel genetic risk factors for AMD, we completed genome-wide association studies in two discovery cohorts followed by replication in 1940 cases and 1955 controls. Testing the full spectrum of disease severity, we examined 361,556 SNPs for association with a 15-step AMD severity scale in 294 individuals from 34 extended pedigrees from the Family Age Related Maculopathy Study and examined 186,807 SNPs for association with case-control status in 586 subjects collected as part of the Age Related Eye Disease Study. We followed up initial findings in five case-control replication cohorts. Strong evidence for association was observed with SNPs at the CFH and ARMS2 loci, with the best findings at rs3753395 (OR = 0.35, $p = 3.7 \times 10^{-64}$) and rs10490924 (OR = 2.79, $p = 2.0 \times 10^{-60}$), respectively. We also observed a protective effect at the C2/BF region, with our strongest result at a previously unreported marker ($p = 9.99 \times 10^{-16}$). Finally, we report the identification of a novel protective locus on chromosome 3 which implicates a pathway previously underappreciated for its potential in contributing to AMD pathogenesis. In summary, we have confirmed existing genes and after conditional analysis have identified novel loci involved in AMD pathogenesis.

## 992/W/Poster Board #650

**Quality assurance of genotypic data for genome-wide association studies.** *C. Laurie[1], T. Bhangale[1], X. Zheng[1], C. McHugh[1], B. Weir[1], L. Bierut[2], J. Rice[2], H. Edenberg[3], D. Mirel[4], E. Pugh[5], K. Doheny[5], E. Harris[6], T. Manolio[7], The GENEVA Investigators.* 1) Dept. Biostatistics, University of Washington, Seattle, WA; 2) Dept. Psychiatry, Washington University School of Medicine, St. Louis, MO; 3) Dept. Biochem. Molec. Biology, Indiana University School of Medicine, Indianapolis, IN; 4) Broad Institute of MIT and Harvard, Cambridge, MA; 5) Center for Inherited Disease Research, Johns Hopkins University, Baltimore, MD; 6) Division of Extramural Research, NIDCR. Bethesda, MD; 7) Office of Population Genomics, NHGRI, Bethesda, MD.

Genome-wide scans of nucleotide variation in human subjects are providing an increasing number of replicated associations with complex disease traits. Most of the variants detected have small effects and, collectively, they account for a small fraction of the total genetic variance. Very large sample sizes are required to validate initial findings and to increase the number of new findings. In this situation, even small sources of bias and noise can cause spurious results or obscure real effects. The need for careful attention to data quality has been appreciated for some time in this field, and a number of strategies for quality control and quality assurance (QA/QC) have been developed. We have extended these methods to include some new approaches, which are illustrated here with examples from the “Gene Environment Association Studies” (GENEVA) program. Some key results: (1) Using probe intensities on the X and Y chromosomes, sex chromosome aberrations (e.g. XXY, XX/XO mosaics) can be detected that might otherwise be considered gender misidentification. (2) Using a measure of allelic imbalance, we detect contaminated samples and autosomal aberrations (in cell lines and blood samples) that may affect genotype calling accuracy. (3) We construct SNP filters based on duplicate concordance measures and suggest duplicate sample sizes appropriate to distinguish among SNPs with different error rates. (4) We have detected significant effects of numerous experimental factors on missing call rate, including tissue type, tissue collection date, DNA extraction method and date, study site, plate, well and genotyping batch. (5) Relatedness is inferred from IBD estimates with consideration of the variance of expected values. Poor quality samples can be detected as those with high relatedness connectivity. (6) We demonstrate sensitivity of principal components to SNP selection. (7) Hardy-Weinberg equilibrium test p-values plotted against minor allele frequency reveal distinct genotyping artifacts.

## 993/W/Poster Board #651

**Identification of 6 Loci for BMI in the Korean Population.** *H. Lee, J. Kim, J. Lee.* Genome Research Center, ASAN institute of life science, Seoul, Korea.

Obesity results from a greater intake of calories than the body requires. This is a serious public health problem which is associated with increase of risk such as type 2 diabetes mellitus, hypertension, dyslipidemia, cardiovascular diseases, sleep apnea and certain cancers. To find out loci associated with obesity in Asians, genome-wide association studies (GWAS) for body mass index (BMI), which is highly heritable and broadly used to diagnose obesity, has been conducted in the Korean population (n= 8842) using Affymetrix 500K SNP chip. In our study, we identified 4 novel BMI loci (FAM124B, RGS12, NFE2L3 and TNR) ($P < 0.00001$) as well as two previously reported BMI loci, FTO and MC4R in Koreans. Our study provides new candidate genes for BMI in Asians, and suggests that FTO and MC4R may be worldwide obesity-risk genes.

## 994/W/Poster Board #652

**A common variant on chromosome 11q13 is associated with atopic dermatitis.** *Y.A. Lee[1,2], J. Esparza-Gordillo[1,2], S. Weidinger[3,4], R. Fölster-Holst[5], A. Bauerfeind[1], F. Rüschendorf[1], K. Rohde[1], I. Marenholz[1,2], F. Schulz[1,2], T. Kerscher[1,2], N. Hübner[1], S. Schreiber[6,7], A. Franke[6], S. Heath[8], N. Novak[9], E. Rodriguez[3,4], T. Illig[10], M. Lee-Kirsch[11], M. Macek[12], A. Rüther[6].* 1) Max-Delbrück-Centrum, Berlin, Germany; 2) Pediatric Pneumology and Immunology, Charité, Berlin, Germany; 3) Department of Dermatology and Allergy, Technische Universität München, Munich, Germany; 4) Division of Environmental Dermatology and Allergy, Helmholtz Zentrum Munich and ZAUM-Center for Allergy and Environment, Technische Universität München, Munich, Germany; 5) Clinic for Dermatology, Venerology and Allergology, University Hospital Schleswig-Holstein, Kiel, Germany; 6) Institute for Clinical Molecular Biology, Christian-Albrechts-University, Kiel, Germany; 7) POPGEN Biobank Project, Christian-Albrechts-University, Kiel, Germany; 8) Centre National de Génotypage, Evry, France; 9) Department of Dermatology and Allergy, University of Bonn, Bonn, Germany; 10) Department of Epidemiology, Helmholtz Zentrum Munich, Germany; 11) Klinik fur Kinder- und Jugendmedizin, Technical University Dresden, Dresden, Germany; 12) Department of Biology and Medical Genetics, Charles University Prague - 2. Medical School and Faculty, Hospital Motol, Prague, Czech Republic.

Atopic dermatitis (AD or eczema) is a chronic inflammatory skin disorder and a major manifestation of allergic disease. In the industrialized countries, the prevalence of AD is approximately 15 % with a steady increase over the past decades. Genetic and environmental factors interact to determine disease susceptibility, and family and twin studies indicate that the genetic contribution is substantial. The molecular mechanisms underlying eczema are not fully understood. To identify genetic variants contributing to AD, we conducted a genome-wide association study in 939 individuals with AD and 975 controls as well as 270 complete nuclear families with 2 affected siblings. SNPs consistently associated with AD in both discovery sets were then investigated in two additional independent replication sets totalling 2637 cases and 3957 controls. Highly significant association was found with a common sequence variant on chromosome 11q13.5 (Pcombined = 7.6 x 10-10) in all 4 study groups. Approximately 13% of individuals of European origin are homozygous for the risk allele, and their risk of developing AD is 1.47 times that of noncarriers. Notably, the same AD risk allele reported here has recently been identified as a susceptibility factor for Crohn’s disease. Crohn’s disease is a complex chronic inflammatory bowel disorder sharing many pathophysiological characteristics with AD such as recurrent inflammation of the epithelial barrier, defective cutaneous/mucosal barrier function, and deficient innate immune responses against bacterial infections. Our data suggest that rs7927894[A] confers susceptibility to AD and Crohn’s disease jointly which may contribute to the higher incidence of AD observed among Crohn’s patients. LD analysis in Hapmap showed that rs7927894 is located in a 200 kb LD block containing C11orf30. C11orf30 encodes the nuclear protein EMSY which has been implicated in chromatin modification, DNA repair, and transcriptional regulation. Finally, we provide a list of additional candidate genes. Further replication in independent cohorts, fine mapping and functional studies will be required to gain a better understanding of the physiological mechanisms underlying this common allergic disorder.

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## 995/W/Poster Board #653

**GWA study of a genetic subisolate identifies variants in C7 and STAT3 predisposing to multiple sclerosis and suggest a positive selection of a "protective"haplotype outside Africa.** *V. Leppä[1,2,3], E. Jakkula[1,2,4], A.-M. Sulonen[1,2], S. Kallio[1,2], S. Purcell[4,10], P. Tienari[5], K. Koivisto[6], I. Elovaara[7], M. Reunanen[8], T. Pirttilä[9], A. Oturai[11], H. Sondergaard[11], H. Harbo[12], I.-L. Mero[12], P. De Jager[13], D. Hafler[13], M.J. Daly[4,10], A. Palotie[1,14], J. Saarela[1,2], L. Peltonen[1,2,14].* 1) Unit of Public Health Genomics, National Institute for Health and Welfare, Helsinki, Finland; 2) Institute for Molecular Medicine Finland, Helsinki, Finland; 3) Helsinki Biomedical Graduate School, University of Helsinki, Finland; 4) The Broad Institute of MIT and Harvard, Cambridge, MA, USA; 5) Dept. of Neurology, Helsinki Univ. Central Hospital, Helsinki, Finland; 6) Central Hospital of Seinajoki, Seinajoki, Finland; 7) Dept. of Neurology, Tampere Univ. Central Hospital, Tampere, Finland; 8) Oulu University Central Hospital, Oulu, Finland; 9) Dept. of Neurology, Kuopio University Central Hospital, Kuopio, Finland; 10) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA; 11) Danish Multiple Sclerosis Research Center, Copenhagen University Hospital, Copenhagen, Denmark; 12) Department of Neurology, Faculty Division Ullevål Universtity Hospital, University of Oslo, Oslo, Norway; 13) Dept. of Neurology, Brigham & Women's Hospital, Boston, MA, USA; 14) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK.

Prevalence of multiple sclerosis (MS) in the Southern Ostrobotnia (SO) sub-isolate region of Finland is 2-fold compared to other populations of Northern European descent. We used genealogical information up to 15 generations back to construct 2 SO megapedigrees. We hypothesize that one or more variants predisposing to MS may be regionally enriched and these pedigrees can be used to identify new, potentially rare MS alleles in GWA. We studied if variants in extended regions of homozygosity might affect MS susceptibility in this megapedigree. 68 MS cases from SO were genotyped using the Illumina 317K chip and 136 IBS matched population controls were selected from a control set of Finnish GWA data. We identified 2 tentative regions (>50 SNPs, >500kb): 1q42.12, 2q24.3. In 1q 16% of the MS (n=11, 9 same allele) and 8% of the controls (n=11, 10 same) and in 2q 40% of MS (n=27, 25 same) and 20% of controls (n=27, all the same) were homozygous. To test if we could identify a shared associated haplotype in this special sample, we performed a 5 SNP sliding window scan over linked regions. One haplotype flanking the C7-FLJ40243 locus on 5p12-14 showed tentative association (p <10-4). The association was replicated in an independent sample from the SO region (p<10-5). Finally to complete the GWA scan in SO we performed single SNP association analysis. Twenty eight loci with liberal p <10-4 were selected for replication in an independent Finnish set of 753 cases and 1029 controls. This identified 2 associated SNPs outside the HLA-region: rs1364194 (p=0.0046) and rs744166 in STAT3 gene (p= 0.0012). The 2 loci were further replicated in 6 GWA meta-analysis study sets (UK, CH, NL and 3 US) and in Nordic (DK, NO) samples (4638 cases, 10279 controls). Analysis of the replication sets using Cochran-Mantel-Haenszel population stratified analysis confirmed the association for STAT3 (p 2.65x10-10). The rs744166 T-allele underrepresented in MS cases tags one haplotype in CEU, CHB and JPT Hapmap populations (>56%) which is less common in YRI (7%). This might indicate positive selection of the "protective" haplotype outside Africa. Using the strength of special population structure, we identified 4 tentative susceptibility regions, 2 confirmed so far. Both genes are relevant biological candidates for MS: C7 is a rate limiting component of the innate immune system and STAT3. In previous studies mice with a knock-out of STAT3 in CD4+ T cells were resistant to EAE.

## 996/W/Poster Board #654

**Genome-wide association study of asthma identifies RAD50-IL13 and HLA-DR/DQ regions.** *X. Li, T.D. Howard, S. Zheng, E.R. Bleecker, D.A. Meyers.* Center for Human Genomics, Wake Forest University School of Medicine, Winston Salem, NC.

Asthma is a heterogeneous and complex disease with strong genetic susceptibility. Genome-wide association study (GWAS) is a powerful tool to study association of genetic markers with disease. To date, few GWAS of asthma have been performed. We performed a GWAS of asthma in a difficult-to-treat asthma population to identify novel genes and confirm previously identified genes involved in asthma.

A GWAS was performed with 292,443 SNPs to test for association with asthma in 473 TENOR cases and 1,892 Illumina general population controls. Asthma-related quantitative traits (total serum IgE, FEV1, FVC, and FEV1/FVC) were also tested for association with the SNPs on identified candidate regions in 473 TENOR cases and 363 controls without a history of asthma to confirm GWAS results. Imputation was performed in identified candidate regions for analysis with denser SNP coverage.

Multiple SNPs in the RAD50-IL13 region on chromosome 5q31.1 were strongly associated with asthma and peaked at rs2244012 in intron 2 of *RAD50* (P = 3.04E-07; Bonferroni adjusted P = 0.089). Several SNPs in *RAD50* and *IL13* were also associated with quantitative traits (P ≤ 0.05). The HLA-DR/DQ region on chromosome 6p21.3 showed consistent association with asthma and peaked at rs1063355 in the 3' UTR of *HLA-DQB1* (P = 9.55E-06). Imputation identified several significant SNPs in the TH2 locus control region (LCR) 3' of *RAD50*. Imputation also identified a more significant SNP, rs3998159 (P = 1.45E-06), between *HLA-DQB1* and *HLA-DQA2*.

Our GWAS confirmed the important role of TH2 cytokine and antigen presentation genes in asthma at a genome-wide level. Furthermore, our findings will stimulate more comprehensive research on these two regions due to their functional importance and structural complexity.

## 997/W/Poster Board #655

**Genome-wide meta-analysis of 38,455 subjects for smoking related traits.** *Z. Liu[1], F. Tozzi[2], V. Mooser[3], L. Cardon[3], C. Francks[4], J. Marchini[1].* 1) Dept Statistics, Oxford Univ, Oxford, United Kingdom; 2) Genetics Division, Drug Discovery, GlaxoSmithKline Research and Development, 37135 Verona, Italy; 3) Genetics Division, GlaxoSmithKline, King of Prussia, PA 19406, USA; 4) Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK.

Smoking is a leading global cause of disease and mortality. The nicotine present in tobacco acts on nicotinic acetylcholine receptors in the brain to release dopamine and other neurotransmitters that sustain addiction. Smoking and Nicotine Dependence are multifactorial, partly heritable traits. We have performed a genomewide meta-analytic association study of smoking-related traits in a total sample of 38,455 individuals drawn from 21 disease, population, and control cohorts (The arc Epidemiology Unit Rheumatoid Arthritis cohort, The Bright Study, The Cambridge GEM Consortium, The GEMS Study, The Bergen COPD case control collection, The GSK/IOP/CAMH bipolar cohort, The GSK/MPI depression cohort, The UK IBD Genetics Consortium, The KORA Study, The KORCULA Study, The LOLIPOP Study, The Medstar Cohort, The Northern Sweden Population Health Study, The ORCADES Study, The PennCATH cohort, The Popgen Biobank Study, The PsyCoLaus cohort, The SardNIA study, The Study of Health in Pomerania, The VIS Study and The WTCCC-CAD cohort). We used imputation based on the CEU HapMap haplotype to combine datasets from different genotyping chips. We attempted replication of associated regions within the TAG and ENGAGE smoking meta-analysis datasets, which are large, independent datasets that have been analysed concurrently with our own. For the cigarettes per day (CPD) phenotype (N=14,952) our analysis confirmed an effect in the chromosome 15 nicotine receptor cluster (P=2.5x10-18 in our own analysis and P=5.19 x 10-73 across all 3 meta-analyses at rs1051730). These results act as a clear positive control for our meta-analysis. A fine mapping analysis of the region suggests other more significantly associated novel variants. Of the 15 regions for the CPD that we took forward for in-silico replication in the TAG and ENGAGE datasets two regions, on chromosomes 8 and 12 (P<10-5), show some evidence of replication in the other studies (P<0.01). Analysis of the smoking initiation phenotype (Ever/Current vs Never smoking status) did not uncover any associations that we could replicate. While confirming that the CHRNA5/A3 region is likely to harbor the strongest common effect on smoking-related traits in the genome, our analysis has identified novel potential loci that await further investigation.

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## 998/W/Poster Board #656

**Modeling genes for astigmatism.** *M.C. Lopes, A. Toby, T.D. Spector, C.J. Hammond.* Department of Twin Research and Genetic Epidemiology, King's College London, London, United Kingdom.

Astigmatism is a complex trait which is influenced by numerous genetic and environmental factors. Recent large twin studies of refractive error have found a high heritability of approximately 60% where the dominant effects explained most of the phenotypical variance. Here we report the first genome-wide association study on astigmatism for 2341 twin UK subjects (1172 DZ), with 318 232 SNPs based on polygenic models. A follow up candidate gene study including fine-mapping analysis was performed after imputing SNPs for the candidate regions. Replication analysis as well as meta-analysis was conducted for 1749 independent twin UK subjects using 537 179 SNPs. We identified significant SNP association ($P<1.05x10^{-5}$) with astigmatism in genes AC011592.5 (SNP rs2727385), DACH1 (SNP rs7328290) and COL13A1 (SNP rs2642609). None of these top hits were replicated. However, after completing the meta-analysis the rs2642609 SNP attained genome-wide significance ($1.14x10^{-5}$). This SNP is upstream of gene COL13A1 and is in a LD block interrupted by recombination hot-spots. Eight informative imputed SNPs in high LD ($r^2>0.8$) with the lead SNP rs2642609 have been identified and will be genotyped. A subset of these will be used for a haplotype study to characterize the common haplotypes associated with astigmatism. COL13A1 is a type of connective tissue, the organization of which affects the refraction of the cornea and then the astigmatism. This study provides new insights into astigmatism disease and advances an understanding of refractive error disease, which affects a considerable part of the world population.

## 999/W/Poster Board #657

**The POCEMON (Point-Of-Care MONitoring and Diagnostics for Autoimmune Diseases) project: building a Lab-On-Chip centered on Rheumatoid Arthritis and Multiple Sclerosis.** *S. Lupoli[1,2], C. Cosentino[2], V. Tieran[2], F. Taddeo[2], S. Atkinson[3], A. Barton[4], A.G. Wilson[3], D. Plant[4], J.R. Maxwell[3], E. Graudens[5], I. Chumakov[5], F. Kalatzis[6], F. Macciardi[2].* 1) INSPE, HsR Scientific Institute, Milan, Italy; 2) University of Milan, Milan, Italy; 3) University of Sheffield, Sheffield, UK; 4) arc Epidemiology Unit, University of Manchester, Manchester, UK; 5) Pharnext S.A., Paris, France; 6) Unit of Medical Technology and Intelligent Information Systems, University of Ioannina, Ioannina, Greece.

POCEMON is a large-scale integrated project founded from the European Commission (FP7-ICT-2007-216088). The aims of the project are to develop an integrated diagnostic platform mainly dedicated to Rheumatoid Arthritis (RA) and Multiple Sclerosis (MS) in a first stage by combining Lab-On-Chip technology, DNA microarray genotyping, microelectronics, mobile devices, intelligent algorithms and wireless communications. MS and RA are two progressive autoimmune diseases and are causes of disability in young adults. Considering the social relevance of MS and RA, it is extremely important to improve the timing and confidence of the diagnosis. According to this vision the POCEMON project can be a real milestone for the improvement of life style of many patients. In a first (discovery) phase, we are performing a case/control whole genome association study, mostly centered to identify HLA (Human Leukocyte Antigens) and other potentially relevant susceptibility genes for the two diseases. For the discovery phase of RA, we are using a homogeneous North-European population (with 800 cases and 800 controls). The discovery is then followed by a confirmatory phase, where the "best" SNPs are evaluated in a second independent sample (2000 cases affected by RA or MS respectively and relative controls), from three separate cohorts. The genotyping phase for RA, using Illumina HumanCNV-370, is concluded. Plink 1.06 is used for QC of genotyping data and Eigenstrat 3 is used for adjusting population structure in genetic association. The analysis is ongoing. Initial findings pointed to several susceptibility genes across the genome involved in the disorder other than confirming a role of HLA.

## 1000/W/Poster Board #658

**GWAS in patients with non-syndromic cleft lip with or without cleft palate (NSCL/P) of European descent.** *E. Mangold[1], S. Birnbaum[1], K.U. Ludwig[2], C. Baluardo[3], H. Reutter[1], S. Herms[2], N.A. de Assis[1], M. Ferrian[3], C. Lauster[4], B. Braumann[5], R.H. Reich[6], A. Hemprich[7], R.P. Steegers-Theunissen[8], F.-J. Kramer[9], T.F. Wienker[10], P.A. Mossey[11], P. Hoffmann[1,2], M. Rubini[3], M. Knapp[10], M.M. Nöthen[1,2].* 1) Institute of Human Genetics, University of Bonn, Bonn, Germany; 2) Department of Genomics, Life and Brain Center, University of Bonn, Bonn, Germany; 3) Department of Experimental and Diagnostic Medicine, Medical Genetics Unit, University of Ferrara, Ferrara, Italy; 4) Department of Cleft Lip and Cleft Palate Surgery, Humboldt University of Berlin, Berlin, Germany; 5) Department of Orthodontics, University of Cologne, Cologne, Germany; 6) Department of Oral and Maxillo-Facial-Plastic Surgery, University of Bonn, Bonn, Germany; 7) Department of Oral and Maxillo-Facial Surgery, University of Leipzig, Leipzig, Germany; 8) University Medical Center, Rotterdam, The Netherlands; 9) Department of Oral and Maxillofacial Surgery, University of Göttingen, Göttingen, Germany; 10) Institute of Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany; 11) Orthodontic Unit, Dental Hospital & School, University of Dundee, Dundee, UK.

Non-syndromic orofacial clefts are considered to have a multifactorial etiology with a strong genetic background. The most frequent form is the non-syndromic cleft lip with or without cleft palate (NSCL/P). We conducted a two-step genome-wide association study involving NSCL/P-patients and controls of Central European origin in order to identify novel susceptibility loci for NSCL/P. The first step of our study involved 224 NSCL/P-patients and 383 controls. A 640-kb region at chromosome 8q24.21 was found to contain multiple markers with strongly significant evidence for association with the cleft phenotype, including three markers which reached genome-wide significance. The 640-kb cleft-associated region was saturated with 146 SNP markers and analyzed in our entire NSCL/P sample of 462 unrelated patients and 954 controls. In the entire sample, the most significant SNP (rs987525) had a P value of 3.34 ×10-24. The OR was 2.57 (95% CI: 2.02-3.26) for the heterozygous genotype and 6.05 (95% CI: 3.88-9.43) for the homozygous genotype. The calculated PAR for this marker is 0.41, suggesting that this is a key susceptibility locus for NSCL/P (Birnbaum et al., Nature Genetics 41, 473-477). Interestingly, we found no evidence of any interaction between the 8q24.21 locus and IRF6, the only generally accepted NSCL/P gene to date. In the second step of our GWAS we extended the sample to 399 patients and 1318 controls. 110 SNPs not mapping to the 8q24.21 region with P values below 10-4 were found. We investigated 47 of the best SNPs in an independent replication sample of 793 triads and found genome-wide significant association in the combined sample for two loci and suggestive evidence for association for three additional loci. Our extension of the GWAS adds two new loci to the list of confirmed NSCL/P susceptibility loci and suggests three loci for independent replication.

## 1001/W/Poster Board #659

**Variational Bayes algorithms for accurate multiple locus and multiple factor genome-wide association (GWA) analysis.** *J. Mezey, B. Logsdon, C. Gao, G. Hoffman, A. Brisbin, L. Omberg.* Biological Statistics (BSCB), Cornell Univ, Ithaca, NY.

Genome-wide association (GWA) studies have successfully identified loci that explain a small percentage of heritable variation in disease susceptibility and other complex aspects of human physiology. GWA algorithms that account for multiple loci and non-genetic factors could be used to explain missing heritability, by discovering associations hidden from standard GWA analysis. Making use of variational Bayes approximate inference techniques, we have developed a class of algorithms that provide a novel solution to the computational constraints and accuracy concerns associated with most multiple locus / multiple factor GWA algorithms. These variational Bayes algorithms simultaneously assess all genetic markers in a GWA study and have excellent scaling properties, such that an analysis of a thousand individuals, genotyped at one million markers, can be run on a desktop in a matter of hours. By analyzing simulated data, we demonstrate these algorithms can reliably identify associations that are too weak to be found by single-marker testing approaches, when using a highly conservative control of false positives. We use these algorithms to identify new locus associations with gene expression, replicated in multiple populations of HapMap, and to identify associations with major heritable disease phenotypes.

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## 1002/W/Poster Board #660

**Genome Wide Association Study of Acute Rejection and Chronic Allograft Nephropathy in Kidney Transplant Donors and Recipients.** *S.L. Musone[1], W. Lin[2], E. Wan[1], M. Akana[1], J. Chen[1], C. Ha[1], S. Horvath[2], D. Salomon[3], P.Y. Kwok[1].* 1) Institute for Human Genetics, University of California San Francisco, San Francisco, CA; 2) Dept. of Biostatistics, University of California Los Angeles, Los Angeles, CA; 3) The Scripps Research Institute, La Jolla, CA.

Purpose: Acute rejection (AR) and chronic allograft nephropathy (CAN) are two important phenotypes contributing to kidney transplant dysfunction and failure. We hypothesize that these forms of allograft rejection are complex traits with genetic signatures that can be identified through a genome-wide association study of single nucleotide polymorphisms (SNPs). Methods: SNPs were genotyped using high-throughput SNP arrays. After rigorous quality control, we have performed logistic regression analysis with multidimensional scaling (MDS) values as covariates to adjust for population structure in 353 recipients versus 114 AR recipients or 72 displaying CAN at a minimum of 1 year post-transplant. We have done the same analysis in 336 donors of well functioning transplants versus 102 AR or 71 CAN subjects. Results: Different regions of the genome reach significance in each of the 4 comparisons, indicating diverse etiologies between AR and CAN as well as contribution by donor and recipient to these outcomes. Significant loci are being validated in an independent cohort with outcome defined in an identical manner.

## 1003/W/Poster Board #661

**Decorrelating genetic association statistics for complex phenotypes: the PCPD method.** *G. Nelson[1], S. Hendrickson[2], S. O'Brien[2].* 1) BRP/LGD-NCI, SAIC Frederick, Frederick, MD; 2) Laboratory of Genomic Diversity, CCR, NCI-Frederick, Frederick MD.

Complex epidemiological phenotypes such as response to HIV are measured by multiple clinical variables collected on study individuals. While this gives more information per subject for association analysis, the interpretation is difficult, since these variables in general are non-independent; when a genetic factor shows significant association with several disease outcomes, is this confirmatory or does it simply reflect the correlation of these measures? In addition this non-independence complicates the problem of correcting for multiple comparisons. We present a general method for decorrelating association statistics using a principal components transformation. Traditionally, biometric measurements on individuals are transformed to independent variables by the principal components method, but this approach is not directly applicable to many of the common epidemiological statistics. On the other hand the population association statistics (e.g. relative hazards or odds ratios) are comparable measures. To quantify the intrinsic correlation of these measures on different outcomes, we consider a population of permutations of the genetic association in question, and for the set of disease measures, compute the principle components over the permuted data (PCPD). Applying this principle component transformation to the actual association data yields a set of uncorrelated association measures. The independent of the measures allows calculating an overall statistical significance, thus the additional information contained in multiple disease measures translates directly to increased power to detect disease associations.

## 1004/W/Poster Board #662

**Genome-wide association study of restless legs syndrome in Korean population.** *M. park, J. Lee, B. Keam, J. Heo, J. Lee.* Center for Genome Science, National Institute of Health, Korea Centers for Disease Control and Prevention, Seoul, Korea.

Background: The restless legs syndrome (RLS) is a common neurologic disorder characterized by an irresistible urge to move the legs. RLS affects near up to one in ten of the adult population and around one-third of patients experience symptoms more than twice weekly causing moderate to severe distress. Despite the high prevalence and social impact of depressive disorder, a little is known about the genetic contribution to the pathogenesis of RLS. The present study was purpose to investigate for sequence variants contributing of RLS in the Korean population. Methods: We conducted a genome-wide association study using 3,265 subjects recruited from a community-based epidemiological study. We used the currently accepted diagnostic criteria according to the National Institutes of Health consensus statement. A total 154 RLS cases and 3,111 controls were genotyped on a single platform using the Affymetrix Genome-Wide Human SNP array 500K chip. Of the SNPs assayed on the chip, 320,942 SNPs were analyzed that showed: 1) a call rate more than 95.0% in cases or controls; and 2) a minor allele frequency (MAF) $\geq$ 1% in the population; and 3) a significant distortion from Hardy-Weinberg equilibrium in the controls ($p \geq 0.05$). Results: In a genome-wide association study, we found 10 SNPs that were significantly associated with RLS. Ten SNPs in genes, PTPRD, ALG14, CPXM2, PAMR1, PELI2, ZFP161, LIMCH1 C2orf40, and C1orf168, exhibited a significant association with significance level of p<10-4. Among these SNPs, SNP (rs1556521) in PTPRD (protein tyrosine phosphatase receptor type delta) on chromosomes 9p showed significant association (p-value= 8.54x10-5), and this association with PTPRD was also confirmed in prior independent case-control study conducted in German, Czech and Canadian population. Conclusion: We have discovered a several SNPs associated with susceptibility to RLS. Further biologic and functional investigations are warranted to confirm these associations.

## 1005/W/Poster Board #663

**Age- and gender-specific genetic variations associated with serum C-reactive protein levels in 8,837 Koreans.** *J.W. Park[1], M.A. Jhun[1], J.Y. Lee[2], T. Park[3], S.H. Jee[4], S. Cho[5], J. Sung[5], J.E. Lee[6], N.H. Cho[7], C. Shin[8], B.G. Han[2], H.L. Kim[9].* 1) Dept Medical Genetics, Hallym Univ, Chuncheon, Korea; 2) Center for Genome Science, Korea Nat'l Institute for Health, Seoul, Korea; 3) Dept Statistics, Seoul Nat'l Univ, Seoul, Korea; 4) Institute for Health Promotion, Yonsei Univ, Seoul, Korea; 5) Dept Epidemiology, Seoul Nat'l Univ, Seoul, Korea; 6) DNA Link, Seoul, Korea; 7) Dept Preventive Medicine, Ajou Univ, Suwon, Korea; 8) Dept Internal Medicine, Korea Univ Ansan Hospital, Ansan, Korea; 9) Korea Nat'l Institute for Health, Seoul, Korea.

Elevated C-reactive protein (CRP) level is considered to be a predictor of a variety of aging-related diseases such as cardiovascular disease. Serum CRP levels vary by age, gender, and ethnicity; and moreover, correlate with adiposity and various biological characteristics. We conducted a genome-wide association study (GWAS) among 8,837 unrelated Koreans to identify age- and gender-specific genetic variants associated with serum CRP level. We initially analyzed the association of serum CRP with 65 variables including environmental and clinical variables to identify possible confounding variables. We analyzed natural log transformed CRP using 352,228 single nucleotide polymorphism (SNP) markers under three genetic models (*i.e.* additive, dominant, and recessive) and three multiple linear regression models stratified by age and sex with adjustments for residence and body mass index (model 1); model 1 plus additional adjustments for nine biochemical variables (model 2); and model 2 plus additional adjustments for five lifestyle variables (model 3). Nine SNPs of the six genes showed the genome-wide level of significance in one of age-gender groups. Among them, SNPs in the GRAMD3 (the lowest $p=2.44 \times 10^{-7}$), EPHA7 ($p=1.43 \times 10^{-7}$), and PIK3R1 ($p=2.8 \times 10^{-7}$) genes were identified in women aged 39-49 years, and ULK4 gene ($p=3.2 \times 10^{-8}$) were identified in women aged 60-70 years; while SNPs of CRP ($p=7.62 \times 10^{-8}$) and KIAA0182 ($p=1.89 \times 10^{-8}$) were significantly associated with CRP level in men aged 39-49 and 50-60 years, respectively. While the results must be confirmed in future studies, the genetic variations observed here might explain, in part, the serum CRP variation with age and gender.

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## 1006/W/Poster Board #664

**The Genetic Architecture of Alopecia Areata.** *L. Petukhova[1], A. Lee[3], J. Freudenberg[3], M. Hordinsky[4], D. Norris[5], V. Price[6], M. Duvic[7], P.K. Gregersen[3], A.M. Christiano[1,2].* 1) Dept of Dermatology, Columbia Univ, New York, NY; 2) Dept of Genetics & Development, Columbia Univ, New York, NY; 3) The Feinstein Institute for Medical Research, North Shore LIJHS, Manhasset, NY; 4) Dept of Dermatology, Univ of Minnesota, Minneapolis, MN; 5) Dept of Dermatology, Univ of Colorado, Denver, CO; 6) Dept of Dermatology, UCSF, San Francisco, CA; 7) Dept of Dermatology, MD Anderson Cancer Center, Houston, TX.

Alopecia Areata (AA) [MIM 104000] is one of the most prevalent and least well understood of the autoimmune diseases. In AA, autoimmunity is acquired against the hair follicle, which causes non-scarring hair loss that is associated with the accumulation of an immune infiltrate around the affected hair follicle. While the pathogenesis of AA remains unknown, we hypothesize that the genetic architecture of AA will be composed of genes expressed in cells of the immune system, as well as genes expressed in the hair follicle that elicit the immune response. We expect that immune response genes are vulnerable to positive selection, which could increase allele frequencies, creating common variants in the population. Alternatively, it is not likely that hair follicle genes would be subject to strong selective forces, so we expect that these alleles will remain at much lower frequencies in the population. Because genome-wide association studies (GWAS) identify common variants, we thus anticipate that the immune component of AA genetic architecture will be particularly amenable to detection with this method. We therefore undertook a GWAS in a discovery sample of 256 unrelated cases and 1152 controls, and replicated in an independent sample of 834 cases and 1260 controls, all of Northern European descent as determined by ancestry informative markers. Joint analysis of the datasets identified 40 SNPs that are associated with AA (p<5x10-7), approximately half of which fall within the HLA region on chromosome 6p. The remaining SNPs cluster in four main regions, implicating immune genes involved in the activation and/or regulation of signaling pathways in T-cells and NK cells. This insight allows us for the first time to place AA within the context of shared genetics and common pathways among autoimmune diseases, and promises to elucidate the underpinnings of disease pathogenesis and illuminate novel therapeutic avenues for the treatment of AA.

## 1007/W/Poster Board #665

**Analysis of GWAS data reveals another locus for Nicotine Dependence.** *J.P. Rice[1], S. Saccone[1], N. Saccone[1], W. Howells[1], A. Goate[1], R. Grucza[1], L. Chen[1], S. Hartz[1], B. Porjesz[2], H. Edenberg[3], T. Foroud[3], J. Nurnberger[3], J. Kramer[4], E. Johnson[5], K. Doheny[6], E. Pugh[6], C. Laurie[7], L. Bierut[1], COGA, SAGE.* 1) Dept Psychiatry, Washington Univ, St Louis, MO; 2) SUNY Downstate Medical Center, Brooklyn NY; 3) Indiana University School of Medicine, Indianapolis, IN; 4) University of Iowa, Iowa City, IA; 5) Research Triangle Institute Research Triangle Park, NC; 6) CIDR Genotyping Lab and JHU SNP Center Johns Hopkins School of Medicine Baltimore, MD; 7) Department of Biostatistics, University of Washington, Seattle WA.

The SAGE addiction project, one of the GENEVA (Gene-Environment Association) GWAS studies, consists of data from three studies (alcohol dependence, cocaine dependence and nicotine dependence). Cases had to have had a diagnosis of alcohol dependence, and controls had neither a diagnosis of alcohol dependence nor cocaine dependence. Genotyping was performed on the Illumina 1M chip at CIDR and data were cleaned following stringent GENEVA standards. We performed logistic regression analysis for the phenotype of nicotine dependence with case-control status defined using a threshold of 4 on the FTND scale, a commonly used scale in tobacco research, where controls had to have ever smoked cigarettes in their lifetime. Covariates consisted of gender, age (coded in four quartiles), ethnicity, co-morbid alcohol dependence with and without cocaine dependence, and SNP genotype coded additively with one degree of freedom. There were 1294 cases and 2071 controls used in analysis. We first analyzed 1,341 SNPs present on the Illumina chip in a set of 56 candidate genes previously identified by a panel of experts (Saccone et al, Hum Mol Genet 2007; 16:36-49). SNPs in the beta nicotinic receptor (CHRNB3) on chromosome 8 were highly significant (p=2.4 x 10-8, OR = 1.54). We next analyzed the full set of GWAS SNPs, and the SNPs in CHRNB3 were the most significant, with the QQ-plot indicating their GWAS significance. Odds ratios were similar for African Americans and European Americans as well as for those with and without a co-morbid diagnosis. This signal was much stronger than that of the alpha5 region of chromosome 15, a highly replicated finding in several studies of nicotine dependence and lung cancer. However, the lung cancer studies, which analyzed cigarettes per day (CPD) as a quantitative phenotype, did not report GWAS significance for CHRNB3. Accordingly, we analyzed CPD and found a significance of 0.001. Interestingly, we found highly significant evidence for interactions with gender and age on chromosome 8, but only with gender for the region on chromosome 15. For CHRNB3, there was a greater SNP effect in males and in those over age 45. The use of CPD, rather than the FTND, may explain why CHRNB3 was not GWAS significant in these other studies. Nicotine dependence represents a major source of health morbidity and mortality, and the identification of risk alleles may lead to therapeutic targets for smoking cessation.

## 1008/W/Poster Board #666

**Common variants at ten loci influence glycated hemoglobin levels via glycemic and non-glycemic pathways.** *S. Sanna[1], N. Soranzo[2,3], E. Wheeler[2], C. Gieger[4], D. Radke[5], J. Dupuis[6], E. Stolerman[7], N. Bouatia-Naji[8], C. Langenberg[9], I. Prokopenko[10,11], M.S. Sandhu[12,13], W.H.L. Kao[14], N.J. Wareham[9], J.C. Florez[7,15,16], M. Uda[1,18], I. Barroso[2,18], J.B. Meigs[17,18] on behalf of MAGIC.* 1) Istituto di Neurogenetica e Neurofarmacologia del CNR, Monserrato, Cagliari, Italy; 2) Human Medical Genetics, Wellcome Trust Sanger Institute, Genome Campus, Hinxton, UK; 3) Department of Twin Research and Genetic Epidemiology, King's College London, UK; 4) Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; 5) Institute for Community Medicine, Ernst Moritz Arndt University Greifswald, Greifswald, Germany; 6) Department of Biostatistics, Boston University School of Public Health, Boston, USA; 7) Center for Human Genetic Research, and Diabetes Research Center (Diabetes Unit), Massachusetts General Hospital, Boston, USA; 8) CNRS-UMR-8090, Institut Pasteur de Lille and Lille 2 University, Lille, France; 9) MRC Epidemiology Unit, Cambridge, UK; 10) WTCHG, Oxford, UK; 11) OCDEM, Oxford, UK; 12) Department of Public Health and Primary Care, Strangeways Research Laboratory, University of Cambridge, Cambridge, UK; 13) Department of Internal Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; 14) Department of Epidemiology and Medicine, Johns Hopkins University, Baltimore, USA; 15) Program in Medical and Population Genetics, Broad Institute, Cambridge, Massachusetts, USA; 16) Department of Medicine, Harvard Medical School, Boston, USA; 17) General Medicine Division, Massachusetts General Hospital, Boston, USA; 18) These authors contributed equally.

Glycated hemoglobin ($HbA_{1C}$) results from the non-enzymatic glycation of hemoglobin molecules carried in erythrocytes, an essentially irreversible reaction whose rate is directly dependent on ambient blood glucose levels. The level of $HbA_{1C}$ reflects the mean blood glucose concentration over the preceding 2-3 months, the average life span of a human erythrocyte. While ambient blood glucose explains ~90% of the variability of $HbA_{1C}$, the relative influence of other factors independent of systemic glycemia, including increased red blood cell turnover, hemoglobinopathies, defects of glucose transport into erythrocytes, or alterations in intracellular glucose metabolism trait, is largely unknown. Though the estimated heritability of $HbA_{1C}$ in populations of European ancestry is relatively high (~47%), the genetic factors thus far identified (*HK1*, *GCK*, *G6PC2*, *SLC30A8*) only account for ~1.5% of the variance. To identify novel loci influencing $HbA_{1C}$ levels, we conducted a meta-analysis of ~2.5M directly genotyped or imputed autosomal SNPs from 10 GWAS (Stage 1 cohorts), totaling 14,898 non-diabetic adults of European descent. By following up the top 20 independent signals in 30,233 additional samples (Stage 2 cohorts) we identified two novel loci and replicated the association at *HK1* ($p=1.5x10^{-50}$), *GCK* ($p=8.8x10^{-20}$), and *G6PC2* ($p=9.3x10^{-18}$). Combining all available data from the Stage 1 and Stage 2 cohorts (45,131 individuals) five additional loci reached genome-wide significance ($p<5x10^{-8}$), including *MTNR1B* ($p=8.3x10^{-11}$), a locus recently associated with fasting glucose and type 2 diabetes risk. Of the ten association signals, five are in or near genes known to regulate systemic glycemia, and two are near genes involved in iron homeostasis. Genes in the remaining loci require further characterization, but may affect $HbA_{1C}$ levels through red blood cell skeletal organization or membrane stability. Measurement of $HbA_{1C}$ has universal significance in the diagnosis and clinical care of diabetes as a time-integrated index of glycemic control and a highly reliable biomarker of diabetes-specific and cardiovascular disease complications. The apparent distinction between loci influencing $HbA_{1C}$ levels via glycemic and non-glycemic pathways highlighted in this study, thus raises important considerations in the clinical interpretation of $HbA_{1C}$ levels.

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## 1009/W/Poster Board #667

**Identification of Systemic Lupus Erythematosus Susceptibility loci by Pooling-based Genomewide Association Scan and Replication Studies.** *T. Tahira[1], K. Masumoto[1], A. Yoshinaga[1], Y. Okazaki[1], Y. Kochi[2], K. Yamamoto[3], T. Horiuchi[4], K. Hayashi[1].* 1) Division of Genome Analysis, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; 2) Laboratory for Autoimmune Diseases, Center for Genomic Medicine, RIKEN, Tokyo, Japan; 3) Department of Allergy and Rheumatology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 4) Department of Medicine and Biosystemic Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Systemic lupus erythematosus (SLE) is an autoimmune disease in which multiple genetic and environmental factors are involved. To identify the genetic background of SLE susceptibility in Japanese, we performed two-stage pooling-based association study, that is, array-based hybridization analysis followed by SSCP analysis, of case pools (sizes 264 and183) and control pools (sizes 426 and 253). Each of the pooled samples was hybridized to six Affymetrix 500K array sets. The disease association was evaluated by silhouette score (calculated by Genepool 0.8.1, http://genepool.tgen-.org/) and interpreted by a sliding-window statistics of median rank in seven different window sizes. SNPs of highest scores in the top 30 regions for each window size, that covers 68 genomic regions were re-examined by quantitative SSCP (http://qsnp.gen.kyushu-u.ac.jp/placeSSCP/), and the associations (estimated $P < 10^{-4}$) for SNPs in 14 regions were confirmed. The association of these SNPs was further verified by individual genotyping using TaqMan assay. We genotyped additional samples (128 cases and 368 controls) and found consistent association in 5 regions. We then examined additional samples (606 cases and 940 controls) as further replication, and significant associations were confirmed for all that were examined (combined values in allelic test: ZPBP-IKZF1, $P = 7.7 \times 10^{-14}$, OR =1.50; PRDM1-ATG5, $P = 5.1 \times 10^{-10}$, OR = 1.4; HLA-G, $P = 3.6 \times 10^{-8}$, OR = 1.34). These three SNPs were all located outside the genic regions and regulatory roles are suspected. Interestingly, the SNP in ZPBP-IKZF1 is in strong linkage disequilibrium with one of the risk variant of Crohn's disease, indicating that this region is shared genetic risk factor for these two autoimmune diseases. This study showed pooling-based GWAS that combines microarray and quantitative SSCP analyses are cost-efficient and effective method in the search for disease related regions.

## 1010/W/Poster Board #668

**Genome-Wide Association Study of Body Mass Index and Follow-up Candidate Genotyping in African Americans: the IRAS Family Study.** *M. Talbert[1, 2], M. Wing[1, 3], N. Allred[1], J. Ziegler[4], C. Langefeld[4], L. Wagenknect[4], K. Taylor[5], J. Norris[6], T. Haritunians[7], M. Bryer-Ash[8], D. Bowden[1, 3, 9].* 1) Center for Human Genomics, Wake Forest University School of Medicine, Winston Salem, NC; 2) Program in Molecular Medicine and Translational Sciences, Wake Forest University School of Medicine, Winston Salem, NC; 3) Program in Molecular Genetics, Wake Forest University School of Medicine, Winston Salem, NC; 4) Department of Public Health Sciences, Wake Forest University School of Medicine, Winston Salem, NC; 5) Medical Genetics Institute, Department of Cardiology, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA; 6) University of Colorado Health Sciences Center, Denver, CO; 7) Departments of Human Genetics, University of California, Los Angeles, CA; 8) Section of Endocrinology and Diabetes, Program for Adult Metabolic Health, Univ of Oklahoma Health Sciences Center, Oklahoma City, OK; 9) Departments of Biochemistry and Internal Medicine, Wake Forest University School of Medicine, Winston Salem, NC.

Obesity's highest prevalence is among ethnic minorities, but little is known about its genetic components in these groups. The Insulin Resistance Atherosclerosis Family Study (IRASFS) has examined 581 African Americans (AAs) from Los Angeles, CA for adiposity traits, including Body Mass Index (BMI;Mean BMI=30.0 kg/m2,Age=42.9,Female %=59.2). A GWAS using the Affymetrix 6.0 platform was performed in a subset of 254 IRASFS AAs (Mean BMI=30.1 kg/m2,Age=45.2,Female %=57.5). Analysis of the GWAS SNPs using the additive model of PLINK on residual BMI (after adjustment for age and gender) revealed a number of genic SNPs among the top 20 associated variants. Among the BMI candidate genes were CYP19A1 (Aromatase, mediates estrogen synthesis from androgens) and the proximal GLDN (Gliomedin,helps form nodes of Ranvier along myelinated axons), TMEFF2 (Tomoregulin,a cell survival and noted neurotrophic factor), and PEPD (Prolidase,metabolizes iminodipeptides containing C-terminal prolines). Genotyping of the most significant BMI-associated genic GWAS SNPs, as well as nearby GWAS-typed SNPs of MAF>5% and P-values<1x10-4, was completed in a replication set (the untyped IRASFS AAs;n= 330). The follow-up genotyping analysis utilized the variance component method of SOLAR, adjusting for age and gender. An intronic SNP in CYP19A1 was associated with BMI (rs7168331;P=5.1x10-7) in the initial GWAS. In the follow-up, rs7168331 of CYP19A1 and rs12148477 of GLDN were associated in the total IRASFS AA cohort (P-values=6x10-4 and .007), but only rs7168331 was associated in both the IRASFS AA GWAS (P=6 x10-4) and Replication (P=0.026) subsets using SOLAR. In the GWAS, TMEFF2 intronic SNPs were associated with BMI (rs4644972,rs2356953,rs4644972;P-values range from 1.1x10-7 to 3.6x10-6). In the follow-up, TMEFF2 SNPs rs7597998 and rs2356953 were associated in the combined IRASFS AAs (P=1.0x10-4 and 2.5x10-5), the GWAS (.007 and 5.7x10-4), and Replication (5.0x10-5 and 2.0x10-3) subsets. Finally, in the GWAS, an intronic SNP of PEPD was associated with BMI (rs4362488;P=3.4x10-6). In the follow-up, PEPD SNPs rs9676705 and rs11084731 showed only trending association with BMI in the combined IRASFS AA cohort (P=.065 and .088). That many of the associated genes have physiologic links to adiposity strongly supports the significance of these findings in AAs. Additional genotyping of tagSNPs in LD blocks of observed association in both the IRASFS AAs and additional populations is ongoing.

## 1011/W/Poster Board #669

**Genome-wide association study of percent body fat.** *B. Tayo[1], S. Kang[2], A. Luke[1], A. Adeyemo[3], C. Rotimi[3], X. Zhu[2], R. Cooper[1].* 1) Loyola University Chicago, Maywood, IL; 2) Case Western Reserve University, Cleveland, OH; 3) NIH Intramural Center for Genomics and Health Disparities, Bethesda, MD.

With the advent of genome-wide association studies, many common genetic variants have been identified to associate with obesity and some of its related traits. However, the identified variants collectively explain only a small fraction of the heritability of these traits, suggesting that some other genetic variants with stronger effect sizes remain to be identified. To identify additional variants accounting for yet unexplained fraction of genetic variation in obesity-related traits, we performed genome-wide association analysis of percent body fat using 823347 single nucleotide polymorphisms (SNPs) from the autosomal chromosomes in a sample of 660 unrelated adult African Americans from Maywood, Illinois who were genotyped on Affymetrix 6.0 platform. Residuals from linear regression of percent body fat on sex, age and body mass index served as phenotype in the fitted additive genetic model for each SNP. After applying genomic control to account for possible inflation in association estimates, we observed 7 SNPs with $P < 1.0 \times 10^{-5}$. We tested these SNPs in another sample of 205 unrelated adult Nigerians both separately and in meta-analysis with the Maywood samples. One of the SNPs, rs1431719 which is located on the CHRM3 gene had $P = 0.0001321$ for the same reference allele and with an effect size in the same direction in the Nigeria samples. Likewise, in the meta-analysis of both Maywood and Nigeria data, rs1431719 attained *p-value* of $6.17 \times 10^{-8}$ after applying genomic control. While additional replication is required, this report however presents evidence of possible association of rs1431719 with body fat in these African-origin populations.

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## 1012/W/Poster Board #670

**PARK2 and SVOPL loci are associated with successful aging in the Amish.** *D.R. Velez[1], J.R. Gilbert[1], J.L. Myers[1], L. Jiang[2], A.C. Davis[2], P.J. Gallins[1], I. Konidari[1], L. Caywood[1], M. Creason[1], D. Fuzzel[1], C. Knebusch[2], R. Laux[2], M.L. Slifer[1], C.E. Jackson[3], M.A. Pericak-Vance[1], J.L. Haines[2], W.K. Scott[1].* 1) Miami Institute for Human Genomics, University of Miami, USA; 2) Center for Human Genetic Research, Vanderbilt University, USA; 3) Scott & White, Temple, TX.

Successful aging (SA) is defined as living to older age with high physical function, preserved cognition, and continued social engagement. Identification of both disease-causing and health-promoting polymorphisms and their interactions with the environment has the potential to greatly improve the health of older adults, the most rapidly growing segment of the U.S. population. Several domains underlying SA have evidence of heritability: longevity, grip strength, lower extremity function, and retention of cognitive ability. In the present study we examined 263 cognitively intact Amish individuals age ≥80 all of whom are part of a single 11-generation pedigree. These individuals were included in a genome-wide association study (GWAS) using the Affymetrix Genome-Wide Human SNP Array 6.0. 74 individuals met criteria for SA (cognitively intact, not depressed, satisfied with life, little self-reported limitation in activities of daily living or musculoskeletal function, in the top 1/3 of the sample on a lower-extremity physical function test) and were compared to the other 189 individuals in a case-control analysis. We also analyzed association with grip strength as an independent marker of musculoskeletal function. After data cleaning, 630,439 autosomal SNPs were analyzed for association with SA using the MQLS method and for association with grip strength using the GRAMMAR-GC method implemented in GenABEL. Both methods use pairwise kinship coefficients to adjust for correlations among related individuals. Chromosome 6q25-q27 (including the FRA6E fragile site and PARK2 locus) contained several SNPs strongly associated with SA (min p = 2 x 10-6) and analyses of grip strength, produced a genome-wide significant result (p = 1 x 10-7) in the SVOPL gene on chromosome 7q34. The associations of genes in the chromosome 6 region are of particular note because PARK2 has been associated with several other aging-related phenotypes including Parkinson's disease, general neuron degeneration, and several forms of cancer. Although the role of SVOPL in aging is unknown, the adjacent gene transcriptional intermediary factor alpha 1 (TRIM24) has been associated with several cancers. Both PARK2 and TRIM24 have been described as tumor suppressor genes, consistent with the theory that preserved DNA repair and tumor suppression activity are essential mechanisms for cancer-free longevity and successful aging.

## 1013/W/Poster Board #671

**A novel efficient genome-wide association study design: Application to glaucoma and age-related macular degeneration.** *K. Wang[1], T.E. Scheetz[2], A.J. Grundstad[3], J.S. Beck[4,5], T.L. Casavant[3], T.A. Braun[6], J.C. Folk[2], J.H. Fingert[2], E.M. Stone[2,5], V.C. Sheffield[4,5].* 1) Dept of Biostatistics, Univ Iowa, Iowa City, IA; 2) Dept of Ophthalmology and Visual Sci, Univ Iowa, Iowa City, IA; 3) Cntr Bioinformatics Comput Biology, Univ Iowa, Iowa City, IA; 4) Dept of Pediatrics, Univ Iowa, Iowa City, IA; 5) Howard Hughes Medical Institute; 6) Dept Biomedical Engineering, Univ Iowa, Iowa City, IA.

Primary open angle glaucoma (POAG) and age-related macular degeneration (AMD) are the two leading causes of irreversible blindness in developed countries. Common genetic variants leading to increased risk of AMD have been identified. Although genetic mutations leading to Mendelian forms of open-angle glaucoma have been identified, genome-wide association studies (GWASs) identifying loci involved in POAG have been lacking. We conducted a GWAS on POAG and AMD with a novel efficient study design. This experiment design maximizes the power to detect disease-associated risk alleles by utilizing both cohorts for discovery: the AMD cohort is the control for the POAG cohort, and vice versa. Allele frequencies in Caucasian populations as determined by the HapMap project are used to infer whether a significant SNP is associated with POAG or AMD. This study involves 400 POAG patients and 400 AMD patients using the Affymetrix 500 K SNP array and the Mapping 5.0 array. Two novel POAG associated loci have been identified. Previously reported AMD associations with CFH gene and ARMS2 gene are confirmed.

## 1014/W/Poster Board #672

**Identification of susceptibility genes/loci of Kawasaki disease in Han-Chinese through genome-wide association study.** *N. Wang[1], F.J. Tsai[2], C.H. Chen[1], L.C. Chang[1], Y.T. Chen[1,3], J.Y. Wu[1].* 1) IBMS, Academia Sinica, Taipei, Taiwan; 2) Col Chinese Medicine, China Medical University, Taichung, Taiwan; 3) Dept Pediatrics, Duke Uni Medical Ctr, Durham, NC.

Kawasaki disease is an acute systemic vasculitis with unidentified etiology, mainly affecting infants and children under 5 years old. The Northeast Asia countries, including Japan, Korea and Taiwan, are found to have the highest incidence rate when compared to other parts of the world. Therefore, a genetic susceptibility of the disease is suggested, although no genes or markers have been consensually confirmed with such role. We carried out a Genome-Wide Association Study with the purpose to identify genes and/or markers that dictate the genetic susceptibility of Kawasaki disease in the Han Chinese population residing in Taiwan. A total of 250 Kawasaki patients and 446 controls have been genotyped with Affymetrix Genome-Wide Human SNP Array 6.0. After filtering with quality control measures, a total of 51 SNPs were found to be significantly associated with P values less than 10-7. Analysis on the location of these SNPs identified 12 loci that contain more than two significantly associated SNPs in vicinity. A candidate gene previously reported to be associated with cardiovascular abnormalities in Kawasaki disease was found in one of these loci. We report here one of the first GWAS studies on Kawasaki based on the Han-Chinese patients and present novel loci in addition to a known candidate, as the putative susceptibility genes of Kawasaki disease.

## 1015/W/Poster Board #673

**Genome Wide Association Study (GWAS) of CT-derived measures of abdominal fat in African Americans of the Insulin Resistance Atherosclerosis Family Study (IRASFS).** *M.R. Wing[1], M.E. Talbert[1], M. Bryer-Ash[2], J.M. Norris[3], Y-D.I. Chen[4], L.E. Wagenknecht[5], D.W. Bowden[1].* 1) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC; 2) Section of Endocrinology and Diabetes, University of Oklahoma Health Science Center, Oklahoma City, OK; 3) Department of Epidemiology, University of Colorado Health Sciences Center, Denver, CO; 4) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 5) Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC.

Excess visceral adipose tissue (VAT), when compared to subcutaneous fat, causes an increased risk for disease (i.e. diabetes), emphasizing the importance of identifying genes associated with VAT and visceral to subcutaneous ratio (VSR). A GWAS focused on adiposity traits was performed in 254 African American (AA) subjects (mean VAT=96.2;mean VSR= 0.32;mean age=45;58% female) from the IRASFS. The Affymetrix 6.0 platform was used for genotyping. Preliminary analysis using a linear regression on residuals adjusting for familial correlation, age, gender, and BMI under the additive model revealed several genic hits among the top 20 associated SNPs for abdominal CT-derived measures (L4/L5 slice) of VAT and VSR. SNPs associated with VAT were in the genes *FETUB* (which regulates the insulin receptor and hepatocyte growth factor receptor), *PTPRD* (which promotes neurite growth and regulates neurons axon guidance), and *STAT3* (a transcriptional activator stimulated by cytokines and growth factors). VSR associated SNPs were in *MYO18B* (which regulates muscle specific genes and intracellular trafficking), *PDSS2* (second subunit of the enzyme that synthesizes Q10 ubiquinone), and *IQGAP1* (which regulates cell morphology and motility). High scoring SNPs and other GWAS variants (MAF>5% and P≤1E-4) in these genes associated with VAT and VSR were subsequently genotyped in additional cohort samples (n=330). Analysis was completed using the variance component approach in SOLAR, adjusting for age, gender, and BMI. SNPs associated in the GWAS with VAT in *FETUB* (rs1047115, rs7999;P<7E-5) and *PTPRD* (rs10977342;P=2.2E-5) were trending or associated in both the replication subset (P≤0.05) and in the total IRASFS AA cohort (P<2.5E-4). The *STAT3* SNP associated in the GWAS (rs3785898;P=1E-5) was only associated in the combined population (P=1E-3). The variants within *MYO18B*, *PDSS2*, and *IQGAP1* were associated with VSR in the GWAS (P<4.4E-4), but were only associated or trending in the total IRASFS AA (P<0.06). Interestingly, the SNPs within these genes were also associated with VAT in the GWAS (P<2.0E-3) and trending or associated in the combined population (1.7E-3<P<0.08). However, only SNPs within *PDSS2* (rs12215105) and *MYO18B* (rs6004901) also showed trends with VAT in the replication subset (P<0.086). Our results illustrate novel VAT and VSR genic hits in African Americans that are functionally relevant. Genotyping of tagSNPs in LD blocks within these genes is currently underway.

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## 1016/W/Poster Board #674

**Treatment-refractory schizophrenia in Han Chinese.** *J.Y. Wu[1], C.J. Hong[2], H.H. Wang[1], Y.J. Liou[2], C.H. Chen[1], Y.T. Chen[1].* 1) Institue of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Taipei Veterans General Hospital.

This genome-wide association (GWA) study aims to identify genes susceptible to treatment refractory schizophrenics (TRS). A total of 418 TRS patients, who showed refractory symptoms for at least two separated 6-week treatments (equivalent to 600mg Chlorpromazine) or who received treatment of Clozapinethe, were recruited from major psychiatric centers in Taiwan. The patients and 446 controls have been genotyped with Affymetrix Genome-Wide Human SNP Array 6.0. More than 20 SNPs showed highly significant signals ($p < 5 \times 10\text{-}8$) in association analysis. Genes located nearby those SNPs and expressed predominantly in the brain and central nervous system were identified. Those genes were found to be involved in neuronal cell-adhesion, neuronal cell surface interaction, adenylate cyclase regulation, circadian regulation, glutamate receptor, cation channel (sodium or calcium), neuromodulator, etc. Most of those genes were not previously reported to be associated with schizophrenia. We report here the first GWAS studies on schizophrenia based on the Han-Chinese patients and present novel loci as the putative susceptibility genes of schizophrenia.

## 1017/W/Poster Board #675

**Genome-wide association study of hematocrit, hemoglobin, and red blood cell indexes.** *J.-P. Lin[1], S. Ganesh[2], CHARGE Consortium.* 1) Office of Biostatistics Research/Devision of Cardiovascular Sciences/National Heart, Lung, and Blood Institute; 2) Nationl Human Genome Research Institute.

Hematocrit, hemoglobin, erythrocyte count, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration are widely reviewed in clinical practice and have a heritability of 40-90%. Hematocrit, hemoglobin and red cell indices are associated with a variety of co-morbid-conditions especially vascular diseases and mortality. We performed a genome-wide association study with 2.5 million genotyped and imputed autosomal SNPs in up to 24,807 subjects of European ancestry from six cohort studies within the CHARGE Consortium to identify loci associated with each of the six traits. We identified associations across the six correlated traits on 22 distinct chromosomal locations (p=1.3E-8 to p=1E-57), confirming several known loci like HBS1L-MYB,6q23.3 (p=1E-57), HFE,6p22.1 (p=1.4E-27) and TMPRSS6,22q12.3 (p=1.1E-30); and identifying many novel ones. Among them, several genes are related to iron homeostasis, erythropoiesis, red blood cell membrane structure and have been reported to be associated with blood pressure and coronary heart disease. This large GWAS provides substantial insight into the genetic architecture of red blood cell traits in the general population revealing promising molecular links between these traits, especially hematocrit, and vascular diseases.

## 1018/W/Poster Board #676

**Genome Wide Association Study of Non-Diabetic End Stage Renal Disease in African Americans using Pooled DNA.** *M.A. Bostrom[1,4,5], L. Lu[2], J. Chou[2], P.J. Hicks[1], J. Xu[4,5], C.D. Langefeld[2], B.I. Freedman[3], D.W. Bowden[1,4,5].* 1) Dept Biochemistry, Wake Forest Univ, Winston-Salem, NC; 2) Biostatistical Sciences, Wake Forest Univ, Winston-Salem, NC; 3) Internal Medicine/Nephrology, Wake Forest Univ, Winston-Salem, NC; 4) Center for Human Genomics, Wake Forest Univ, Winston-Salem, NC; 5) Center for Diabetes Research, Wake Forest Univ, Winston-Salem, NC.

African Americans have increased susceptibility to non-diabetic (hypertensive) end stage renal disease (ESRD) and previous studies have demonstrated a genetic component to the disease in this population. To identify genes associated with non-diabetic ESRD in African Americans, we performed a genome wide association study (GWAS) on 1000 African American non-diabetic ESRD cases and controls. Sample DNA was genotyped as pools for the GWAS and significant associations were confirmed with individual genotyping. DNA from 500 case and 500 control samples was quantified using gel electrophoresis and spectrophotometric analysis. Each DNA sample was normalized to a concentration of 50ng/μl. Samples were pooled to create 10 case pools and 10 control pools with each pool containing 50 samples. DNA pools were genotyped in duplicate on the Illumina HumanHap550-Duo BeadChip. Channel intensities were extracted from the Bead Studio data and channel means were normalized. Allele frequencies were calculated from the normalized channel intensities. A t-test was used to compare allele frequencies from case and control pools. Fourteen SNPs had p-values less than $1.0\times10^{-5}$. To confirm association, twenty-five of the most significantly associated SNPs were individually genotyped on 662 AA non-diabetic ESRD cases and 740 controls. Five SNPs were significantly associated after correction for multiple testing ($p < 0.002$). Three of these SNPs, rs9610448 ($p = 8.0\times10^{-8}$, odds ratio (OR) =0.64, confidence interval (CI) = 0.54-0.75; additive model), rs4821467 ($p = 1.13\times10^{-10}$, OR=1.66, CI= 1.42-1.94, additive model) and rs713753 ($p = 1.9\times10^{-13}$, OR=.41, CI=.32-.52, dominant model) are located on chromosome 22 near MYH9 gene which has been shown previously to be associated with non-diabetic ESRD. The remaining SNPs (rs4733947 and rs4658749) are located on chromosome 8 in the TACC1 gene ($p = 1.9\times10^{-5}$, OR=0.67, CI=0.56-0.8, additive model) and on chromosome 1 in the KIF26B gene ($p = 4.5\times10^{-4}$, OR=0.73, CI=0.62-.86, additive model). We have performed the first GWAS for non-diabetic ESRD in an African American population using pooled DNA samples. Through individual genotyping we have confirmed association at five SNPs, rs9610448, rs4821467, rs713753, rs4733947, and rs4658749. Additional populations will be needed for replication studies. In addition, by using other normalization methods and individual genotyping, we are identifying additional SNPs associated with non-diabetic ESRD.

## 1019/W/Poster Board #677

**Genomewide Association Study of Generalized Vitiligo in a European Founder Population Identifies *SMOC2*, in the Vicinity of *IDDM8*.** *S. Birlea, P. Fain, R. Spritz.* Human Medical Genetics Program, Univ Colorado Denver, Aurora, CO.

Generalized vitiligo is a disorder in which patchy loss of skin and hair pigmentation results from autoimmune loss of melanocytes in skin and hair. We previously characterized an isolated founder population in Romania with greatly elevated prevalence of generalized vitiligo (2.9%) as well as other autoimmune diseases, including autoimmune thyroid disease, rheumatoid arthritis, and type 1 diabetes mellitus. Here, we describe a genome-wide association (GWA) study of 310,598 SNPs in 32 distantly related villagers with generalized vitiligo and 50 healthy controls from surrounding villages. After data-cleaning and correction for relatedness among cases, vitiligo was significantly associated with SNPs in a 30-kb LD block on chromosome 6q27, in the immediate vicinity of a linkage region (*IDDM8*) previously identified for type 1 diabetes mellitus and rheumatoid arthritis, autoimmune diseases that are epidemiologically associated with generalized vitiligo in this founder population. The region of association contains only one gene, *SMOC2*, within which SNP rs13208776 attained genome-wide significance for association with generalized vitiligo ($P = 3.13\text{E-}08$) at odds ratio 7.45 (95% CI = 3.56 - 15.53) for the high-risk allele and population attributable risk 28.00. *SMOC2* encodes a modular extracellular calcium-binding glycoprotein of unknown function. Our findings indicate that *SMOC2* is a novel risk locus for generalized vitiligo and perhaps other autoimmune diseases.

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## 1020/W/Poster Board #678

**Genomewide Association Study of Generalized Vitiligo.** *Y. Jin, S. Birlea, S. Riccardi, P. Holland, K. Gowan, P. Fain, R. Spritz, the VitGene Generalized Vitiligo Consortium.* Human Medical Genetics Program, Univ Colorado Denver, Aurora, CO.

Generalized vitiligo is a common autoimmune disease, in which patches of depigmented skin and hair result from loss of melanocytes from the involved regions. Generalized vitiligo is the 4th or 5th most frequent autoimmune disease, and the consequent white patches can result in significant psychosocial trauma, particularly in patients from highly pigmented ethnic groups. About 20-30% of vitiligo patients develop other autoimmune diseases, most often autoimmune thyroid disease, psoriasis, adult-onset type 1 diabetes, rheumatoid arthritis, pernicious anemia, lupus, and Addison's disease. We have carried out a genomewide association (GWA) study of generalized vitiligo. We initially genotyped 610,000 SNPs in 1509 unrelated Caucasian (CEU) generalized vitiligo patients from the USA, Canada, and UK, and compared the results with a dataset of 3505 CEU controls obtained from dbGaP. We first specifically analyzed data for several candidate genes that have previously been implicated in generalized vitiligo. We observed exceedingly significant association with numerous SNPs in the MHC, including several that are associated with autoimmune susceptibility. We also observed highly significant association with rs2476601 in *PTPN22* and a SNP that tags rs231775 in *CTLA4*, both SNPs that are thought to be causally related to autoimmune disease susceptibility. Data for *NLRP1*, which has been associated with generalized vitiligo in multiplex families, were equivocal. We next analyzed the data genomewide to prioritize SNPs for replication testing. We are currently conducting a replication study of several hundred SNPs that were highly ranked in the genomewide screen, genotyping these SNPs in over 250 CEU trios and over 250 CEU multiplex families, together comprising ~1000 trios, as well as an additional 750 unrelated CEU generalized vitiligo cases versus controls. We anticipate that the results of these analyses will identify novel loci that are associated with genetic susceptibility to generalized vitiligo, including susceptibility loci shared with other autoimmune diseases.

## 1021/W/Poster Board #679

**Joint analysis of genome-wide association studies of multiple related phenotypes.** *K.I. Morley[1,2], J.C. Barrett[1], UK IBD Genetics Consortium and Wellcome Trust Case Control Consortium.* 1) Wellcome Trust Sanger Institute, Cambridge, United Kingdom; 2) Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, School of Population Health, The University of Melbourne, Melbourne, Australia.

Genome-wide association (GWA) studies of autoimmune disorders have identified over 50 associated genomic regions, many of which appear to be involved in pathogenesis of more than one condition and suggest the presence of common disease mechanisms. One example of this is Crohn's disease (CD) and ulcerative colitis (UC); GWA studies have identified numerous genomic regions associated with these diseases, many of which are shared between the two disorders. Finding shared genes is, however, complicated by the fact that a risk allele for one autoimmune disorder may be protective against another (e.g. the protective allele of PTPN22 in CD confers risk for type 1 diabetes). We report two efforts to identify shared genes for autoimmune disorders using data on CD, rheumatoid arthritis, type 1 diabetes, and UC from the Wellcome Trust Case Control Consortium. First we have conducted a large-scale joint GWA study of CD and UC, using 4,176 cases (1,748 CD, 2,428 UC) and 5,539 controls. This simple approach, when applied to closely related traits replicates previous findings of genes associated with both CD and UC such as IL23R and TNFSF15, some of those associated with more numerous autoimmune disorders such as PTPN2, as well as highlighting potentially novel associations that require further investigation. Second, in order to search for loci with allelic heterogeneity among diseases, we have implemented tests that pool information from multiple SNPs in a gene- or locus-centric approach.

## 1022/W/Poster Board #680

**Genome-wide association study of Invasive Pneumococcal Disease.** *A. Rautanen[1], T. Mills[1], I. Prokopenko[1], D. Crook[2], TN. Williams[3], A. Scott[3], SJ. Chapman[1], AV. Hill[1].* 1) WTCHG, University of Oxford, Oxford, United Kingdom; 2) Department of Microbiology, John Radcliffe Hospital, Oxford, United Kingdom; 3) Kenya Medical Research Institute/Wellcome Trust Programme, Centre for Geographic Medicine Research, Coast, Kilifi District Hospital, Kilifi, Kenya.

Infection with *Streptococcus pneumonia* is a major global problem and a common cause of death even in Western Countries. Colonisation of the nasopharynx by pneumococcus is widespread, yet only a minority of patients develop invasive disease, which is defined by the isolation of pneumococcus in a normally sterile site. Current understanding of the host genetic factors which influence the development of Invasive Pneumococcal Disease (IPD) is limited. Earlier studies have identified several susceptibility genes for IPD, but only a minority of these have been replicated. No genome-wide association studies for IPD have been reported to our knowledge.

We have conducted a genome-wide association study of 400 severe IPD cases and 1,300 publicly available controls from the UK. This is one of the largest collections of DNA samples from patients with IPD available worldwide. The genotyping of both cases and controls was performed using the Affymetrix 6.0 SNP chip, which consists of nearly 1 million SNPs. Following stringent QC criteria and association analysis, 4 loci showed association at $p<5x10^{-6}$, and a further 19 loci at $p<5x10^{-5}$. Three highly significant association signals in 1q32, 6p21 and 7q11 are located in genes that are directly involved in immune function. Others, such as those encoding for transmembrane ion channels might be involved in immune-related processes. Follow-up replication of the tagSNPs, capturing common variation within the most interesting loci, is ongoing in the sample set that consists of Kenyan children with pneumococcal bacteraemia, a severe manifestation of IPD, and healthy Kenyan children as controls. Investigation of the selected loci in the sample of African descent may allow us to define the boundaries of the IPD susceptibility loci with greater precision due to lower LD observed in Africans.

Furthering knowledge of the pathogenesis of invasive pneumococcal infection by identifying new susceptibility genes is extremely important, given the current limited understanding of the genetic basis of invasive bacterial infection and the huge global burden of the disease. The identification of functional genetic variants that predispose individuals to IPD is likely to have major implications for the study of other infectious and inflammatory disease processes as well.

## 1023/W/Poster Board #681

**Whole-genome association studies of severe malaria.** *K. Small, Malaria Genomic Epidemiology Network.* WTCHG, Oxford Univ, Oxford, United Kingdom.

Malaria kills approximately a million children each year, but a much larger number survive despite repeated infections. Human genetic factors have been estimated to determine 25% of severe malaria risk, of which known genetic variants account for a small fraction. The Malaria Genomic Epidemiology Network (partner institutions listed at www. malariagen.net) was established to carry out multicenter studies investigating the genetic basis of resistance to malaria. We present the results of genome-wide association studies of severe malaria in West and East Africa and discuss the implications for further genome-wide association studies in Africa.

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## 1024/W/Poster Board #682

**Genome-wide association study identifies novel loci associated with endometriosis in a Caucasian population.** *H. Albertsen, K. Ward, G. Frech, P. Farrington, S. Dintelman.* Juneau Biosciences, LLC, Salt Lake City, UT.

Endometriosis is a complex condition of uncertain etiology in which endometrial glands, stroma, and/or tissue normally confined to the inner lining of the uterus is found outside the uterus, most often in the pelvic area. Endometriosis is further characterized by inflammation, cysts, and scarring at affected sites, as well as by pelvic pain, dysmenorrhea, and infertility. Endometriosis affects up to 10% of reproductive-age women. Using a large population-based genealogy database, we have demonstrated a significantly elevated kinship coefficient between endometriosis patients compared to matched population control sets. Family studies have also determined the relative risk of developing endometriosis among first-degree relatives to be 4-8 fold higher than the risk in the general population. Genetic association studies have cumulatively tested association between endometriosis and over 50 genes from numerous pathways, including cytokine signaling and cellular differentiation and regulation. To further elucidate the genetic underpinning of endometriosis, we undertook a genome-wide association study that involved 588 endometriosis patients and 1534 controls. Genotyping was performed using the Affymetrix 6.0 GeneChip. Of the 906,600 SNPs present on the 6.0 chip, 673,708 SNPs remained after filtering. Participants were initially evaluated for ethnicity using principal components analysis as implemented in the Eigenstrat package and subsequently analyzed for association using the Armitage Trend test as implemented in the PLINK package. In all, a total of 90 SNPs were identified with p-Trend values less than $10^{-4}$. We identified 12 autosomal SNPs with p-values between $9.40x10^{-6}$ and $1.56x10^{-8}$ and odds ratios between 0.23 and 1.86. The genes most closely located to these SNPs include *FSTL5*, *ZNF366*, *HLA-G*, *TBL2*, *FOXP2*, *SNX16*, *MPDZ*, *PAPPA* and *KCTD12*. The results presented here strengthen the evidence for a genetic component in endometriosis. As our case population expands, we expect these findings to strengthen further.

## 1025/W/Poster Board #683

**Association of the Protocadherin gene cluster on chromosome 5q31 with extreme obesity in the NHLBI Family Heart Study (FamHS).** *I.B. Borecki[1], M.F. Feitosa[1], R.H. Myers[2], J.B. Wilk[2], Q. Zhang[1], S. Ketkar[1], D.M. Becker[3], L.C. Becker[3].* 1) Division of Statistical Genomics, Washington University School of Medicine, St Louis, MO; 2) Department of Neurology, Boston University, Boston, MA; 3) Division of General Internal Medicine, The Johns Hopkins University School of Medicine.

The prevalence of obesity is increasing in the United States, particularly extreme obesity with its profound morbidities. We sought associations of common variants with extreme obesity (E_OB: BMI≥35.0 kg/m$^2$; N = 63) versus controls (18<BMI<27.0 kg/m$^2$; N=421) in the FamHS. Genotypes were assessed using the Illumina HumMap550 chip, and we imputed up to ~2.5 million SNPs based on HapMap Release 22 CEU phased haplotypes. Logistic regression, adjusting for age, sex, and field center effects that account for population stratification, was carried out, assuming additive SNP effects. The strongest association meeting genomewide significance occurred within the protocadherin (PCDH) gene cluster on 5q31 (p = 1.72e-08), in a broad region of linkage disequilibrium. In all, 48 SNPs in a vicinity spanning ~110 kb, showed evidence of association (P<1e-06); the minimum pairwise LD was D'=1 ($R^2$=0.75). For the SNP with strongest association, the minor allele was associated with an increased risk of E_OB (OR=4.43, CI:2.517.85, for each copy of the risk allele). Referring to the broader cohort of 1,000 subjects from which this sample of cases and controls was selected, the mean BMI was 27.5, 29.7, and 35.6 kg/m$^2$ for genotypes with 0, 1, and 2 copies of the risk allele, respectively, and the SNP accounts for 2.2% of BMI variability. We attempted to validate these associations in an independent sample of 1,251 Caucasian subjects from the Genetic Study of Aspirin Responsiveness (GeneSTAR) study. We tested all 48 PCHD SNPs for association with either E_OB (N=163) or Obesity (N=403; OB: defined as BMI ≥ 30.0 kg/m$^2$). While only 3/48 SNPs replicated for E_OB (p<0.01), virtually the entire region replicates with the OB trait with 37/48 SNPs significant at nominal levels; 9 additional SNPs within the focal LD region also were significant in the GeneSTAR data alone. This is a complex region including several closely-linked members of the protocadherin family, with overlapping reading frames, and with strong LD throughout, thus additional dissection of the region and the signal is needed. The protocadherins are members of the cadherin superfamily. While cadherins have a known role in cell adhesion, evidence suggests protocadherins are highly expressed in the central nervous system, and are involved in the modulation of synaptic transmission and the generation of specific synaptic connections, suggestive of a neuronal pathway influencing obesity.

## 1026/W/Poster Board #684

**Genetic variants in the RELN gene are associated with otosclerosis in populations of European origin.** *G. Van Camp[1], I. Schrauwen[1], M. Ealy[2], M.J. Huentelman[3], M. Thys[1], N. Homer[3,4], K. Vanderstraeten[1], E. Fransen[1], J.J. Corneveaux[3], D.W. Craig[3], M. Claustres[5], C.W.R.J. Cremers[6], I. Dhooge[7], P. Van de Heyning[8], R. Vincent[9], E. Offeciers[10], R.J.H. Smith[2].* 1) Dept Medical Genetics, Univ Antwerp, Antwerp, Belgium; 2) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, 200 Hawkins Drive, Iowa City, IA 52242, USA; 3) Neurogenomics division, Translational Genomics Research Institue (TGen) Phoenix, Arizona 85004, USA; 4) Department of Computer Science, University of California, Los Angeles CA 90095-7088, USA; 5) Université Montpellier 1, Faculté de Médecine et CHU, Laboratoire de Genetique Moleculaire, IURC, Montpellier, F-34000 France; 6) Department of Otorhinolaryngology, Donders Centre for Brain, Cognition and Behaviour, University Medical Center St.-Radboud, Philips van Leydenlaan 15, 6500 HB Nijmegen, The Netherlands; 7) Department of Otorhinolaryngology, University Hospital of Ghent, De Pintelaan 185, 9000 Ghent, Belgium; 8) Department of ORL, University Hospital of Antwerp, Wilrijkstraat 10, 2650 Edegem, Belgium; 9) Jean Causse Ear Clinic, Traverse de Béziers, 34440 Colombiers, France; 10) University Department of Otolaryngology, St.-Augustinus Hospital Antwerp, Oosterveldlaan 24, 2610 Antwerp, Belgium.

Otosclerosis is a common form of hearing loss characterized by abnormal bone remodelling in the otic capsule. The etiology of the disease is largely unknown, and both environmental and genetic factors have been implicated. To identify genetic factors involved in otosclerosis, we used a case-control discovery group (604 samples) to complete the first genome-wide association (GWA) study on otosclerosis with 555,000 single nucleotide polymorphisms (SNPs) utilizing pooled DNA samples. By individual genotyping of the top 250 SNPs in a stepwise strategy, we identified two highly associated SNPs on chr7q22.1 and chr11q13.1 that replicated in two additional independent populations (replication set 1: 784 and replication set 2: 935 samples). In addition, this study also highlights the success of pooling DNA samples as the first screening step in a GWA setup, as approximately 85% of top highly ranked SNPs identified by pooling were validated as true differences between cases and controls by individual genotyping of the discovery group. We then genotyped 79 tagSNPs to fine map the genomic regions defined by the associated SNPs. The region with the strongest association signal (pcombined=$6.23x10^{-10}$; OR:1.52), is on chromosome 7q22.1 and is located in the gene Reelin (RELN), a gene known for its role in neuronal migration. Furthermore, evidence for allelic heterogeneity was found in this region. We confirmed expression of RELN in the inner ear and in stapes footplate specimens. Subsequently, we genotyped several SNPs in this region in 4 additional small European populations (1141 samples total), which replicated again in the same direction when combining all 4 populations (pcombined= $4.25x10^{-6}$). In conclusion, we provide evidence that implicate RELN in the pathogenesis of otosclerosis. These results point towards a possible new function for RELN in bone metabolism.

## 1027/W/Poster Board #685

**A genome-wide association study in sarcoidosis reveals a new risk locus at 6p12.1.** *S. Hofmann[1], A. Fischer[1], A. Till[1], H. Schaarschmidt[1], A. Franke[1], M. Nothnagel[2], J. Müller-Quernheim[3], M. Schürmann[4], S. Schreiber[1].* 1) Inst Clinical Molec Biol, Kiel, Germany; 2) Institute of Medical Informatics and Statistics, Kiel, Germany; 3) Department of Pneumology; University of Freiburg, Freiburg, Germany; 4) Institute of Human Genetics, University of Lübeck, Lübeck, Germany.

Sarcoidosis is a complex systemic inflammatory disease with a strong genetic component. To identify sarcoidosis susceptibility loci, we genotyped 116,204 SNPs in 381 sarcoidosis patients and 392 control individuals of German ancestry. We followed up the 25 most strongly associated SNPs in 1,582 sarcoidosis cases and 1,783 controls. Our results provide strong support for the association of one locus on chromosome 6p12.1 and sarcoidosis (nominal $P$ value = $2.64x10^{-4}$ in the GWAS, and $P$ = $1.17x10^{-3}$ in the validation panel). Extensive fine mapping of the novel locus and expression studies of the corresponding genes points to yet unidentified variants in the *XXX*[*] gen as the most likely risk factor. Genetic and functional association of the gene with several kinds of cancer has been shown. Moreover, the gen is a key member of a pathway that has been implicated to play a role in chronic lung fibrosis and immune system communication. Our results extend the list of genetic loci implicated in sarcoidosis susceptibility and suggest a further promising target for study in related complex immune disorders. -------------------------------------------------- [*]anonymized, the gene name and corresponding SNPs will be presented at the meeting.

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## 1028/W/Poster Board #686

**A combined genome-wide association scan and proteomics approach for the identification of genetic modifiers of pseudoexfoliation syndrome.** *K. Burdon[1], S. Sharma[1], P. Danoy[2], P. Leo[2], A. Hewitt[1], T. Chataway[3], M. Brown[2], D. Mackey[4], J. Craig[1], Wellcome Trust Case Control Consortium.* 1) Dept Ophthalmology, Flinders Univ, Bedford Park, SA, Australia; 2) Musculoskeletal Genetics Group, UQ Diamantina Institute, The University of Queensland, QLD, Australia; 3) Department of Human Physiology, Proteomics Laboratory, Flinders University, Adelaide, SA, Australia; 4) Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, Australia.

Pseudoexfoliation (PEX) syndrome is an age-related disorder characterized by accumulation of fibrillar extracellular deposits in the anterior structures of the eye. It is the most common identified cause of glaucoma and may lead to severe visual impairment or blindness. Recently, common coding variants in the *LOXL1* gene were associated with PEX in a genome-wide scan conducted in Iceland. This genetic association has now been replicated worldwide. Although a strong association between *LOXL1* and PEX is observed in Australia, the prevalence of the disease is significantly lower at around 5% in those aged over 80 years, compared to approximately 40% in Iceland. The *LOXL1* risk allele frequencies are very similar between the countries. Therefore the penetrance appears to be lower in Australia. Thus we hypothesised that there are additional factors that modify the risk of PEX. We have undertaken a genome-wide association scan on 243 PEX cases from Australia and 1436 individuals from the 1950 British Birth Cohort from the Wellcome Trust Case-Control Consortium as well as 62 Australian control samples. Australian samples were typed on either the Illumina HumanHap 370 or 610 arrays and the WTCCC controls on the 550 array. Analysis was conducted on the subset of SNPs common to all three arrays. After quality control screening, 329422 SNPs were analysed in 243 cases and 1496 controls. Three SNPs around the *LOXL1* gene survived Bonferonni correction for multiple testing with the most significantly associated SNP being rs2165421, p= 1.6x10-20. This confirms that the *LOXL1* gene is the most highly associated gene in the Australian population, as well as in Scandinavia where it was first reported. Multiple other SNPs also survived correction for multiple testing and clusters of highly associated SNPs are observed in many functional candidate genes. These are undergoing further investigation to confirm the results. In parallel with the genetic study, we have undertaken a proteomics approach to dissect the molecular pathology of PEX. PEX material isolated at the time of cataract surgery was chemically cleaved and subjected to mass spectrometry. Multiple novel proteins were identified in the material and are currently undergoing validation by immunohistochemistry. The genome-wide association results will be presented in relation to the mass spectroscopy results and those proteins previously reported to be found in PEX material, or differentially expressed in the disease.

## 1029/W/Poster Board #687

**A PRELIMINARY COMPARISON OF THE DISTAL GUT MICROBIOME IN HIV-INFECTED AND HEALTHY SUBJECTS.** *L.M. Bull[1], B. Youmans[2], Y. Shang[2], S.K. Highlander[2], J.F. Petrosino[2], K.C. Worley[1], L. Armitige[3], R.A. Gibbs[1].* 1) Human Genome Sequencing Ctr, Baylor College Med, Houston, TX; 2) Department of Molecular Virology and Microbiology, Baylor College Med, Houston, TX; 3) Department of Infectious Disease, Internal Med, University of Texas-Health Science Center-Houston, Houston, TX.

Metagenomics has begun to revolutionize our perception of health and disease. Within the last decade the requisite role of the GI tract in acute HIV infection has become increasingly evident and potentially implicates the importance of the microbiome in maintaining homeostasis. To examine the relationship between HIV infection and the distal colon's microbiome, we evaluated the difference in microbial community structure and diversity in HAART-naïve HIV infected patients (CD4 cell count <100 cells/ml) and compared their microbiomes to HIV-negative healthy controls. All subjects were matched by sex, race and age. 16S rDNA from fecal samples was amplified and sequenced using standard Sanger methods (1536 clones, 3072 reads per subject), and the taxonomic groups were compared. We found that in all samples, the predominant bacterial classes were the Clostridia (Phylum Firmicutes) and Bacteroidetes (Phylum Bacteroidetes), and to a lesser extent Actinobacteria (Phylum Actinobacteria). There was greater variation in the number of minor groups found in the control, including representatives from Betaproteobacteria and Erysipelotrichidae, which were not identified in the HIV-infected cases. We also found that in both case groups there was an average reduction of the Bacteroidetes to half of the community membership representation that was observed in the controls and a substantial increase in Clostridia and Bacilli when compared to the controls. Most significantly, there was a clear trend in the differences between the subjects. Group A had a slightly more advanced disease and almost a log greater HIV viral load (average 5.26 log10 copies/ml) than Group B (average 4.32 log10 copies/ml). While both case groups showed drastic changes at the phylum level compared to the controls, Group A also showed both a greater reduction in Bacteroidetes and a greater increase in Firmicutes than Group B, doubling the membership of Bacilli in comparison to Group B. Similarly there was greater variation in the number of minor class groups found in Group B compared to Group A, although there was less variation than found in the controls. This suggests that the shift in the GI microbial structure maybe correlated with HIV viral load in a dose-dependent manner. This is the first study to examine the relationship between HIV infection and the human distal colon's microbiome, and the first to report a possible association between HIV viral load and key microbial community membership.

## 1030/W/Poster Board #688

**Dairy intake associates with the IGF2 rs680 polymorphism to height variation in Greek children. The GENDAI study.** *GV. Dedoussis, E. Louizou, C. Papoutsakis, KP. Skenderi, M. Yannakoulia.* Dietetics-Nutrition, Harokopio University, Athens, Greece.

Objective: Height is a classic polygenic trait with a number of genes underlying its variation. We evaluated the prospect of gene to diet interactions in a children cohort for the IGF2 rs680 polymorphism and height variation. Methods: We screened 795 peri-adolescent children (424 females) aged 10-11 years old from the (Gene and Diet Attica Investigation; GENDAI) paediatric cohort for the IGF2 rs680 polymorphism. Results: Children homozygous for common allele (GG) were taller (148.9 ± 7.9 cm) comparing to those with the A allele (148.1 ± 7.9 cm), after adjusting for age, sex, and dairy intake ($\beta$±SE: 2.1± 0.95, p=0.026). A trend for interaction for the Igfrs680xdairy intake is also revealed (p=0.09). Stratification by IGF2 rs680 genotype revealed a positive association between dairy products intake and height only in A allele carriers, adjusted for the same confounders (standardized $\beta$=0.111, p=0.014). When dairy intake was classified, based on the median value, into two equal groups of low (1.9 ± 0.7 servings/day) and high dairy products intake (4.4 ± 1.5 servings/day), it was found that in A allele children high dairy eaters were significantly taller (p=0.05) compared with low dairy eaters (148.8 ± 7.9 cm vs 147.4 ± 7.7 cm respectively, adjusted for age and sex). Conclusion: A higher consumption of dairy products associated with increased height depending on the rs680 IGF2 genotype. Thus, exploring height variants and elucidating possible interactions with environmental factors like diet could help us to design effective strategies for the prevention of height associated diseases.

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## 1031/W/Poster Board #689

**Interaction between maternal smoking and TNF, GSTP1, ADRB2 variants in childhood wheezing.** *F. Nyberg[1, 2], S. Panasevich[1], C. Lindgren[3], J. Kere[4, 5, 6], M. Wickman[1, 7, 8], G. Pershagen[1, 9], E. Melen[1, 10].* 1) Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden; 2) Epidemiology, AstraZeneca R&D, Molndal, Sweden; 3) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 4) Clinical Research Centre, Karolinska University Hospital, Huddinge, Sweden; 5) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 6) Department of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden; 7) Sachs Children's Hospital, Stockholm, Sweden; 8) Centre for Allergy Research, Karolinska Institute, Stockholm, Sweden; 9) Department of Community Medicine, Karolinska University Hospital, Stockholm, Sweden; 10) Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden.

**Background:** Children exposed to tobacco smoke early in life have higher risk of wheeze. Individual susceptibility may depend on genetic factors. We studied gene-environment interactions between single nucleotide polymorphisms (SNPs) in the *tumor necrosis factor* (*TNF*), *glutathione S transferase P1* (*GSTP1*), $\beta$*2-adrenoreceptor* (*ADRB2*) genes and tobacco smoke exposure in relation to asthma, childhood wheeze and sensitisation.

**Methods:** In the Swedish prospective birth cohort BAMSE (n=4089), data on tobacco smoke exposure and clinical outcomes up to age four were collected by questionnaires, specific IgE measurements and medical examinations. Early tobacco smoke exposure was defined as maternal smoking during pregnancy and/or shortly after childbirth. We investigated 5 *TNF*, 6 *GSTP1*, and 3 *ADRB2* SNPs in 982 selected wheezers and non-wheezers.

**Results:** Significant interaction with tobacco smoke exposure was found for 3 *TNF* SNPs (-857C/T, Intron 1, Intron 3) with respect to early wheeze (up to 2 years of age). For example, the odds ratio (OR) for developing early wheeze related to early tobacco smoke exposure in children with wild type CC homozygote genotype of the *TNF* -857 SNP was 2.3 (95%CI 1.5-3.6), while no risk related to tobacco smoke exposure was seen in carriers of the rare T allele. A clear dose-response was observed, with an OR of 1.2 (95%CI 1.1-1.5) per each additional pack per week smoked by the mother during pregnancy, in children with the common CC genotype. Suggestive interaction was also seen for 3 *GSTP1* SNPs (Intron 5, Intron 6 and Ile105Val) with respect to transient wheeze, but not for *ADRB2* and wheeze. No interactions were suggested for allergic sensitisation.

**Conclusions:** Our results suggest that the risk of early childhood wheeze associated with tobacco smoke exposure may be modified by TNF and GSTP1 polymorphisms.

## 1032/W/Poster Board #690

**Association between *FTO* polymorphism, adiposity peak and adiposity rebound in the Northern Finland Birth Cohort 1966.** *U. Sovio[1], N.J. Timpson[2,3], N.M. Warrington[4], L. Briollais[5], D. Mook-Kanamori[6], M. Kaakinen[7], A.J. Bennett[2], J. Molitor[1], M.I. McCarthy[2], M.-R. Jarvelin[1,7,8].* 1) Imperial College London, UK; 2) University of Oxford, UK; 3) MRC CAiTE Centre, Bristol University, UK; 4) The University of Western Australia, Australia; 5) Mount Sinai Hospital, Canada; 6) Erasmus Medical Center, the Netherlands; 7) University of Oulu, Finland; 8) National Public Health Institute, Finland.

A common variant in the fat mass and obesity related gene (*FTO*) has a strong effect on adult body-mass index (BMI) but its association with BMI development throughout childhood is less well studied. Early age of adiposity rebound (AR), i.e. the point where BMI reaches its minimum in childhood, is associated with higher level of cardiovascular disease risk factors such as obesity, hypertension and diabetes in adulthood. The association between timing of infant adiposity peak (AP) and these risk factors is unclear. This study investigated the association between the *FTO* SNP rs9939609 and both age and BMI at AP and AR using longitudinal data from the Northern Finland Birth Cohort 1966. AP and AR were derived from random effects models fitted at >0-1.5 years and >1.5-13 years (N=2,782 infants and N=3,413 children with minimum 3 measurements per age window). Additionally, correlations between derived parameters and BMI at age 31 were studied. Mean age at AP was 8.8 months and at AR was 5.7 years, and mean BMI at AP was 18.0 kg/m$^2$ and at AR was 15.4 kg/m$^2$. Age at AP correlated weakly with BMI at AP (r=0.04) and at AR (r=0.18) but not at 31 (r=0.02). Age at AR had a weak inverse correlation with BMI at AP (r=-0.05), and a modest inverse correlation with BMI at AR (r=-0.50) and at 31 (r=-0.44). BMI at AP and BMI at AR predicted adult BMI (r=0.20 and r=0.45, respectively). The *FTO* risk allele associated with slightly later AP (3 days per allele, p=0.0002) but it did not associate with BMI at AP (p=0.67). The risk allele associated with earlier AR (1.4 months per allele, p<0.0001) and with higher BMI at AR (0.12 kg/m$^2$ per allele, p<0.0001). The *FTO* effect on BMI increased gradually throughout childhood. This study shows the *FTO* effect is apparent by the time children reach AR.

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## 1033/W/Poster Board #691

**Response to microRNA therapy in a model of the commonest human blood cancer.** *N. Zanesi, A. Bottoni, M. Kaou, R. Visone, A. Burch, C. Taccioli, M. Fabbri, S. Costinean, A. Efanov, E. Gaudio, Y. Pekarsky, A. Cimmino, C. Croce.* The Ohio State University, Columbus, OH.

Background: Chronic lymphocytic leukemia (CLL) is the most frequent hematopoietic cancer in adults in the Western world. Previously, we generated transgenic mice over-expressing the TCL1 oncogene in B cells. These mice develop a disease very similar to human CLL. Recent studies showed that CLL harbors also alterations in the microRNAs genes miR-15a and miR-16-1 that are frequently deleted and/or down-regulated in CLL patients. Both microRNAs negatively regulate Bcl2, an anti-apoptotic protein over-expressed in human as well as mouse CLL B cells. Therefore, miR-15 and -16 are natural antisense Bcl2 interactors that could be used for therapy of Bcl2-overexpressing malignancies. Since mouse CLL cells, like in humans, are mostly resting CD5+ B cells, in a previous study we did transplantation experiments of mouse malignant lymphocytes into syngeneic mice in order to expand CLLs in vivo. Purpose: We exploited the in vivo transplants to verify the potential ability of miRs 15/16 to delay or stop mouse CLL. Experimental Design: Ex vivo treatments of CLL cells with miRs 15/16 were performed as follows. White cells with expanded CD5+/IgM+ populations (70 to 95%) were isolated from the enlarged spleen of two syngeneic mice previously transplanted with CLL # 450 and 504 and incubated in vitro in 10% FBS in RPMI medium 1640 in three different conditions: a) regular medium, b) transfected with pSR-GFP-Neo expression lentiviral vector containing both miRNAs (TWEEN-miR15/16), and c) transfected with control empty lentiviral vector. After 2-3 days incubation, fifteen million cells were injected intraperitoneally into new syngeneic mice to monitor the evolution of the disease over time. Results: Eight mice were used as controls (injected with untreated or empty virus-treated cells) and eight more animals were injected with miR-treated cells. After at least 10 months from CLL cells injection, seven control mice were dead (87%) while only one miR-treated mouse died (13%; Fisher's exact test, $p = 0.01$). These findings show a significant delay in the disease due to miR-treatment. Conclusions: This is the first study showing an inhibitory effect of specific microRNAs on CLL phenotype with a therapeutic potential. These promising results from the ex vivo experiments encourage us to try now a direct in vivo approach to demonstrate the tumor suppressor effect of miR 15/16 in the TCL1 mouse model of CLL. Supported by the Sidney Kimmel Foundation for Cancer Research.

## 1034/W/Poster Board #692

**Gorham Stout Syndrome of the skull base.** *E. Prera, N. Lüblinghoff, W. Maier, R. Laszig, R. Birkenhäger.* Otorhinolaryngology H&N Surg, University Medical Center Freiburg, D-79106 Freiburg, Killianstr. 5, Germany.

Background: The Gorham Stout Syndrome (GSS) is exceedingly rare; as fewer as 150 cases have been described worldwide, 31 of which involve the facial skeleton and skull. GSS is characterised as a benign but aggressive angiomatosis of the bone with predilection sites of the shoulder and pelvic girdle. Alterations in the MMP2 and TNFRSF11A genes are potentially involved in the Gorham Stout Syndrome. Case History: A 40-year-old male patient presented himself in 2000 with a right-sided hearing loss and tinnitus for about nine month. He also developed dysphonia and dysphagia followed by headache and neck pain with nausea and emesis. The CT scan showed a pronounced osseous destruction of the skull base. The tip of the right petrous bone was destroyed reaching the semicircular canals. On the left side the inner auditory canal, the jugular foramen and the carotid canal were partly differentiated. The histological finding of an open biopsy showed an unspecific inflammation. Therapy was radiation with 40 Gy. In 2004 the clinical examination proved a now bilateral close to deafness hearing loss. The CT scan showed progression of the osteolytic lesions at the posterior and middle skull base, the petrous bone with complete destruction of the left inner ear. The nystagmography revealed a vestibular failure on the right side with spontaneous nystagmus to the left side. The radiological picture and reanalysing the biopsy (angiomatosis of the bone) supported the suspicion of a GSS. As a differential diagnosis a Schwannoma was considered. Methods: For the background of the patient history we performed direct sequencing of the encoding exons, intron-transitions and the promoter region of the NF2, MMP2 and TNFRSF11A gene. Also a whole genome analysis was accomplished with the Affymetrix 500K SNP array to identified additional factors witch maybe involved in that rare disease. Results: We detected no mutation in the NF2, MMP2 and TNFRSF11A gene. We further identified seven polymorphisms in MMP2 and TNFRSF11A gene that do not seem to cause GSS. Conclusion: The clinical, histological and molecularbiological findings lead to the 3 following differential diagnosis: Gorham Stout Syndrome, NF2 and enlarged chronic osteomyelitis . This case may represent a combination of these three diseases or a new entity because the findings do not match the typical profile of one of the three. Until now it is not conclusively settled if additional genes are involved in this rare disease.

## 1035/W/Poster Board #693

**Investigation of the NPAS3 gene as a candidate in gliomas.** *M.S. Rana[1], F. Morin[1], D. Kamnasaran[1,2].* 1) Pediatrics Research Unit, Laval Hospital Research Centre, Quebec, Canada; 2) Department of Pediatrics, Laval University, Quebec, Canada.

Gliomas are among the most common tumors occurring in ~50-60% of adults and ~30% of children with brain tumors. Overall, the prognosis is still poor especially with those patients diagnosed with a high grade glioma such as Glioblastoma multiforme (GBM). Moreover, there is a current paucity in our knowledge of relevant genetic pathways that contribute towards gliomagenesis. We previously cloned and discovered the NPAS3 gene, a transcription factor mapping to human chromosome 14q13, is mutated in patients with schizophrenia (J Med Genet. 2003 40(5):325-32). In our quest to understand the roles of NPAS3 in human diseases, we investigated this gene as a candidate for gliomas. With the use of RT-PCR, western blot analyses and immuno-histochemistry, we identified NPAS3 expression in human astrocytes. To investigate the link between NPAS3 and gliomagenesis, we identified loss/reduction of NPAS3 protein expression in a panel of human glioma cell lines (n=3/5), and in ~68% of human adult GBM surgical specimens (n=31/46), compared to levels of expression in normal astrocytes. With the use of FISH analyses, we identified both homozygous and heterozygous loss of function deletions of the NPAS3 gene in our panel of human glioma specimens with mutant NPAS3 expression. Furthermore, we discovered that an elevated expression of NPAS3 in a human malignant cell line could significantly suppress the in-vitro and in-vivo transformation potential. The function of the NPAS3 gene is relatively unknown to date. However, our data is the first to suggest a potential functional link between NPAS3 and gliomagenesis, with a potential tumor suppressive role.

## 1036/W/Poster Board #694

**Evidence for a shared CASP8 haplotype associated with multiple common cancers.** *R. Abo[1], Z. Cai[1], L. Wei-Yu[2], G. Elliott[2], SH. Rigas[2], DT. Bishop[3], LA. Cannon-Albright[1], DE. Neal[4], JL. Donovan[5], FC. Hamdy[6], A. Cox[2], NJ. Camp[1].* 1) Biomedical Informatics, Univ Utah, Salt Lake City, UT; 2) University of Sheffield, Sheffield, UK; 3) University of Leeds, Leeds, UK; 4) University of Cambridge, Cambridge, UK; 5) University of Bristol, Bristol, UK; 6) University of Oxford, Oxford, UK.

Recent studies identified a CASP8 variant to be associated with breast cancer in Caucasian women (Cox et al. 2007). Subsequently this was refined to a risk haplotype (Shephard et al. 2009). Given these findings and the key biological role of CASP8 in the apoptosis pathway, it is hypothesized that other common cancers may share CASP8 genetic risk factors. For this study, we analyzed previously generated case-control genotype data for 14 tagging SNPs across CASP8 for breast cancer (brca; n=2,277), prostate cancer (prca; n=2,618) and colon cancer (coca; n=2,178) using hapConstructor (Abo et al. 2008). The 3 cancers were analyzed separately to identify cancer-specific risk haplotypes, as well as together to determine the possibility for shared risk alleles across common cancers. HapConstructor is a haplotype-mining technique that can be used to identify risk haplotypes, which also allows for meta analyses. Very simply, it begins by testing single SNP associations and builds haplotypes SNP-by-SNP based on significance. The initial separate analyses indicated significant haplotypes in all three datasets. Although the haplotypes identified were not identical from each analysis, similarities were evident. The risk direction for brca and coca were in the same direction; however, for prca the direction of the effect appeared to be reversed. To investigate the possibility of shared CASP8 haplotypes across the three common cancers, we performed three hapConstructor meta-analyses: brca and coca: brca and prca (allowing the direction of risk to be reversed for prca); and for all three cancers (again, reversed for prca). Inspection of the most significant haplotypes from each of the three meta analyses indicated extreme consistency with each other and with the previous results from Shephard et al. (2009). From the brca and coca analyses, the most significant haplotype achieved a p-value of $9.0 \times 10^{-5}$. The most significant haplotype from the brca and prca analysis was $p=1.6 \times 10^{-4}$. From the combined three-cancer meta analyses the most significant haplotype was $p=3.0 \times 10^{-5}$. Similarities in these haplotypes results were quite striking and indicative of single underlying risk variant. Whilst these results require replication, the strong concordance observed suggests that a shared genetic factor in CASP8 may be associated with multiple common cancers.

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## 1037/W/Poster Board #695

**Molecular characterization of P2X7 gene encoding polymorphisms in the Omani patients with gastric cancer.** *A. Alsaegh[1], A. Al-Shukaili[2], M. Al-Moundhri[3].* 1) University of Calgary/Sultan Qaboos University. 903, 80 Point McKay Cres.NW. Canada, Alberta, Calgary T3B 4W4; 2) Sultan Qaboos University, Department of Microbiology and Immunology. PO Box 35, Muscat 123, Oman; 3) Sultan Qaboos University, Department of Medicine. PO Box 35, Muscat 123, Oman.

Links between cancer and inflammation were first established in the nineteenth century, on the basis of observations that tumour arose at sites of chronic inflammation. The hallmarks of cancer-related inflammation include the presence of inflammatory cells and inflammatory mediators (for example, cytokines and prostaglandins) in tumour tissues, tissue remodelling and angiogenesis similar to that seen in chronic inflammatory responses, and tissue repair. One of the inflammatory mediators that have been proven to be a key mediator in inflammation is interleukin 1 beta (IL-1β). P2X7 receptor plays an important role in the production of IL-1 β. P2X7 is a member of P2X family of nucleotide-gated channels, which in the presence of ATP forms a non-selective channel that allows the passage of molecules. Arg-307 to glutamine (946 G →A ) that lies within the ATP binding pocket of the extracellular domain and if found abolishes the binding of ATP to the receptor. In our study, we are investigating the Arg-307 to glutamine (946 G →A ) polymorphism in exon 9 of the P2X7 gene, in patients with gastric cancer (n=25) and healthy control (n=25). PCR and direct sequencing methods were used to detect this polymorphism. Results: the SNP of P2X7 gene (946 G →A) in exon 9 was not detected in both groups. Conclusion: the loss of function of SNP (946 G→A) of P2X7 does not appear to be a common polymorphism in Omani population and does not seem to be a susceptibility gene locus for the development of gastric cancer in the Omani patients.

## 1038/W/Poster Board #696

**Variation in the adiponectin (ADIPOQ) and adiponectin receptor (ADIPOR1) genes and prostate cancer in African American men.** *J. Beebe-Dimmer[1], K. Zuhlke[2], A. Ray[2].* 1) Karmanos Cancer Institute, Detroit, MI; 2) University of Michigan Medical School, Ann Arbor MI.

Background and Purpose: Prostate cancer is the most common invasive cancer diagnosed among men in the United States and the second leading cause of cancer death. The incidence of prostate cancer is 60% higher among African American (AA) men and they are approximately two times more likely to die from their disease compared to Caucasian men. Adiponectin, a protein derived from adipose tissue is suspected to play an important role in prostate carcinogenesis. A genetic influence on circulating levels of adiponectin has been demonstrated in a number of studies. Variants in the adiponectin (ADIPOQ) and its type I receptor (ADIPOR1) genes have been recently linked to an increased risk of both breast and colorectal cancer. Therefore, we set out to examine the role of polymorphisms in these genes and risk of prostate cancer in AA men. Methods: Ten haplotype-tagging SNPs in ADIPOQ and ADIPOR1 were genotyped in DNA samples from 134 AA prostate cancer cases and 355 controls participating in the Flint Men's Health Study. A logistic regression modeling approach was used to test the association between these SNPs with risk of prostate cancer and obesity. Results: No significant associations were detected between any of the tested SNPs and prostate cancer. However, a single SNP in ADIPOQ (rs1501299) was associated with increased body mass. The +276G→T SNP was more commonly observed among obese men (BMI >30.0 kg/m2) compared to non-obese men (p=0.02). Conclusion: Based on these findings, genetic variation in ADIPOQ and ADIPOR1 does not predict prostate cancer in AA men. However, obese men were more likely homozygotes for the variant allele (TT) in rs1501299, a finding supported by studies linking this SNP to circulating adiponectin. Further investigation is warranted to determine if racial differences exist in the influence of the adiponectin pathway on prostate cancer risk.

## 1039/W/Poster Board #697

**Large-scale fine mapping of *HNF1B* reveals a more complex association with prostate cancer risk.** *S.I. Berndt[1], M. Yeager[1,2], K.B. Jacobs[1,2,3], R.B. Hayes[4], D.J. Hunter[5,6], R.N. Hoover[1], G. Thomas[1], S.J. Chanock[1] for the CGEMS Initiative.* 1) Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, MD; 2) Core Genotyping Facility, Advanced Technology Program, SAIC-Frederick, Inc., NCI-Frederick, Frederick,MD; 3) Bioinformed Consulting Services, Gaithersburg, MD; 4) Division of Epidemiology, New York University Medical Center, New York, NY; 5) Department of Epidemiology, Harvard School of Public Health, Boston, MA; 6) Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Previous genome-wide association studies revealed an association between a locus in the *HNF1B* gene (rs4430796) and both prostate cancer and diabetes risk. Subsequently, a second locus (rs11649743) separated from the first by a recombination hot spot was reported to be associated prostate cancer risk. In order to fine-map genetic variation in this important prostate cancer region further, we genotyped 87 SNPs in the region harboring *HNF1B* in 10,272 prostate cancer cases and 9,123 controls of European ancestry from 10 case-control studies as part of the Cancer Genetic Markers of Susceptibility (CGEMS) initiative. SNPs were chosen from a region defined using the 0.2cM HapMap recombination data flanking the most significant SNP. Tag SNPs were selected using the HapMap CEU population with D'=0.6; notable SNPs ($P<10^{-3}$) from the initial genome-wide scan served as obligate-includes. TagSNPs were included if they were observed to be correlated with an $r^2 \geq 0.8$ in the HapMap CEU, YRI, and JPT+CHB populations with the obligate-includes. Of the SNPs evaluated, ten were significantly related to prostate cancer risk ($P < 1x10^{-7}$) with the most significant association being observed with rs4430796 ($P = 2.5x10^{-23}$). However, risk within this first locus was not entirely explained by rs4430796. Although modestly correlated ($r^2$=0.64), rs7405696 was also associated with risk ($P = 1.3x10^{-21}$) even after adjustment for rs4430769 (P = 0.009). As expected, rs11649743 was also related to prostate cancer risk ($P = 5.1 \times 10^{-8}$); however, the association within this second locus was stronger for rs4794758 ($P = 3.3 \times 10^{-14}$), which explained all of the risk observed with rs11649743 (P = 0.41 and P = 0.001 for rs11649743 and rs4794758, respectively, in the joint model). After mutual adjustment, all three SNPs (rs4430796, rs7405696, and rs4794758) remained associated the prostate cancer risk (P < 0.02). Compared to men without any risk alleles, men with six *HNF1B* risk alleles had a 1.8-fold increased risk of prostate cancer (95% CI: 1.47-2.19). Haplotype analysis also revealed a multifaceted pattern of association. In conclusion, this study demonstrates a complex association between genetic variation in the *HNF1B* gene and prostate cancer risk. Additional studies are needed to further characterize this important region and to assess the functional impact of genetic variation in this gene.

## 1040/W/Poster Board #698

**Association of chromosome 8q variants with prostate cancer risk in Caucasian, Hispanic, and African American Men.** *J. Beuten[1,2], J. Gelfond[3], M. Martinez-Fierro[5], K. Weldon[2], A. Crandall[2], A. Rojas-Martinez[5], I. Thompson[4], R. Leach[1,2,4].* 1) Department of Pediatrics; 2) Department of Cellular and Structural Biology; 3) Department of Epidemiology and Biostatistics; 4) Department of Urology, The University of Texas Health Science Center, San Antonio, TX; 5) Biochemistry and Molecular Medicine, School of Medicine, Universidad Autonoma de Nuevo Leon, Monterrey, Mexico.

Multiple independent studies have demonstrated compelling evidence that genetic variations in at least 3 regions of 8q24 independently influence the risk of prostate cancer. We evaluated the association between prostate cancer (PCa) risk and variants within a 615kb region within 8q24 using a customized Illumina panel on a BeadXpress system. Genotyping of 49 haplotype tagged single nucleotide polymorphisms (SNPs) in 2380 samples (879 cases and 1501 controls) of three ethnic/racial groups found SNPs which are significantly associated with the risk for PCa. The highest significance in Caucasian men was found for rs6983267; the AA genotype reduced the risk for PCa (OR=0.48, 95%CI=0.35-0.65, p=2.74x10-6). This SNP also had a significant independent effect from other SNPs in the region in this group. In Hispanic men, rs7837328 and rs921146 showed independent effects (OR=2.55, 95%CI=1.51-4.31, p=4.33x10-4, OR=2.09, 95%CI=1.40-3.12, p=3.13x10-4, respectively). In African American men rs6985419 had an independent effect (OR=3.85, 95%CI=1.50-9.86, p=0.005). Significant synergist effects for increasing numbers of high-risk alleles were found in all three ethnicities. Haplotype analysis revealed major haplotypes, containing the non-risk alleles, that conferred protection against PCa. We found high linkage disequilibrium between significant SNPs within the region and SNPs within the Cub and Sushi Multiple Domains 1 gene (CSMD1), on the short arm of chromosome 8 in Caucasians and Hispanics. These data suggest that multiple interacting SNPs within 8q24, as well as different regions on chromosome 8 far beyond this 8q24 candidate region, may confer increased risk of PCa. This is the first report to investigate the involvement of 8q24 variants in the susceptibility for PCa in Hispanic men.

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## 1041/W/Poster Board #699

**Investigation of innate immunity genes CARD4, CARD8 and CARD15 as germline susceptibility factors for colorectal cancer.** *S. Buch[1, 2], N. Möckelmann[1], w. v. Schönfels[1], A. Franke[4], M. Brosch[1], H. Kalthoff[5], M. Krawczak[6], C.D. Bröring[3], C. Schafmayer[3], J. Hampe[1].* 1) Medical Dept, Inst Clinical Molec Biology, Kiel, Germany; 2) POPGEN Biobank project, Kiel, Germany; 3) Department of General and Thoracic Surgery, Kiel, Germany; 4) Institute of Clinical Molecular Biology, Kiel, Germany; 5) Institute for Experimental Cancer Research / Comprehensiv Cancer Center North, Kiel, Germany; 6) Institute of Medical Informatics and Statistics, Kiel, Germany.

Background: Variation in genes involved in the innate immune response may play a role in the predisposition to colorectal cancer (CRC). Several polymorphisms of the CARD15 gene have been reported to be associated with an increased susceptibility to Crohn disease. Since the CARD15 gene product and other CARD proteins function in innate immunity, we investigated the impact of germline variation at the CARD4, CARD8 and CARD15 loci on the risk for sporadic CRC, using a large patient sample from Northern Germany. Methods: A total of 1044 patients who had been operated with sporadic colorectal carcinoma (median age at diagnosis: 63 years) were recruited and compared to 724 sex-matched, population-based control individuals (median age: 68 years), Genetic investigation was carried out following both a coding SNP and haplotype tagging approach. Results: No significant differences were observed between the patient and control allelic or haplotypic spectra of the three genes under study. Conclusion: Variation in the innate immunity genes CARD4, CARD8 and CARD15 is unlikely to play an important role in the susceptibility to CRC in the German population.

## 1042/W/Poster Board #700

**Comprehensive resequence analysis of a 97kb region containing the MSMB gene associated with prostate cancer.** *L. Burdett[1,2], X. Deng[1,2], J. Boland[1,2], C. Matthews[1,2], J. Bacior[1,2], V. Lonsberry[1,2], A. Hutchinson[1,2], L. Qi[1,2], K. Jacobs[2,3], J. Gonzalez-Bosquet2[2], S. Berndt[2], R. Hayes[2], R. Hoover[2], G. Thomas[2], D. Hunter[4], M. Dean[5], S. Chanock[2], M. Yeager[1,2].* 1) Core Genotyping Facility, NCI Frederick, Bethesda, MD; 2) Division of Cancer Epidemiology and Genetics, Center for Cancer Research, NCI, NIH, Bethesda, Maryland 20892, USA; 3) Bioinformed Consulting Services, Gaithersburg, Maryland 20877, USA; 4) Program in Molecular and Genetic Epidemiology, Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts 02115, USA; 5) Laboratory of Experimental Immunology, Cancer and Inflammation Program, NCI-Frederick, National Cancer Institute, Frederick, MD 21702.

Genome-wide association studies of prostate cancer have identified single nucleotide polymorphism markers in a region of chromosome 10q14, harboring the microseminoprotein-$\beta$ (MSMB) gene. Both the gene product of MSMB, the prostate secretory protein 94 (PSP94) and its binding protein, PSPBP have been previously investigated as serum biomarkers for high-grade prostate cancer risk. Recent functional work has shown that the different alleles of the promoter SNP, rs10993994, influence MSMB expression. Since it plausible that additional variants in this region contribute to the risk of prostate cancer, we have used next-generation sequencing technology to resequence a ~97kb region that includes the area surrounding MSMB (chr10:51168025-51265101) in 36 prostate cancer cases and 26 controls of European origin in order to nominate additional variants that may be investigated in functional studies. We identified 242 novel polymorphisms within this region, including 143 within the 51kb block of linkage disequilibrium (LD) that contains rs10993994 and the proximal promoter of MSMB. No sites were observed to be polymorphic within the exons of MSMB.

## 1043/W/Poster Board #701

**Fine-Mapping of the Prostate Cancer Locus on Chromosome 11q13 Reveals Three Independent Common Variants in CGEMS.** *C.C. Chung[1], J. Ciampa[1], N. Chatterjee[1], M. Yeager[1], K. Jacobs[1], J. Gonzalez-Bosquet[1], R.N. Hoover[1], D.J. Hunter[2], G.D. Thomas[1], S.J. Chanock[1].* 1) LTG/DCEG, NCI/NIH, Bethesda, MD; 2) Havard School of Public Health, Boston, MA.

Based on the second stage of the CGEMS genome-wide association study, we fine mapped a region of chromosome 11q13 marked by rs10896449 (p= 1.76E-09 in a combined analysis of stage 1 and 2). SNPs were chosen from a region defined using the 0.2cM HapMap recombination data flanking the most significant SNP. Tag SNPs were selected using HapMap CEU with D'=0.6; notable SNPs ($p < 1.00E-03$) served as obligate-includes. Tags were chosen with r2 of ≥ 0.8 in HapMap CEU, YRI, JPT+CHB with obligate-includes. In stage three, 94 SNPs passed quality control metrics and were analyzed in ten studies, totaling 10,272 cases and 9,123 controls, all of European background. Combined joint analysis adjusted for age, study, center, and population stratification indicated that 18 SNPs on chromosome 11q13.2 (Chr11q13.2:68660041-68765690) exceeded genome-wide significance (p values: 3.91E-09 to 7.94E-19). 14 of 18 SNPs segregates into three correlation bins; bin1: rs10896438, rs2924538, rs11228551, and rs11228553, bin2: rs4255548, rs4495900, and rs7950547, bin3: rs4620729, rs7931342, rs10896449, rs9787877, rs7939250, rs10896450, and rs11228583 ($r2 > 0.8$ threshold); 4 SNPs (rs10896437, rs2924540, rs12793759, and rs12281017) were singletons. To test for possible independent signals, we examined: 1) the linkage disequilibrium pattern by study, continental origin and all controls; 2) assessment of recombination hotspots using SequenceLDhot; and 3) multi-locus models conditioned on the initial signal, rs10896449. We detected two independent signals, marked by rs10896449 and rs12793759, that showed statistically significant effect after the conservative Bonferroni adjustment for multiple testing (p=4.76E-05). Other SNPs, notably rs10896438, also showed promising associations after accounting for both rs10896449 and rs12793759, but none reached global significance for the whole region. LD between rs10896449 and rs10896438, rs12793759, and between rs10896438 and rs12793759 in all controls (r2, 0.11, 0.17, and 0.14, respectively). Of three observed distinct recombination hotspots, two encompass all 18 SNPs and one separates bin1 from the other bins; notably, one of the new signals, rs12793759 is a singleton. Our study has identified a more complex architecture underlying the common variants contributing prostate cancer risk in this segment of 11q13. Further studies are required to investigate the biological basis of the observed multiple association signals.

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## 1044/W/Poster Board #702

**Genetic interaction contributing to prostate cancer risk identified in 8q24.** *R. Culverhouse[1,2], P. Pal[3], R. Deka[3], C.H. Jin[4], W.J. Catalona[5], B.K. Suarez[4,6].* 1) Department of Internal Medicine, Washington University School of Medicine, St Louis, MO; 2) Division of Biostatistics, Washington University School of Medicine, St. Louis, MO; 3) Department of Environmental Health, Center for Genome Information, University of Cincinnati Medical Center, Cincinnati, OH; 4) Department of Psychiatry, Washington University School of Medicine, St. Louis, MO; 5) Department of Urology, Robert H Lurie Comprehensive Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, IL; 6) Department of Genetics, Washington University School of Medicine, St. Louis, MO.

Few genetic findings in the field of prostate cancer (PC) have been as significant and persuasively replicated as the association signals observed in the region of chromosome 8q24. We recently reported the results of a case/control study of 49 SNPs spanning the 3 regions of 8q24 in 596 men of European descent with histologically verified PC and 567 unrelated men of European descent. These cases were ascertained from patients seen at Washington University School of Medicine between 1989 and 2001. The control subjects were recruited from the same catchment area as the cases and were followed for many years as part of a long-term prostate cancer screening study in which PSA blood tests and digital rectal examinations (DRE) were performed at 6 to 12 month intervals and who met the following criteria: (a) at least 65 years old, (b) never had a DRE suspicious for PC, (c) never had registered a PSA level >2.5 ng/ml, and (d) had no known family history of prostate cancer. In these data, 4 SNPs (rs1016342, rs1378897, rs871135 and rs6470517) were found to be significantly associated with PC after adjusting for multiple comparisons using an allele frequency test. We report here an interaction analysis of SNP genotypes (or tightly linked genetic elements) explaining trait variation in PC. These interactions were identified with the Restricted Partition Method (Culverhouse et al. Genet Epidemiol 2004) and validated using logistic regression. The most significant 2-way interaction involves rs6983267 and rs871135, which individually account for 0.5% and 2.0% of the trait variation for PC, respectively, but which jointly account for 9.7% of the variance. The most significant 3-way interaction involves the same 2 SNPs plus rs1016342 which, by itself, accounts for 1.0% of the trait variation. However, the full three-way interaction model accounts for 12.7% of the variation in susceptibility to PC. In both the 2-way and 3-way models, the addition of the interaction terms decreases the p-value of the respective models by over 5 orders of magnitude.

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## 1045/W/Poster Board #703

**Association of polymorphisms in the TGF Beta pathway with breast cancer risk and breast density.** *E. Dean[1], L. Fejerman[1], I. Romieu[2], E. Lazcano-Ponce[2], S. Huntsman[1], K. Beckman[3], E. Burchard[1], G. Torres-Mejia[2], E. Ziv[1,4].* 1) Dept of Medicine, Inst for Human Genetics, Medical Effectiveness Res Cntr and Helen Diller Family Comp Cancer Cntr, University of California, San Francisco, CA; 2) Inst Nacional de Salud Publica, Cuernavaca, Morelos, Mexico; 3) University of Minnesota, Minn, MN; 4) Dept of Epi and Biostats, University of California, San Francisco, CA.

Background: Transforming growth factor beta 1 (TGFB1) is a regulator of cellular proliferation that suppresses early tumor formation, but, paradoxically, also promotes tumor invasion. A polymorphism in TGFB1 has been associated with breast cancer in a meta-analysis. We evaluated the association between polymorphisms in the TGFB ligands (TGFB1, TGFB2, TGFB3) and the types 1 2 and 3 TGFB receptors (TGFBR1, TGFBR2 and TGFBR3) and breast cancer in a case-control study of breast cancer among Mexican women. We also evaluated the association between these polymorphisms and mammographic breast density, a strong predictor of breast cancer which is highly heritable. Methods: Our study included 579 breast cancer cases and 1057 controls ascertained from clinics and hospitals in 3 districts of Mexico (Mexico City, Veracruz and Monterey). Cases and controls were matched by ascertainment site and by age. We selected tag SNPs using hap map data (CEU and Asian populations) and successfully genotyped 133 SNPs. We also included 106 ancestry informative markers which were used to estimate genetic ancestry. Associations with breast cancer and breast density were tested using logistic regression models and linear regression models, respectively and adjusted all models for genetic ancestry. Results: We found a nominally significant association between 10 of the 133 polymorphisms and breast cancer. The strongest association with breast cancer was with a SNP in the 3' end of TGFBR3 (p=0.002). Only 2 polymorphisms were nominally significant for association with breast density. The strongest association with breast density was with a SNP in TGFBR2 (p= 0.001). We found no polymorphisms that were associated with both breast density and breast cancer. Conclusions: Polymorphisms in the TGFBeta pathway do not clearly explain the association between breast density and breast cancer risk. The nominal associations we identified should be retested in larger studies.

## 1046/W/Poster Board #704

**Association of UGT2B17, UGT2B7 and UGT2B28 gene copy number with prostate cancer risk.** *F. Demichelis[1,2], S.R. Setlur[3], R.R. Hossain[1], C.X. Chen[1], V.E. Van Doren[1], B. Stenzel[4], D.A. Oldridge[1], N. Katabayashi[1], J.S. Ha[3], J.Y. Chen[3], G. Schäfer[4], C. Lee[3,5], M.A. Rubin[1,5], H. Klocker[4].* 1) Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, NY; 2) Institute for Computational Biomedicine, Weill Cornell Medical College, New York, NY; 3) Pathology Department, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 4) Department of Urology, University Hospital Innsbruck, Innsbruck, Austria; 5) Broad Institute of MIT and Harvard, Boston, MA, USA.

Statement Of Purpose: Prostate cancer has been known to have a strong germline component. Recently, several publications have examined the role of germline deletions of the gene, UDP glucuronosyltransferase 2 family, polypeptide B17 (UGT2B17) for association with prostate cancer risk. This gene belongs to a family of UDP glucuronosyltransferases that are involved in the catabolism of steroids. UGT2B17 in particular catalyzes the conjugation of DHT, testosterone and 3a-Diol, thereby regulating androgen homeostasis. Two recent studies reported an association between copy number loss of UGT2B17 with an increased risk of developing prostate cancer while two other studies failed to find association. Methods: The current study takes advantage of a carefully screened population of men at risk of having prostate cancer to explore for associations with copy number variation of UGT2B17, UDP glucuronosyltransferase 2 family, polypeptide B7 (UGT2B7) (both sharing the same substrate specificity) or its neighboring gene UDP glucuronosyltransferase 2 family, polypeptide B28 (UGT2B28). We examined 427 individual (214 cases and 213 controls) drawn from the Tyrol Early PCA Detection Program (Austria), which includes PSA testing on men between 45 and 75 years of age. The PSA threshold is age dependent and is a low as 1.25 ng/ml. The cases are men with prostate cancer and the controls are defined as men with PSA levels below 0.5 ng/ml for 3 years following a negative biopsy. We characterized all samples for copy number changes of the genes UGT2B17, UGT2B7 and UGT2B28. Copy number status was validated for a subset of samples by QPCR. Summary of Results: We did not detect copy number changes in UGT2B7. The overall incidences of homozygous and hemizygous deletion for UGT2B17 were 10.1% and 41.9%, respectively and UGT2B28 were 2.6% and 23.2%, respectively. We found no association of either gene with conferring risk of prostate cancer when examined both individually and in combination. We therefore conclude that these two genes do not play a major role in prostate carcinogenesis. We hypothesize that the discordance between independent studies may be driven by either different criteria in case selection (PSA levels) or by non-specificity of primers for the evaluation of copy number status of the gene of interest (UGT2B17 and UGT2B15 show 95% homology).

## 1047/W/Poster Board #705

**Association of CRYBB2 Gene Polymorphism with Prostate Cancer in African Americans.** *M. Faruque[1, 4], C. Ahaghotu[2], L. Ricks-Santi[3], E. Jingwi[1], T. Mason[1], C. Mouton[4], M. Fidelia-Lambert[5], G. Dunston[1].* 1) National Human Genome Center, Howard University College of Medicine, Washington, DC; 2) Department of Surgery, Howard University College of Medicine, Washington, DC; 3) Howard University Cancer Center, Washington, DC; 4) Department of Community and Family Medicine, Howard University College of Medicine, Washington, DC; 5) Department of Pathology, Howard University College of Medicine, Washington, DC.

Prostate cancer shows disproportionately higher incidence and disease associated mortality rate in African American men, compared to their counterpart in other population groups. Human crystallin beta B2 (CRYBB2) gene has been reported as one tumor signature gene that differentiated between African-Americans and European-American by gene expression profiling and comparison of prostate tumors tissue, thereby suggesting differences in tumor biology between the two groups. Mutations in CRYBB2 gene have been found to also be associated with cataracts. We investigated the role of CRYBB2 variants in prostate cancer in a case-control study of African Americans, consisting of 145 prostate cancer cases and 155 controls (n= 300). Six haplotype-tagged SNPs spanning a region of 6.7 kb upstream of the transcription start site and thus containing the putative promoter were selected for genotyping and association studies. Genotyping was performed by pyrosequencing. One SNP (rs16979774) was found monomorphic in the study population and thus excluded from further analyses. Association was examined by logistic regression analyses with adjustment for age. We found a significant association of prostate cancer with rs9620497 (-1873G>T) in a dominant model. The odds ratio (OR) of T allele for prostate cancer was estimated to be 0.375 (95% CI = 0.157-0.894; P = 0.027). None of the other four SNPs (rs12167370, rs2213882, rs8136087 and rs2267078) was statistically significantly associated with the disease risk. Additional studies are warranted to further confirm the association as well as to examine the role of this variant in the biology of prostate cancer.

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## 1048/W/Poster Board #706

***PDE11A*, a dual-specificity phosphodiesterase that is frequently polymorphic in the general population and conveys risk to adrenal and testicular tumors, may also be a susceptibility gene for prostatic cancer.**

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Despite significant advances in molecular genotyping, Prostate cancer (PC) does not appear to have a major single gene responsible for the disease. Among the genomic loci that have been suggested to harbor potential candidate genes is the 2q31-35 chromosomal area. The gene encoding phosphodiesterase 11A (*PDE11A*) is located on 2q31-32; it belongs to the family of phosphodiesterases (PDE)-enzymes that catalyze the hydrolysis of the second messengers cAMP and cGMP. Four different isoforms are known for *PDE11A*: *PDE11A4* is the isoform with the highest expression in prostate, compared to all other studied endocrine tissues. We recently reported *PDE11A*-inactivating mutations to be associated with a predisposition to endocrine neoplasm, including adrenocortical (ADT) and testicular germ-cell tumors (TGCT), which also express the *PDE11A4*. In the present study we screened 50 unrelated PC patients of Brazilian descent for PDE11A sequence defects. We identified 8 different coding alterations in a total of 16 patients (32%): 7 missense and one stop-codon mutation. Three of the missense were novel (R202C, Y658C and E840K); constructs containing the mutations were tested *in vitro* in HEK293 human cells. All mutant transfectants impaired the ability of the protein to degrade cAMP *in vitro*; an additional four mutants (Y727C, R804H, R867G and M878V) and the R307X stop-codon mutation that had also been found in association with ADT and TGCT, have been tested and published elsewhere as impairing the ability of the enzyme to mediate PDE activity. We compared the type of the mutations and their frequencies with those in 287 healthy control individuals: the newly identified substitutions were not found in the control group and a significantly higher number of *PDE11A* missense and nonsense variations were seen in the PC patients (allelic frequency 0.16 vs 0.043 in the controls, $\chi^2$=18.23, p<0.0001, OR=4.18, 95%CI 2.14 - 8.16). Immunostaining and additional genetic studies aim at further clarifying the role of these sequence changes in PC. We conclude that these preliminary data point to *PDE11A* genetic changes as a potential susceptibility factor for PC; this is consistent with PDE11A's essential role in the regulation of cAMP signaling responses in other endocrine tissues and its involvement as a risk factor for ADT and TGCT. Interestingly, *PDE11A* is inhibited by some of the PDE-inhibitors used for erectile dysfunction by patients of the same age as those that are at risk for PC.

## 1049/W/Poster Board #707

**Analysis of the breast cancer-associated gene PALB2 in familial and sporadic pancreatic cancer.**

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PALB2 (Partner and Localizer of BRAC2) plays a major role in localizing the breast cancer susceptibility gene BRCA2 in the nucleus, thus facilitating DNA repair. PALB2 is itself a modest contributor to breast cancer susceptibility and truncating germline mutations have been observed to segregate with breast cancer in several families. Recently, Jones et al screened 96 familial pancreatic cancer patients and truncating PALB2 mutations were identified in three patients (3.1%) (Science 2009;324:217). We sought to examine a larger cohort of pancreatic cancer cases, including both familial and sporadic types, to determine the wider contribution of PALB2 mutations in pancreatic cancer. We are currently screening the exons and intron-exon boundaries of PALB2 using a combination of HRM (High Resolution Melt), direct sequencing and MLPA (Multiplex Ligation-dependent Probe Amplification) in 276 individuals with pancreatic cancer of which 84 have a family history of pancreatic cancer, 45 have a family history of breast/ovarian cancer and 24 have a history for both. The remaining 123 cases have no family history for either cancer type. Using MLPA we detected a heterozygous, germline deletion including exons 12 and 13 of PALB2 in a patient who was affected by both breast and pancreas cancer (ages 47 and 61, respectively) and whose mother died of pancreas cancer at age 83. There are no other reported cases of cancer in this family. We confirmed the presence of the deletion in the patient by long-range PCR and were able to estimate its size to approximately 6.7kb. Our findings to date support previous observations that germline PALB2 mutations predispose to pancreas cancer in a small fraction of all familial cases.

## 1050/W/Poster Board #708

**The impact of maternal genetic effects in the initiation of childhood acute lymphoblastic leukemia.**

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Acute lymphoblastic leukemia (ALL) is the most common cancer in children but the underlying determinants of this disease remain poorly understood. ALL is a complex disease that involves the combination of multiple genetic and environmental factors during fetal life and early childhood. In terms of genetics, both the affected individual's inherited genotype and parental-mediated mechanisms (i.e. parental genotypes) can affect the initiation of this type of cancer. In particular, a mother can influence the offspring's risk of disease not only as a genetic donor but also because she provides the fetal environment. To investigate the role of maternal genotypes in ALL onset, we genotyped 28 promoter SNPs from 12 cell-cycle genes in a sample of 203 child-parent triads, 118 unrelated cases and 329 unrelated controls. A log-linear, likelihood-based approach was used in a stepwise procedure to assess child and maternal genetic effects and combined feto-maternal effects. Our results reveal novel maternal-mediated effects at CDKN2A rs36228834 (P= 0.017) and CDKN2B rs36229158 (P= 0.022) that were shown to act in combination with the child's genotype to increase susceptibility to disease. Further experimental analysis showed that the promoter variant rs36228834 influences transcriptional activity and alters DNA-protein complex formation in an allele-specific manner. A protective feto-maternal combination effect was also identified at CDKN2B rs2069416 with maternal/child carriage of at least one T allele leading to reduced susceptibility to childhood pre-B ALL in offspring (P= 0.027). Our study suggests that regulatory SNPs in cyclin-dependent kinase inhibitor genes CDKN2A and CDKN2B could influence maternal and fetal gene expression. Disregulated cell division and disruption of tissue homeostasis during gestation could disrupt the maternal-fetal interface and interfere with important physiological processes such as the growth of the fetus and/or normal haematopoiesis and potentially lead to increased susceptibility to ALL. Unraveling offspring from maternal genetic effects will provide invaluable insights into the genetic mechanisms underlying childhood ALL and help refine our understanding of the etiology of this and perhaps other pediatric cancers.

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## 1051/W/Poster Board #709

**Expression and activity of carbonyl reductase 1 and hydroxysteroid dehydrogenase11B1; two enzymes responsible for the conversion of NNK to NNAL.** *A.K. Hull[1], A.L. Knipe[2], C.J. Gallagher[2], G. Chen[2], D. Marshall[1], P. Lazarus[2], J.E. Muscat[2].* 1) Biology, Lincoln University, Lincoln University, PA; 2) Penn State Cancer Institute, Cancer Control & Population Science, Public Health Sciences and Pharmacology,The Milton S. Hershey Medical Center, Penn State College of Medicine, Hershey, PA.

Lung cancer is the most common cause of cancer related deaths in the US. Smoking is estimated to account for 87% of all lung cancer cases. However, only some smokers develop cancer, indicating that they may be more susceptible to the effects of tobacco smoke carcinogens. One of the major carcinogens in tobacco smoke is the nicotine derived nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). NNK is reduced to another potent lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), which can be glucuronidated by the uridine glucuronosyltransferase (UGT) enzyme family and excreted. Metabolism of NNK to NNAL is predominantly carried out by carbonyl reductase 1 (CBR1) and hydroxysteroid dehydrogenase 11B1 (HSD11B1). The current study focuses on discovering individual differences in DNA sequence, mRNA expression, and enzyme activity of CBR1 and HSD11B1. These differences may result in an increased risk of cancer in smokers. The coding region of the HSD11B1 gene was analyzed for nonsynonymous single nucleotide polymorphisms (SNPs). SNP data has previously been published for CBR1; only low prevalence alleles (<2%) were found. Human liver cDNA from 24 individuals were sequenced to identify coding changes in HSD11B1. No coding SNPs were observed in these samples. mRNA expression levels of CBR1 and HSD11B1 from 110 adjacent normal tissue liver samples were found to vary significantly between individuals. Carbonyl reductase activity assays performed with either cytosol (to measure CBR1 activity) or microsomal extracts (to measure HSD11B1 activity) demonstrated no correlation between expression levels of CBR1 mRNA levels and enzymatic activity in cytosol or between HSD11B1 and enzymatic activity in microsomes. The current data shows that variation in activity levels cannot be explained by either DNA sequence variation or mRNA expression. The lack of correlation between mRNA expression and enzymatic activity suggests a complex picture in which either post-transcriptional regulation of protein expression or post-translational regulation of enzyme activity are involved. Alternatively, other enzymes capable of NNK reduction may play a greater role than previously suggested. Currently, variation in CBR1 and HSD11B1 protein levels among the same individuals is being measured.

## 1052/W/Poster Board #710

**Developing an Innovative High-Throughput MALDI-TOF MS Based 40-Plex Assay for Simultaneous Mutational Analysis of Breast Cancer-Linked Genes in Breast Cancer Cell Lines.** *C. Kohler[1], R. Radpour[1], Z. Barekati[1], R. Asadollahi[1], XC. Fan[1], W. Holzgreve[2], XY. Zhong[1].* 1) Department of Biomedicine, Woman`s Hospital Basel, Basel, Switzerland; 2) University Medical Centre of Freiburg,University Freiburg, Freiburg, Germany.

Developing innovative high-throughput techniques for mutational analysis of cancer-related genes may provide the basis for a new dimension of high-throughput diagnostics. Recently we developed a novel high-throughput MALDI-TOF MS based assay, which enables simultaneous detection of up to 40 single-nucleotide variants on various genes in one single reaction. We explored the feasibility of our approach using the newly developed MALDI-TOF MS based 40-plex assay for the investigation of the mutational status of candidate cancer genes in 6 human breast cancer cell lines. These candidate cancer genes have been shown to be mutated at a significant frequency in breast cancer, what could make them an ideal candidate for a breast cancer screening approach. By analyzing assay quality parameters like call rate and call probability and by visually analyzing the spectrograms we could confirm a good performance of the 40-plex assay; with mean call rates ranging between 85 and 100, what is suggested as optimal and with a mean call probability score ranging from 0,78 to 0,91 (1=high quality). Using the MALDI-TOF MS based 40-plex assay we were able to show the possibility of reliable and robust analysis of up to 40 allelic variants simultaneously in a single reaction.

## 1053/W/Poster Board #711

**Rapid detection of sequence variation in the NAT1 3' UTR by Pyrosequencing® technology.** *D. Koontz, J. Huckins, S. Nikolova, M. Gallagher.* Dept DLS, CDC, Atlanta, GA.

N-acetyltransferase 1 (NAT1) mediates activation and detoxification pathways for several known carcinogens. Polymorphisms in this gene have been postulated to modify human risk to a variety of cancers by altering the rate at which potentially carcinogenic compounds are either neutralized or activated. Several allelic variants occur in the 3' untranslated region (UTR) of the gene. These variants are widely studied for their potential to affect mRNA stability, which in turn affects NAT1 enzyme levels. Considerable effort has been invested in the development of genotyping methodologies to detect NAT1 gene variants for pharmacogenomic and epidemiological applications. Most NAT1 genotyping approaches are gel based and consist of PCR-restriction fragment length polymorphism (RFLP) analysis or allele-specific PCR. However, these approaches are slow, labor intensive, and not amenable to the automation needs of large population studies. We developed a novel Pyrosequencing assay, which allows for simultaneous interrogation of the most common variants in the 3' UTR region. The initial assay validation was performed on 90 DNA samples from the Coriell Polymorphism Discovery Resource (PDR) and results confirmed by cycle sequencing. In addition to the wild-type allele, NAT1*4, four variant alleles (NAT1*3, *10, *11, and *26) were detected. These variant alleles consisted of two SNPs, a 9bp deletion, and the rare 3bp insertion. We were able to detect the most frequent combinations of these alleles (*4/*4, *4/*10, *10/*10, *3/*4, *3/*11, and *4/*11), which capture >90% of the variation in this region that affects NAT1 activity. Genotype calls were derived from quantitative peak height ratios within signature regions of the Pyrogram traces that give predictive values for each allelic combination. We genotyped 4 ethnic groups: Hispanic (N=32), Pacific Rim (N=54), African American (N=47), and European Caucasian (N=48) from the Coriell Human Variation Collection. Despite small sample sizes, NAT1*10, designated as the at-risk allele for various cancers by numerous investigators, was considerably less prevalent in European Caucasians than in the other ethnic groups. This method could be of great value to epidemiological studies for screening NAT1 gene variation in large populations to detect informative biomarkers of susceptibility to cancers.

## 1054/W/Poster Board #712

**Identification of a candidate tumor suppressor gene for breast and colorectal cancer from the chromosomal region 11q23.** *A.S.G. Lee[1,2], D.C.T. Ong[1], Y.M. Ho[1].* 1) Div Med Sci, National Cancer Ctr, Singapore, Singapore; 2) Yong Loo Lin School of Medicine, National University of Singapore, Singapore.

The chromosomal region 11q23-q24 is frequently deleted in breast and colorectal cancer, as well as in a diverse range of malignancies. By utilizing loss of heterozygosity (LOH) analysis and microarray comparative genomic hybridization (Array-CGH), a 6-Mb region on 11q23 was examined. A candidate gene *LARG* (also known as *ARHGEF12*), identified from the region, was found to be frequently underexpressed in primary breast (32%; 12/38) and colorectal (54%; 20/37) cancers, and also breast (100%; 11/11) and colorectal (50%; 5/10) cell lines. Underexpression of the *LARG* transcript was significantly correlated with genomic loss (p = 0.00334). A lack of *LARG* promoter methylation was observed in primary breast (n=24) and colorectal cancers (n=26) by qualitative high-throughput analysis of DNA methylation by bass-specific cleavage and mass spectrometry. *In vitro* treatment of four breast and four colorectal cell lines with 5-aza-2'-deoxycytidine and/or trichostatin A did not result in reactivation of LARG. Growth suppressive activity of the breast and colorectal cancer cell lines, MCF-7 and SW620, stably transfected with constructs containing *LARG* or empty vector were assessed using colony formation, cell proliferation and wound healing assays. Reduced cell proliferation and colony formation in both breast and colorectal cancer cells, and a markedly slower cell migration rate in colorectal cancer cells were observed, suggesting that *LARG* has functional characteristics of a tumor suppressor gene.

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## 1055/W/Poster Board #713

**Genome-wide Linkage Analysis of Nasopharyngeal Carcinoma in 223 Multiplex Families.** *X. Liang[1], F. Kuo[2], XR. yang[1], KF. Kerstann[3], C. Huang[2], AM. Goldstein[1], A. Hildesheim[4], C. Chen[5], SR. Diehl[2].* 1) Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, MD; 2) Center for Pharmacogenomics and Complex Disease Research, UMDNJ, Newark, NJ; 3) Winship Cancer Institute, Emory University, Atlanta, GA; 4) Infections and Immunoepidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, MD; 5) Genomics Research Center, Academia Sinica, Taipei, Taiwan.

Nasopharyngeal carcinoma (NPC) is one of the most common malignant tumors in southern China and Southeast Asia. Genetic susceptibility has been suggested to play an important role in the pathogenesis of NPC, but no major susceptibility genes have been identified so far. Power of previous studies has been limited by small numbers of cases and inclusion of few multiplex families. Therefore we conducted a genome-wide linkage scan in 223 multiplex NPC families by genotyping 400 microsatellite markers (10cM spacing) on 2800 individuals (including 515 cases). We analyzed NPC status and anti-Epstein-Barr virus (EBV) seropositivity (absent/present) separately using both parametric and non-parametric linkage analysis. Since shared environmental/viral factors are likely to contribute to the observed familial clustering, we used an ordered subset analysis (OSA) to define a more genetically homogeneous subset of families based on covariates including age at onset (AAO), number of affecteds in a family, anti-EBV seropositivity among unaffected individuals, and cigarette smoking. A region on chromosome 2 (chr2) showed suggestive evidence for linkage at marker D2S364 (non-parametric two point (NPL_2pt) LOD score=2.88, p=0.0001). This marker also had NPL_2pt LOD>1.0 in 4 subset analyses (families with more than 3 affecteds: LOD=1.27, p=0.0078; AAO≤40 yrs: LOD=2.35, p=0.0005; cigarette smoking duration ≥20 yrs: LOD=1.86, p=0.0017; more than 3 anti-EBV seropositive unaffecteds in a family: LOD=2.00, p=0.0012). Multipoint analyses confirmed findings on chr2 in both the overall and subset analyses. The same region on chr2 was also linked to EBV seropositivity. The region defined by both traits was 18.5 cM. In addition to the region on chr2, our study identified a potential linkage region on chr4. D4S2964 (88.4cM) had 2pt LOD=2.72 in families with more than 3 affecteds; 1.72 in early-onset subset; 1.40 in families with more than 3 anti-EBV seropositive unaffecteds, under a recessive model assuming heterogeneity. D4S392 (79.0cM) showed evidence for linkage to EBV status with OSA 2pt LOD=2.25 in younger group using average AAO as a covariate. LOD scores were more modest with NPC as the outcome (LOD=1.06 in the overall set and 1.26 in the EBV positive subset). This marker was close to the peak region identified by a previous study. Additional fine mapping is being conducted to further refine the linkage findings and to narrow down these regions for candidate gene sequencing.

## 1056/W/Poster Board #714

**RUNX3 polymorphisms associated with intestinal-type gastric cancer susceptibility.** *B. Lim, C. Kang.* Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, Korea.

Genetic association of human RUNX3 gene polymorphisms with gastric cancer susceptibility was examined in this case-control association study using 925 unrelated Korean subjects, who were individually genotyped for nine single-nucleotide polymorphisms (SNPs), covering the entire HapMap-enlisted SNPs in the RUNX3 gene. A SNP around the distal promoter of RUNX3 was significantly associated with intestinal-type gastric cancer susceptibility (P = 0.0028) but not with diffuse-type cancer and found to affect CREB1 binding and regulate the promoter activity. Because the intestinal-type is characteristically more differentiated than the diffuse-type, expression and cell growth function of p44-RUNX3 were tested in differentiated and undifferentiated gastric cell lines. RUNX3 expression level was negatively correlated with differentiation status of gastric cancer, and restoration of p44-RUNX3 inhibited proliferation of differentiated gastric cancer cells more prominently than that of undifferentiated cells. Because the intestinal-type gastric cancer is known to develop through chronic inflammation, several inflammation-related phenotypes such as NF-kB activity and cytokine levels were measured in the cells established to stably produce p44- or p33-RUNX3. While p44-RUNX3 reduced NF-kB activity and mRNA levels of IL-1$\beta$ and IL-6, p33-RUNX3 enhanced them. In conclusion, a SNP in the RUNX3 distal promoter is associated with intestinal-type gastric cancer susceptibility, and the risk allele enhances the promoter activity allowing for CREB1 binding and increases production of p33-RUNX3. Increased p33-RUNX3 enhances NF-kB activity and IL-1 abundance, possibly operating a dominant negative role against p44-RUNX3, and finally confers intestinal-type gastric cancer susceptibility.

## 1057/W/Poster Board #715

**Structural variant discovery in a liver cancer-related 39Mb region using capture-sequencing approach.** *C.H. Lin[1], J.S. Su[1], K.M. Wu[1], Y.H. Chang[1], J.C. Li[1], Y.F. Lin[1], Y.M. Liu[1,2], T.T. Liu[2], S.F. Tsai[1,2].* 1) National Health Research Institutes, Zhunan, Taiwan; 2) Genome Research Center, National Yang-Ming University, Taipei, Taiwan.

There are lots of DNA sequence variants, including single-nucleotide polymorphisms, indels and structural variants (SVs) in the human genome. SVs can affect expression of nearby and distant genes, and some of them might cause certain phenotypic differences. SVs vary slightly in location and frequency among different populations. Currently, sequencing personal genome is feasible using high-throughput sequencing systems, and it provides complete genomic information at the base-pair resolution. As an alternative, capture sequencing is an inexpensive strategy to enrich target DNA for sequencing specific regions of interest with acceptable sequence coverage. Chromosome 4q13.3-25 is a well-characterized region for DNA loss-of-heterozygosity in hepatocellular carcinoma (HCC). Genomic sequences (from 76Mb to 115Mb) were used to design a high-density hybridization array (Roche/NimbleGen) to enrich target DNA for sequencing. The 39Mb target sequencing was carried out in 10 HCC tumors and 2 paired non-tumor samples using the Illumina/Solexa system. DNA sample was sheared, ligated to adaptors and purified to obtain 150bp for pair-end sequencing (2x36) in two lanes. In average, the number of mappable reads for each sample was ~9.3 millions, which cover ~84% of the target region. Besides 12 larger-scale chromosomal abnormalities (>1Mb), 42 small SVs (241bp~178kb, average size: 8kb) were identified based on the numbers and pair-end positions of reads. Twenty-one SVs (50%) were found in at least two cases, and some were somatically acquired SVs. Twenty-nine SVs (69%) had been reported in the database of genomic variants. Nineteen protein-coding genes, e.g., UBE2D3 and PDLIM5, are affected by SVs. This study provided the candidate genomic variants for studying the HCC tumorgenesis, and also demonstrated the application of capture sequencing to SV discovery. Although a large proportion of the identified SVs might not be related to HCC tumorgenesis, they can be used as informative and powerful markers for future genetic studies, e.g. copy number variation association studies.

## 1058/W/Poster Board #716

**A Large Scale Genetic Association Study of Esophageal Adenocarcinoma Risk.** *C. Liu[1], G. Liu[2], D. Christiani[1].* 1) Dept Environmental Hlth, HSPH, Boston, MA; 2) Department of Medical Biophysics, Princess Margaret Hospital, University of Toronto, Canada.

The incidence of esophageal adenocarcinoma (EA) is increasing rapidly, particular among white males, over the past few decades in the United States. However, the etiology of EA and the striking male predominance is not fully explained by known risk factors. To identify susceptible genes for EA risk, we conducted a pathway-based genetic association study on 335 Caucasian EA cases and 319 Caucasian controls. A total of 1330 SNPs selected from 354 genes were analyzed using an Illumina GoldenGate assay. The selected candidate genes were in the pathways of cell growth, inflammation, angiogenesis, apoptosis, cell cycle control, sex hormone signal, DNA repair, and carcinogens /procarcinogens metabolism. The genotyped common SNPs include missense and exonic SNPs, SNPs in the promoter and UTR regions, and tagSNPs for genes with little functional information available. Logistic regression adjusted for age, sex, body mass index (BMI) at age 18, and smoking status was used to assess the genetic effect of each SNP on EA risk. We also tested gene-gender interactions using the likelihood ratio tests. SNPs in three genes that play a critical role in apoptosis (*CASP9*, *CASP7*, and *CHEK1*) were found to be associated with an increased risk of EA after correcting for multiple comparisons. Strong gene-gender interaction effect was observed on SNP rs572483 in *progesterone receptor* (*PGR*) gene (nominal p=0.0001; FDR p=0.07). Protective effect was observed among women carrying the variant *G* allele (adjusted OR= 0.27; 95%CI=0.12-0.57) but was not observed among men (adjusted OR= 1.27; 95% CI=0.95-1.69). In conclusion, we found the genetic variants of *CASP9*, *CASP7*, and *CHEK1* in the apoptosis pathway may be associated with EA risk and *PGR* in the sex hormone signaling pathway may be associated with the gender differences of EA risk.

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## 1059/W/Poster Board #717

**Predisposition for TMPRSS2:ERG fusion in prostate cancer by variants in DNA repair genes.** *C. Maier[1], M. Luedeke[1], C.M. Linnert[1], R. Kuefer[2], H.M. Surowy[1], A.E. Rinckleb[1], J. Hoegel[1], M.A. Rubin[3], W. Vogel[1].* 1) Inst of Human Genetics, Univ Ulm, Ulm, Germany; 2) Dept of Urology, Univ Ulm, Ulm, Germany; 3) Dept of Pathology and Laboratory Medicine, Weill Cornell Medical Center, New York, NY.

The oncogenic TMPRSS2:ERG fusion is a common event in prostate cancer. We hypothesized that defects in repair genes lead to an increase of chromosomal rearrangements and thus to the occurrence of the TMPRSS2:ERG fusion. We have previously conducted a genome wide linkage analysis in fusion positive prostate cancer families, revealing potential susceptibility loci on chromosome 5q14, 9p13, 9q21, 10q26, 11q24, 12q15, 13q12, and 18q. In the present study we investigated candidate genes related to DNA damage response and repair from these regions. A total of eleven candidate genes were screened for mutations in TMPRSS2:ERG positive families. We found 17 non synonymous variants (6 had a MAF < 0,05). Fourteen variants were investigated in a case control analysis, including 507 controls versus 327 sporadic and 203 familial cases. A polymorphism in RMI1 (RECQ-mediated genome instability) was associated with prostate cancer in general (OR = 1.33; 1.04 - 1.70; p = 0.02) and an uncommon variant of BRCA2 was associated to familial prostate cancer (OR = 6.16; CI95% 1.19 - 32; p = 0.03;), both however were independent of the TMPRSS2:ERG fusion status. Two variants in ESCO1 (Establishment of Cohesion 1, OR = 4.27; 1.62 - 11.3; p = 0.0034) and POLI (Polymerase iota, OR = 4.62; 1.84 - 11.6; p = 0.0011;) were highly significant associated with TMPRSS2:ERG fusion positive prostate cancer cases, and withstood the correction for multiple testing (Bonferroni threshold p = 0.0038). These results need validation and the variants with high OR may become useful in risk estimates for prostate cancer.

## 1060/W/Poster Board #718

**MATRIPTASE-2 (TMPRSS6) AS A BREAST CANCER RISK FACTOR.** *A. Mannermaa[1,2,3], J. Kauppinen[1,2,3], R. Sironen[1,2,3], Y. Soini[1,2,3], M. Eskelinen[4], V. Kataja[5,6], V.-M. Kosma[1,2,3].* 1) Institute of Clinical Medicine, Pathology and Forensic Medicine, University of Kuopio, Kuopio, Finland; 2) Department of Pathology, Kuopio University Hospital, Kuopio, Finland; 3) Biocenter Kuopio, Kuopio, Finland; 4) Department of Surgery, Kuopio University Hospital, Kuopio, Finland; 5) Department of Oncology, Kuopio University Hospital, Kuopio, Finland; 6) Department of Oncology, Vaasa Central Hospital, Vaasa, Finland.

*TMPRSS6* encodes for matriptase-2, a member of the type II transmembrane serine protease family that has been implicated in tumor progression. Low expression of matriptase-2 has been reported to predict poor patient survival.

We have previously reported chromosomal region 22q12-q13 and further a SNP rs733655 in matriptase-2 gene (*TMPRSS6*) to be associated with breast cancer. Here we report extensive case-control association analysis of the *TMPRSS6* gene by using 13 SNPs across the gene region in an Eastern Finnish population sample of 497 breast cancer cases and 458 controls. Genotyping was done using Taqman chemistry. In a total sample set markers rs733655 and rs2543519 were significantly associated with breast cancer (p=0.041, OR 1.36 and p=0,027, OR 1.38) and a 6-marker haplotype analysis of a 15kb region with high linkage disequilibrium showed a permutated p value of 0.001. In a more homogeneous subpopulation altogether 6 individual markers showed significant association with breast cancer with p-values ranging between p=0.05 and p=0.0002 and in a haplotype analysis with 8 markers a p-value of 0.01 was reached.

Our results provide further evidence for *TMPRSS6* as a risk factor in breast cancer and urges for additional studies on the role of matriptase gene family in breast cancer.

## 1061/W/Poster Board #719

**GSTM1, GSTT1 genes polymorphisms in esophageal squamous cell carcinoma: a case-control study in North-East IRAN.** *H. Marjani[1], F. Biramijamal[1], A. Hossein-Nezhad[1], R. Malekzadeh[2].* 1) National Institute of Genetic Engineering & Biotechnology (NIGEB); Tehran-Iran; 2) Digestive diseases research center-tehran university of medical siences .Tehran -IRAN.

From the early years of 1970's Golestan province in North-east Iran have been recognized as one of the most prevalent areas for esophageal squamous cell carcinoma (ESCC) in the world. Smoking, opium consumption, poor oral hygiene, low nutritional quality and hot tea drinking are thought to be the most important environmental factors in esophageal cancer risk. Exposure to polycyclic aromatic hydrocarbons (PAH) is an evident risk factor for many human cancers specially gastrointestinal tract malignancies.PAHs are generated from incomplete combustion of organic materials such as cigarette and broiled foods. PAHs' electrophilic nucleuses can invade DNA and impose many random mutations in its sequence so make the cells prone to undergo malignant changes. We have studied the genetic polymorphisms of two PAH (xenobiotics) metabolizing enzymes; GSTT1, GSTM1, because of their biologic contributions in detoxification and elimination of many kinds of PAHs.The mentioned two proteins belong to phase II xenobiotic metabolizing enzymes which can reduce electrophilic chemical radicals and make them conjugated with some natural compounds such as glucoronids and finally help them to be excreted in water soluble forms in liver. This study was performed on ESCC patients and healthy controls in Golestan province. For GSTM1 no significant difference was found between cases and controls group but we have noticed that GSTT1 null genotype was significantly high among the case group so there was a statistically meaningful relation between ESCC and GSTT1 null polymorphism (pvalue=0.04).

## 1062/W/Poster Board #720

**Non-sense mediated mRNA decay and microRNA array analysis of Finnish HPCX-linked families.** *H. Mattila[1], T. Ikonen[1], M. Vihinen[2], J. Isotalo[1], H. Oja[3], T. Tammela[4], T. Wahlfors[1], J. Schleutker[1].* 1) Laboratory of Cancer Genetics, Institute of Medical Technology, University of Tampere and Tampere University Hospital, Tampere, Finland; 2) Bioinformatics, Institute of Medical Technology, University of Tampere, Tampere, Finland; 3) Tampere School of Public Health, University of Tampere, Tampere, Finland; 4) Division of Urology, Tampere University Hospital and Medical School, Tampere, Finland.

Several predisposition loci for hereditary prostate cancer (HPC) have been suggested, including HPCX at Xq27-q28. In Finnish population HPCX seems to be very significant, but the susceptibility gene for the locus is still not identified, as the region itself is extremely complicated by structure. Gene identification by non-sense mediated mRNA decay inhibition has proven to be a useful technique for genome-wide discovery of genes containing truncating mutations. Six brother pairs (prostate cancer patient and healthy brother) were selected from families showing linkage to HPCX locus and total mRNA was isolated from cells after pharmacological treatment. Altered levels of gene-specific mRNA expression were measured using Agilent 44K oligoarrays and selected genes were screened for mutations by direct sequencing. For microRNA expression analysis the number of families was increased by five and miRNA expression levels were measured by Agilent Human miRNA V2 Oligo microarrays. MiRanda algorithm was used to identify genomic targets for miRNAs. Seventeen genes were selected for resequencing based on NMD array results but no truncating mutations were found. The most interesting identified variant for follow-up was MAGEC1 Met1Thr and a borderline association was seen between the variant and unselected PRCA (odds ratio (OR), 1.99; 95 % confidence interval (CI), 0.98-3.88) and HPC (OR, 2.86; 95 % CI 0.83-7.55). miRNA profiles were also analysed in HPCX-linked families and 16 miRNAs were found to have altered expression between patients and controls. In further analysis, hsa-miR-770-5p, was found to have highest expression in the group of healthy individuals and lowest expression in the group of aggressive cancer cases. The miRNA target analysis by MiRanda algorithm revealed that 12 of the 16 miRNAs having significantly altered expression also had possible targets site in the found variant sites. Interestingly, hsa-miR-770-5p has a possible target site in the MAGEC1 gene. The role of MAGEC1 Met1Thr variation and hsa-miR-770-5p and their possible involvement in the HPC risk in Finnish population need to be studied further. Also joint profiling and analysis of genes and miRNAs in the HPCX region may be required to fully understand their genetic contribution to prostate cancer risk.

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## 1063/W/Poster Board #721

**Genetic variants encoding carcinogen-metabolizing enzymes may modify the association between breast cancer risk and active cigarette smoking.** *L. Mirea[1,2], M. Cotterchio[1,2], N. Kreiger[1,2], H. Ozcelik[2,3].* 1) Population Studies and Surveillance, Cancer Care Ontario, Toronto, Canada; 2) Dalla Lana School of Public Health, University of Toronto, Toronto, Canada; 3) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada.

The evidence regarding breast cancer risk and cigarette smoking is inconclusive. To evaluate the possible interaction between genetic susceptibility and active smoking, we conducted a case-control study and examined a number of candidate gene variants encoding carcinogen-metabolizing enzymes. DNA and smoke exposure (active and passive) data were obtained from a subset of breast cancer cases (N=1399) and controls (N=1492) that participated in the "Ontario Women's Diet and Health Study" during 2002-2003. Associations between breast cancer risk and genetic main effects (36 SNP's in carcinogen-metabolizing candidate genes CYP1A1, CYP1A2, CYP1B1, CYP2E1, EPHX1, NAT1, NAT2, UGT1A7, SOD2, SULT1A1, GSTM1, GSTT1), active smoking (former/current status and pack-years versus "never active and never passive" referent group), and all gene*smoking interactions were evaluated using multivariate logistic regression models adjusted for confounders. Analyses were also performed separately in pre- (PRM) (cases/controls = 541/626) and post-menopausal (PSM) (cases/controls = 1173/1148) subgroups. Several gene variants were significantly associated with increased breast cancer risk: GSTT1 locus (Deletion vs Present: OR (95%CI)=1.24 (1.05-1.54)), CYP2E1 rs6413432 (AA/AT vs TT: 1.23 (1.03-1.46)), and UGT1A7 rs17868323 (TT vs GG: 1.24 (1.01-1.53)) in the pooled data, CYP1A1 rs4646903 (CC vs TT: 2.64 (1.24-5.58)) in PSM and CYP2E1 rs 2480259 (TT vs CC: 1.70 (1.01-2.85)) in PRM women. Preliminary results suggest no statistically significant association between active smoking and breast cancer risk; however, several gene*smoking interactions were significant. Strongest evidence for effect modification was observed for active smoking status with NAT1 rs4921880 (p=0.01) in PRM and SOD2 rs4880 (p=0.01) in PSM women. A significant interaction was also seen between CYP1B1 rs1056836 and pack-years in the pooled data (p=0.03) and retained in both PSM (p=0.04) and PRM (p=0.05) women. Additional significant interactions were detected in PSM women for GSTT1 (p=0.03), CYP1A2 rs2470890 (p=0.04) and CYP1A1 rs2646903 (p=0.04) with smoke status. Specific to PRM, borderline interactions were seen for UGT1A7 rs11692021 (p=0.05) with smoke status, and for CYP1B1 rs2855658 (p=0.05) with pack-years. Our findings suggest that genetic variants may play a role in modifying the association between breast cancer risk in both pre- and post-menopausal women with active cigarette smoking.

## 1064/W/Poster Board #722

**Sequencing, phylogenetic analysis, and multipopulation studies suggest ABCB5 SNPs may be associated with diverse cancer subtypes.** *K. Moitra, M. Scally, K. McGee, M. Nickerson, G. Lancaster, J. Gould, B. Gold, M. Dean.* Cancer & Inflammation Program, NCI-FCRDC, Frederick, MD.

ABCB5 is a member of the ATP binding cassette (ABC) superfamily of transporters which function in the ATP dependent transport of structurally diverse molecules. Previously, ABCB5 was demonstrated to be a melanoma progression marker predominantly expressed in melanoma stem cells. Cancer stem cells are characterized by their capacity for self renewal and enhanced drug efflux capability thus protecting them from chemotherapeutic regimes. In this context it is crucial to determine the role of genetic variations in *ABCB5*. A phylogenetic analysis indicated that ABCB5, along with ABCB1, ABCB4, and ABCB11, shared a common ancestor which began duplicating early on in the evolutionary history of chordates. ABCB1, ABCB4 and ABCB11 are all full transporters with two nucleotide binding domains (NBDs) and two transmembrane domains. A comparative sequence analysis of the full length ABCB5 transporter indicates that the N-terminal transmembrane and NBD domains have been conserved during mammalian evolution indicating that the gene encodes a full transporter. *ABCB5* contains several relatively common non-synonymous polymorphisms which lie in functionally significant regions of the ABCB5 protein. The functional effect of these variations were predicted using Panther and SIFT programs combined with our knowledge base of ABC transporter function. Results from the in silico studies were applied to predict cSNPs which may crucially impair protein function. From genotyping studies of these SNPs in 52 human populations (HGDP) we found that the SNPs rs34603556, rs2301641, rs6461515 and rs58795451, would be important targets for cancer stem cell association studies. The SNPs rs2301641 and rs6461515 were also present in a number of the melanoma cell lines included within the NCI 60 panel. These cell lines have abundant data available on the effect of over 100,000 compounds on growth, and the genotype information can be used for functional studies.

## 1065/W/Poster Board #723

**Nonsense-mediated decay escaping mutations in microsatellite-unstable colorectal cancer.** *I. Niittymäki[1], A. Gylfe[1], L. Ahonen[2], H.J. Lehtonen[1], M. Laakso[3], J. Sirkiä[1], K. Nousiainen[3], J. Pouwels[2], H. Järvinen[6], K. Nuorva[7], J-P. Mecklin[8], A. Ristimäki[4], T.F. Ørntoft[5], S. Hautaniemi[3], A. Karhu[1], M.J. Kallio[2], L.A. Aaltonen[1].* 1) Department of Medical Genetics, Genome-Scale Biology Research Program, University of Helsinki, Finland; 2) VTT Technical Research Centre of Finland, Medical Biotechnology, Turku, Finland; 3) Computational Systems Biology Laboratory, Institute of Biomedicine and Genome-Scale Biology Research Program, Biomedicum Helsinki, University of Helsinki, Finland; 4) Department of Pathology, HUSLAB and Haartman Institute, and Genome-Scale Biology Research Program, University of Helsinki, Finland; Department of Pathology, Institute of Diagnostics, University of Oulu and Oulu University Hospital, Oulu, Finland; 5) Molecular Diagnostic Laboratory, Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark; 6) The Second Department of Surgery, Helsinki University Hospital, Finland; 7) Department of Pathology, Jyväskylä Central Hospital, Jyväskylä, Finland; 8) Department of Surgery, Jyväskylä Central Hospital, Jyväskylä, Finland.

Genomic instability drives tumorigenesis by allowing the accumulation of genetic alterations that provide cells with growth advantage. Microsatellite-instability (MSI) and the underlying mutator phenotype caused by a defect in mismatch repair (MMR) functions is the hallmark of Lynch syndrome, and is also observed in a subset of all colorectal cancers (CRC). In cells with a defective MMR system, spontaneous length changes of repetitive microsatellite sequences accumulate all over the genome at highly increased rates. At coding regions instability may lead to frameshift mutations and altered protein products. Genes that mutate this way under MMR deficiency giving selective advantage to cells in tumorigenesis are called MSI target genes. It is generally anticipated that the frameshift mutation-containing transcripts that lead to prematurely terminated proteins undergo nonsense-mediated decay (NMD), followed by a reduction in gene expression levels. However, when a premature stop occurs in the carboxyl-terminal end of the gene it might escape decay mechanisms, which may lead to either dominant-negative or oncogenic effects. Aim of this study was a genome-wide unbiased identification of new MSI CRC target genes that escape NMD. By combining bioinformatic search to expression profiling, we created a list of 330 genes that contained mononucleotide repeats from 6 to 10 base pairs and were likely to be translated despite potential mutations. A novel frameshift predictor software was developed to search all repeat-containing transcripts in the human genome that would escape NMD after one nucleotide deletion. To enhance the odds of identifying oncogenic mutants, the analysis was restricted to genes that were overexpressed in MSI CRC versus normal colonic mucosa. All of these genes were screened initially by sequencing the given repeat in a panel of 30 MSI CRCs. Whenever the mutation frequency exceeded 20% in the tumor set, which was considered evidence for possible selection in MSI tumorigenesis, an additional set of 70 MSI CRCs was sequenced. The great majority of the successfully sequenced genes had no mutations. Altogether four genes were mutated in over 20% of the samples in the extended 100 MSI tumor panel. These candidate driver target genes are being evaluated further by various methods, including sequencing of MSI CRC cell lines and microsatellite-stable (MSS) CRCs, statistical analyses, and functional *in vitro* experiments.

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## 1066/W/Poster Board #724

**Differential biallelic expression of PTEN and mRNA degradation in Cowden syndrome patients with heterozygous germline PTEN mutations.** *M. Orloff[1, 2], H. Zhu[1], C. Eng[1, 2, 3, 4].* 1) Genomic Med Inst, Cleveland Clinic, Cleveland, OH; 2) Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH; 3) Department pf Genetics, Case Western Reserve University, Cleveland, OH; 4) CASE Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH.

Heterozygous (het) germline mutations in PTEN are associated with Cowden Syndrome (CS), a dominant disorder characterized by a high risk of breast and thyroid cancers. Previous studies on het PTEN mutation-positive patients showed unexplainable discordance between PTEN genotype and their protein and transcript expression. This led us to hypothesize that some het PTEN mutations may have differential bi-allelic expression that cannot be explained by nonsense-mediated decay (NMD). We also postulated that this differential bi-allelic expression may play a pathogenic role in PTEN mutation-positive CS patients. To test our hypothesis, we used allele-specific quantitative (q) RT-PCR to compare the expression of allele-specific transcript levels in 51 unrelated patients carrying 3 different classes of PTEN mutations. In parallel, we measured PTEN protein expression by q-Western blot in all patients. Relative to the mean total transcript levels of controls (set at 1.0, 99%CI 0.86-1.14), all 3 nonsense mutations (NS) studied, R130X, R233X and R335X, from 39 individuals, showed decreased total transcript levels ranging from 0.22 (0.16-0.29) to 0.56 (0.52-0.60). Relative to mean control PTEN protein levels (set at 1.0, 99%CI 0.91-1.1), normalized PTEN protein levels from these 3 NS groups ranged from 0.22 (0.19-0.25) to 0.3 (0.26-0.34). Of note, het germline R335X was associated with almost no wildtype (WT) transcript (0.1 that of control levels) but relatively higher expression of the mutant allele (2-fold higher than their paired WT allele). All 8 individuals bearing 3 different missense mutations at codon 130 had equal bi-allelic transcription and either normal or slightly decreased protein levels. Unexpectedly, a synonymous variant, G44G found in CS but not controls, showed low (0.23+/-0.04) PTEN mRNA levels with a relatively high expression of the variant-bearing allele. Measurement of mRNA half-lives revealed that all the NS had mutant transcripts with a shorter half-life compared to normal transcripts. Moreover, both the mutant and WT transcripts from G44G had a significantly lower half-life compared to normal transcripts. While we have no direct evidence, our observations may suggest that specific mutations and variants, illustrated by R335X and G44G, can promote degradation of their counterpart WT-allele transcript. This may therefore represent a novel “double whammy” type of mechanism of PTEN dysfunction with decreased dosage of WT PTEN.

## 1067/W/Poster Board #725

**Family-based Association Analysis of 42 Hereditary Prostate Cancer Families Identifies a 15 Kb Gene Locus on Chromosome 22q12.3.** *E.A. Ostrander[1], B. Johanneson[1], S.K. McDonnell[2], D.M. Karyadi[1], P. Quignon[1], L. McIntosh[3], S.M. Riska[2], G. Johnson[1], K. Deutsch[4], G. Williams[1], J.L. Stanford[3,6], D.J. Schaid[2], S.N. Thibodeau[5].* 1) Cancer Genetics Branch, Bldg 50, National Human Genome Research Institute/NIH, Bethesda, MD; 2) Dept. of Health Sciences Research, Mayo Clinic, Rochester, MN; 3) Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle WA; 4) Institute for Systems Biology, Seattle, WA; 5) Depts. of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; 6) Dept. of Epidemiology, School of Public Health, Univ. of WA., Seattle, WA.

Dozens of genome-wide scans for hereditary prostate cancer (HPC) susceptibility loci have been published to date, with putative loci identified on nearly every chromosome. However, few studies have been replicated with statistically significant results. One exception is chromosome 22q, for which five independent studies, including our own, identified strong linkage evidence for a susceptibility locus. Previously, we refined this region to a 2.53 Mb interval using recombination mapping in a set of 42 linked HPC pedigrees from the Mayo Clinic and the PROGRESS family collections. In the present study, we refine this locus to a 15 kb region spanning one major gene by performing family-based association analyses. Prostate cancer cases from 42 families linked to the 22q12.3 region were compared to a set of 533 unrelated population controls. This allowed exclusion of potential phenocopies and unaffected risk-variant carriers, thus increasing the power and accuracy of the overall analysis. The two family collections independently identified SNPs within a 15 kb locus at the 5’ end of the same gene as having the strongest associations, with the most robust results coming from a combined analysis of cases from all 42 families. Analysis of 15 tagSNPs across the 5’ end of the locus identified six SNPs with P-values ≤ 1.5x10-4 (200,000 simulations). Additional analyses using the LAMP program provided consistent results. Two independent family collections, highlighting the same two linkage disequilibrium (LD) blocks within the same gene strongly suggest that a variant within the minimal 15 kb interval, or one in strong LD with it, contributes to prostate cancer risk. This work highlights the values of combined approaches for follow-up of initial findings of linkage for complex traits.

## 1068/W/Poster Board #726

**Are Endothelial Nitric Oxide (NOS3) Gene Polymorphisms Important in Chronic Myeloid Leukemia (Ph+)?** *S. Pehlivan[1], M. Pehlivan[2], L. Kaynar[3], T. Sever[1], M. Yilmaz[2], B. Eser[3], V. Okan[2], M. Cetin[3].* 1) Medical Biology and Genetics, Gaziantep University, Medical Faculty, Gaziantep, Turkey, PhD; 2) Hematology, Gaziantep University, Medical Faculty, Gaziantep, Turkey, MD; 3) Hematology, Erciyes University, Medical Faculty, Kayseri, Turkey, MD.

Purpose: It was aimed to investigate whether there was an association between Nitric Oxide (NOS3) gene polymorphisms and Philadelphia positive (Ph+)-Chronic Myeloid Leukemia (CML). Method: DNA was isolated from the bloods of 59 patients diagnosed with CML and 100 healthy controls. NOS3 (+894) gene polymorphism was analyzed by PCR-RFLP whereas NOS3 (intron 4 VNTR) gene polymorphism was analyzed by means of PCR. The association between these polymorphisms and CML was analyzed using chi-square test and de-Finetti program. Conclusion: When the control and CML groups were compared in terms of Hardy-Weinberg Equilibrium (HWE), no deviations were observed in NOS3 (+894) polymorphism in CML whereas a deviation was detected in the control group (p:0.033). When the control and CML groups were compared in terms of HWE, no deviations were observed in NOS3 (intron 4 VNTR) polymorphism in the control group whereas a deviation was detected in CML patient group (p:0.015). A significant association was detected between the control and CML groups in terms of both allele and genotype frequencies in the 2 polymorphisms analyzed. In terms of haplotype frequency, it was found out that haplotype 13 (AATT) increased significantly while haplotypes 23, 31, 32 and 33 (ABTT, BBGG, BBGT, BB, TT) were present only in CML. Discussion: It is considered that the significant increase in TT allele of NOS3 (+894) polymorphism in CML patients has 7.8 times (OR:4.382/0.499) more risk for the etiopathogenesis of CML in comparison to the healthy group. It is considered that the significant increase in BB allele of NOS3 (intron 4 VNTR) polymorphism in CML patients has 3.2 times more risk for the etiopathogenesis of CML in comparison to the healthy group (OR:1.839/0.580). 2 polymorphisms of NOS3 gene in CML patients were simultaneously analyzed for the first time in this study and it was demonstrated that these polymorphisms could play a role in the etiopathogenesis of CML.

## 1069/W/Poster Board #727

**Updated genome-wide analysis identifies colon neoplasia susceptibility locus at chromosome 7q36.** *L.S. Phillips[1,2], R.C. Elston[1,2], S. Lewis[3], S.D. Markowitz[4,5], G.L. Wiesner[3,6].* 1) Epidemiology/Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Case Comprehensive Cancer Center, Case Western Reserve Univ, Cleveland, OH; 3) Genetics, School of Medicine, Case Western Reserve University, Cleveland, OH; 4) Molecular Biology and Microbiology, School of Medicine, Case Western Reserve University, Cleveland, OH; 5) Howard Hughes Medical Institute, Cleveland, OH; 6) Clinical Genetics, University Hospitals of Cleveland, Cleveland, OH.

Heritable factors account for ~20% of all colorectal cancers, but only a few genes associated with highly penetrant colon cancer syndromes have been identified to date. The Colon Neoplasia Sibling study (CNSS) identified 9q21-23 as a susceptibility locus in a genome-wide linkage analysis of non-HNPCC, non-FAP kindreds with two or more siblings affected with colon adenomatous polyps, advanced adenomas or colon cancer (Wiesner, 2003; Daley, 2008). Since then, several genome-wide association and linkage studies have suggested many other loci of interest, including 8q21 and 8q24, 10p14, 11q23, 14q22, 15q13, 16q22, 18q21, 19q13, 20p12, and most recently 7q31. Our initial results did not find significant linkage signals at any of these loci except for a minimally significant linkage signal at 7q36 (P=0.0011), which was telomeric to the 7q31 region identified in a family based study of colon cancer sibpairs (Neklason, 2008). We then sought to test whether an updated analysis of the CNSS dataset would confirm a putative susceptibility locus on chromosome 7. Phenotypic information for the entire CNSS dataset was updated by additional review of medical records for colon neoplasia in the index cases and siblings. Colon screening results changed affection status for some participants from unknown to either affected or unaffected. Three kindreds were excluded because subsequent tumor microsatellite instability testing identified them as possibly having HNPCC. Haseman-Elston linkage analyses on the updated set of 191 pedigrees (884 sib pairs, including 369 concordantly affected, 427 discordant, 88 concordantly unaffected, and 360 uninformative) showed a strengthened linkage signal at marker D7S3070 at 163 cM on 7q36 (P=0.0004). Removing families containing affected sib pairs with the most severe diagnoses (advanced adenomas and/or colon cancer) lessened but did not eliminate the peak. The region spans 15 cM and is 37 cM from the 7q31 signal found by Neklason et al. This updated analysis strengthens our initial linkage signal as a possible susceptibility locus in familial colon neoplasia and lessens the likelihood of phenotypic misclassification. Further, this putative locus is separate and distinct from the region previously identified at chromosome 7q31. Our results can be used to further identify the genetic factors that cause a predisposition to familial colon cancer and adenomatous polyps.

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## 1070/W/Poster Board #728

**Association of FPGS and MLL variants with childhood acute lymphoblastic leukemia in different ethnicities.** *D. Piwkham[1,4], J. Gelfond[3], B. Rerkamnuaychoke[4], S. Pakakasama[5], G. Tomlinson[1,2], J. Beuten[1,2].* 1) Greehey Children's Cancer Research Institute; 2) Department of Pediatrics; 3) Department of Epidemiology and Biostatistics, The University of Texas Health Science Center, San Antonio, TX; 4) Department of Pathology; 5) Department of Pediatrics, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. Genetic variation may explain the risk of developing childhood ALL. We present data on 2 genes that were analyzed as part of an ongoing study investigating the association of 8 candidate genes with ALL risk. The gene encoding folylpolyglutamate synthetase (FPGS), an important enzyme in the folate metabolic pathway, plays a central role in intracellular accumulation of folate and antifolate in the cell. Loss of FPGS activity results in decreased cellular levels of antifolates and consequent drug resistance in leukemia. The second gene, the mixed lineage leukemia (MLL) gene, is involved in many chromosomal translocations at 11q23 leading to a diverse subset of hematologic disorders, including childhood ALL. We investigated possible associations between single nucleotide polymorphisms (SNPs) and haplotypes of the FPGS and MLL genes with ALL susceptibility in different ethnic groups. Sixteen tagged SNPs, including SNPs with potential functional effects, were genotyped in ethnicity and age matched case-controls of Caucasian, Hispanic, African-American and Thai children using a customized Illumina panel on the BeadXpress platform. Significant differences in allele frequencies between cases and controls were observed for rs525549 ($p<0.001$) and rs6589664 ($p=0.02$) (MLL) in Hispanics and rs629470 ($p<0.01$) (MLL) in African-Americans. Three SNPs within FPGS (rs7039798, rs1544105 and rs10106) showed a significant association with ALL risk in the Thai sample ($p=0.014$, $p=0.010$, $p=0.026$, respectively). SNP rs1544105 (FPGS) was also significantly associated with ALL risk in the Caucasians ($p=0.041$). Four MLL variants significantly conferred ALL risk; rs573971 and rs6589664 in Caucasians, rs525549 and rs6589664 in Hispanics and rs629470 and rs6589664 in African-Americans (p=0.004-0.042). In addition, a significant gene-dosage effect for increasing numbers of potential high-risk genotypes was found in the three ethnic groups (OR=4.91-7.41; p= 0.001-0.002). Haplotype analysis further showed major haplotypes of the MLL gene that were significantly associated with ALL in our sample (p= 0.001-0.015). This study suggests that common genetic variants in the FPGS and MLL genes are associated with the ALL risk and that susceptibility shows population specific differences.

## 1071/W/Poster Board #729

**Fine-mapping of JAZF1 region associated with prostate cancer susceptibility.** *P. Porter-Gill[1], M. Tarway[1], A. Burrell[1], W. Tang[1], G. Thomas[2], M. Yeager[2], D. Albanes[3], L. Prokunina-Olsson[1], CGEMS Initiative.* 1) LTG/DCEG, NCI/NIH, Bethesda, MD; 2) CGF/DCEG, NCI/NIH, Bethesda, MD; 3) NEB/DCEG, NCI/NIH, Bethesda, MD.

The single nucleotide polymorphism (SNP), rs10486567, located in intron 2 of JAZF1 has been identified as a novel risk factor for prostate cancer (PC) susceptibility (Thomas et al., Nat Gen, 2008). We performed additional genotyping and show that this SNP is associated with prostate cancer susceptibility based on an adjusted model using the genotype test with 2 degrees of freedom; the observed p-value is 1.83x10-11 in 9,331 cases and 10,558 controls of European origin in the Cancer Genetic Markers of Susceptibility (CGEMS) Initiative. The rs10486567 is located 203 Kb from and has low correlation (D'=0.34, r2=0.038) with another JazF1 SNP, rs864745, associated with susceptibility to type 2 diabetes (Zeggini et al., Nat Gen, 2008). To explore the genetic landscape surrounding the PC-associated SNP, we first sequenced a region of 56 Kb in genomic DNA from 5 individuals (3 prostate cancer cases and 2 HapMap controls) and genotyped 11 SNPs (novel and variants from dbSNP with unknown frequencies) in three HapMap populations (European, Asian and African). Based on LD pattern observed in the HapMap samples of European descent (CEU), we selected 15 SNPs in r2>0.5 with the original SNP rs10486567 and genotyped this set of markers in 500 controls and 500 prostate cancer cases from Finland (ATBC study). We observed that the pattern of LD in this region differed from the HapMap samples substantially. To further explore the LD structure and genetic variation in this region, we are conducting deep resequencing with the next-gen technology (454 Roche) over a 127 Kb region (chr7:27,899,757-28,027,351) in 96 samples representing several ethnic groups. The fine mapping will help to refine the genetic landscape of the region in relevant sample sets and to select variants for follow up with larger scale association studies.

## 1072/W/Poster Board #730

**The breast cancer-associated SNP, rs11249433, is associated with expression of NOTCH2 splicing forms in human tissues.** *L. Prokunina-Olsson[1], J.P. Arhancet[1], A. Shah[1], J.L. Hall[2].* 1) LTG/DCEG, NCI/NIH, Bethesda, MD; 2) Lillehei Heart Institute, University of Minnesota, Minneapolis, MN.

A recent Genome-Wide Association Studies (GWAS) in breast cancer identified a novel locus at a pericentromeric region of chromosome 1p11.2, associated with susceptibility to breast cancer (BC) (p-value of 6.74x10-10) for SNP rs11249433 (Thomas et al., Nature Genetics, 2009). Since the NOTCH2 gene is located within this region, it represents an attractive candidate for investigation of the underlying mechanism related to the association signal. The NOTCH2 family of receptors plays a significant role in regulation of cell-cell communication, cell proliferation and differentiation. We identified and cloned two splicing forms of NOTCH2, one form encodes a full-length protein and the second form encodes a truncated protein that is predicted to have a dominant-negative function compared to the full-length protein. We examined the mRNA expression of both splicing forms in a set of human tissues and NCI 60 cell lines. In monocytes (n=64), liver (n=62) and pancreas (n=41), mRNA expression of the full-length form was significantly increased, while expression of the truncated form was decreased proportionally to the number of alleles of rs11249433. At the same time, expression of NOTCH2 splicing forms was not significantly affected by another NOTCH2 SNP, rs10923931, recently associated with susceptibility to type 2 diabetes (Zeggini, et al., Nature Genetics, 2008); these SNPs are located 662 Kb from each other and the correlation between them is D'=0.05, r2=0.0 in HapMap CEU samples. It is possible that the BC-associated variant rs11249433 might affect the ratio in expression of two splicing forms of NOTCH2. Overexpression of the full-length form together with decreased expression of the truncated form might favor cell proliferation and development of cancer in expense of cell cycle arrest and differentiation. The ongoing studies are focused on the expression of NOTCH2 splicing forms in normal and cancer breast samples. These studies form the basis of the biological exploration of the signal on chromosome 1 associated with breast cancer risk. Further studies are warranted to pursue these initial observations.

## 1073/W/Poster Board #731

**High-throughput hacking of the methylation profiles of 22 candidate genes in breast cancer using in vitro transcription and thymidine-specific cleavage mass array on MALDI-TOF silico-chip.** *R. Radpour, C. Kohler, Z. Barekati, R. Asadollahi, X.Y. Zhong.* Department of Biomedicine, University of Basel, Basel, Switzerland.

Alterations of DNA methylation patterns have been suggested as biomarkers for diagnostics and therapy of cancers. Every novel discovery in the epigenetic landscape and every development of an improved approach for accurate analysis of the events may offer new opportunity for the management of patients. Existing methods for quantitative analysis of methylation are limited, and are often too laborious for high throughput or inadequate. Recently, a MassCLEAVETM assay has been developed using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to analyze base-specific methylation patterns after bisulfite conversion. To find an efficient and more cost effective high-throughput method for analyzing the methylation profile in breast cancer, we developed a method that allows for the simultaneous detection of multiple targets CpG residue by employing thymidine-specific cleavage mass array on MALDI-TOF slico chips. Using this novel high-throughput mass spectrometry method, we determined semi-quantitative methylation changes of 22 candidate genes (APC, BIN1, BMP6, BRCA1, BRCA2, CADHERIN 1, CST6, DAPK1, EGFR, ESR2, GSTP1, NES1, Nm23-H1, P16, P21, Progesterone receptor, Prostasin, RAR-b, RASSF1, SRBC, TIMP3, TP53) in breast cancer tissues. For the first time we analysed the methylation status of a total of 42,528 CpG dinucleotides on 22 genes in 96 different paraffin-embedded tissues (48 breast cancerous tissues and 48 paired normal tissues). A two-way hierarchical cluster analysis was used to classify methylation profiles. In this study, 10 hypermethylated genes (APC, BIN1, BMP6, BRCA1, CST6, ESRb, GSTP1, P16, P21 and TIMP3) were identified to distinguish between cancerous and normal tissues according to the extent of methylation. Individual assessment of the methylation status for each CpG dinucleotide indicated that cytosine hypermethylation in the cancerous tissue samples was mostly located near the consensus sequences of the transcription factor binding sites. These hypermethylated genes may serve as biomarkers for clinical molecular diagnosis and targeted treatments of patients with breast cancer.

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## 1074/W/Poster Board #732

**Genetic Variation in Adiponectin and Adiponectin Receptor 1 and Prostate Cancer.** *A. Ray[1], K. Zuhlke[1], S. Chen[2], J. Douglas[2], J. Beebe-Dimmer[3], K. Cooney[1,4].* 1) Dept of Internal Medicine, Univ of Michigan Medical School, Ann Arbor, MI; 2) Dept of Human Genetics, Univ of Michigan Medical School, Ann Arbor, MI; 3) Barbara Ann Karmanos Cancer Center, Wayne State Univ, Detroit, MI; 4) Dept of Urology, Univ of Michigan Medical School, Ann Arbor, MI.

Prostate cancer (PCa) is the most commonly diagnosed cancer and the second leading cause of cancer death among American men. In addition to age and African American ancestry, family history is a leading risk factor for developing PCa suggesting that germline genetic variation plays a role in PCa initiation and/or progression. Recently, common variants in the adiponectin (*ADIPOQ*) and adiponectin receptor 1 (*ADIPOR1*) genes have been identified as being associated with breast and colorectal cancer risk. Circulating adiponectin, an adipokine that plays a role in cell proliferation and insulin resistance, is associated with PCa risk and grade, and adiponectin receptor 1 expression is decreased in PCa tissue compared to normal prostate tissue. Here we examine the role of *ADIPOQ* and *ADIPOR1* genetic variants in PCa risk. Ten haplotype-tagging SNPs in *ADIPOQ* and *ADIPOR1* were genotyped in a set of discordant sibling pairs (676 PCa affected and 583 unaffected men) from the University of Michigan Prostate Cancer Genetics Project. Using family-based association tests, 3 of these SNPs were found to be associated with early onset (EO) PCa. SNPs rs753942 and rs10920531 in *ADIPOR*, which were in strong linkage disequilibrium ($r^2$=0.71), were both associated with EO PCa (138 affected men) ($p<0.05$) under an additive model. Notably, the G allele of rs7539542, which is associated with decreased expression of the ADIPOR1 mRNA, was under-transmitted to affected men with EO PCa. SNP rs266729 in *ADIPOQ* was associated with EO PCa ($p<0.05$) under a dominant model. Interestingly, rs7539542 and rs266729 have been previously associated with breast and colon cancers respectively, but both in the opposite directions seen here. These results suggest that germline genetic variation in *ADIPOQ* and *ADPOR1* may be involved in PCa susceptibility, and further investigation into the exact role of these genes is warranted.

## 1075/W/Poster Board #733

**NUP98-PHF23 fusion gene impairs TPA-induced cellular differentiation and alters normal PHF23 protein distribution.** *J. Reader[1], Q. Leng[2], Y. Ning[1,2].* 1) Program in Human Genetics, University of Maryland, Baltimore, Baltimore, MD; 2) Department of Pathology, University of Maryland, Baltimore, Baltimore, MD.

*NUP98* is a promiscuous fusion partner gene linked to hematological malignancies. We identified a cryptic 11;17 translocation in an acute myeloid leukemia (AML) patient creating a novel in-frame fusion between *NUP98* exon 13 with *PHF23* exon 4. *NUP98* encodes a nucleoporin and has been involved in more than 20 different fusions. *PHF23* encodes a protein containing a plant homeodomain (PHD) involved in chromatin remodeling. The fusion partners of NUP98 can be categorized into two distinct groups: homeobox (HOX) genes and non-homeobox (non-HOX) genes. The non-HOX fusion partner genes, which include *NUP98-PHF23*, are diverse in function and are only related by possessing and retaining coiled-coil domain(s). The majority of research has focused on the mechanism of NUP98-HOX genes in leukemogenesis; therefore, our interests lie in further characterizing this novel non-HOX fusion gene. In order to test if *NUP98-PHF23* and normal *PHF23* are able to confer an oncogenic phenotype and to further characterize the gene, we cloned the full length fusion gene and normal *PHF23* and generated deletion constructs using an expression vector, and expressed it in fibroblast cell line NIH-3T3 and myeloid cell line K562 for localization and differentiation analyses, respectively. The PHF23 protein consists of only a coiled-coil and PHD domain. Taking advantage of this simple structure, we generated a series of deletion constructs, by removing the coiled-coil domain (ΔCoil), the PHD domain (ΔPHD) or both of the domains creating a double deletion mutant (ΔCoil-PHD), to delineate the function of these domains. We have shown that the NUP98-PHF23 fusion protein has a unique nuclear localization pattern, which is different from normal PHF23. The mutant proteins also demonstrated altered distribution within the cell. In addition, we demonstrated that NUP98-PHF23 fusion protein and the ΔCoil mutant protein partially blocked TPA-induced differentiation in K562 cells indicating that the PHD domain in the fusion gene is required to inhibit differentiation. Preliminary chromatin immunoprecipitation and real time quantitative PCR analysis of the fusion gene exhibited increased binding of the fusion protein to the promoter of *HOXA9*. These results indicate that NUP98-non-HOX fusions may function through HOX dependent pathways. Elucidation of the leukemogenic mechanism of NUP98 fusion proteins could lead to greater understanding of leukemogenesis and development of targeted therapy.

## 1076/W/Poster Board #734

**Interrogating genomic signatures of health disparities in prostate cancer.** *L. Ricks-Santi[1], V. Apprey[2], T. Mason[2], R. Kittles[4], M. Faruque[2], G.M. Dunston[2,3].* 1) College Med, Howard Univ Cancer Ctr, Washington, DC; 2) National Human Genome Center at Howard University, Washington, DC; 3) Department of Microbiology, Howard University, Washington, DC;; 4) Department of Medicine, The University of Chicago, Division of Biological Sciences, Chicago, IL.

Background: Prostate cancer (PCa) is a common malignancy and a leading cause of cancer death among men in the US. With regards to populations, the incidence and mortality rate of PCa among African American (AA) men is higher compared to Caucasians. The molecular mechanisms underlying this disparity, in addition to PCa's development remain poorly understood. Methods: For this study, SNPs (n= 79) within 15-35Mb of genes previously associated with PCa in AAs(1q, 7q, and 12p) and with significant allele frequency differences between Africans and Europeans were genotyped in a cohort of AA men enrolled in PCa genetic association studies by the Howard University Cancer Genomics Core. Pairwise linkage disequilibrium (LD) techniques were applied and strong LD was found among SNPs, within each chromosomal region, resulting in analysis of SNPs selected to represent each region. Results: Of the 34 SNPs analyzed, 9 SNPs (on 7q and 12p) were significantly associated with PCa risk. The level of significance varied according to the genetic effect model used. Using the additive genetic model, rs1993973 (IVS) had the strongest association with PCa (p=0.006). With the dominant genetic model, rs1993973 remained significant (p=0.03). However, rs1963562 and rs585224 had the strongest association when applying the dominant genetic model (p=0.008 for both SNPs). With the general model, only one SNP, rs615382 (RacGAP1), emerged as being associated with PCa risk (p=0.03). We also tested the independent effect of each significantly associated SNP and rs1561131 (p=0.04), rs1963562 (p=0.04), rs219821 (TRRAP) (p=0.0025), and rs883403 (CPSF4) (p=0.05) remained significantly associated with PCa. Intergenic SNPs were evaluated for pairwise association with other SNPs within 50kb using HapMap and rs1993973 was found to be in high LD (r2>0.80) with SSPN, an oncogene. Sequence analysis also revealed that transcription factor binding could be affected in 6 of the 9 SNPs. Methylation could also be potentially affected due to creation or ablation of CpG methylation sites in 2 of the SNPs. Conclusions: For this study, genetic markers with significant allele frequency differences between African and Caucasian populations where used to identify genetic variants associated with PCa in African Americans. We believe that this novel approach could help us determine if allele frequency differences in populations could be linked to the biology of disease in health disparities.

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## 1077/W/Poster Board #735

**Assessment of genetic diversity of EpHB2 and its role as a genetic risk factor for prostate cancer among African American men.** *C.M. Robbins[1], W. Hernandez[2], S. Beckstrom-Sternberg[1], W. Tembe[1], S. Hooker[2], T.Y. Moses[1], J. Beckstrom-Sternberg[1], J. Pearson[1], R.A. Kittles[2], J.D. Carpten[1]*. 1) TGen, Phoenix, AZ; 2) Section of Genetic Medicine, Department of Medicine, The University of Chicago Pritzker School of Medicine, Chicago, IL.

Our group was the first to implicate the EpHB2 tyrosine kinase receptor as a tumor suppressor in prostate cancer (PC), where we reported somatic inactivating mutations occurring in 5% - 10% of sporadic tumors. With its role in maintaining normal epithelial architecture and functional data suggestive of a tumor suppressor in prostate cancer, EphB2 is an attractive candidate genetic risk factor for PC. Following previous findings, we hypothesized that there might be other common variants in EpHB2 that confer risk of sporadic prostate cancer among AA men. To test this hypothesis, 490 AA sporadic prostate cancer cases and 567 matched controls were genotyped with 335 genetic variants mapping within the EpHB2 locus. SNPs genotyped included 1) haplotype tagging SNPs with minor allele frequencies >5% based on the Yoruban population, International HapMap II data, 2) known coding variants, and 3) novel coding variants discovered by our group through sequencing of 24 AA cases and controls using Sanger sequencing. Logistic regression has revealed ten EpHB2 variants showing statistically significant association with prostate cancer risk after correction for multiple testing. Eight of these SNPs are located within the intron 1 region of which four are in strong linkage disequilibrium along with near identical odds ratios (1.5 or 1.6). Furthermore, to supplement these data, we used Next Generation Sequencing to interrogate the entire EpHB2 genomic locus in a pool of 400 African American cases and controls for discovery of all EpHB2 common variants in our cohort. This represents ~200,000 basepairs of total genomic sequence in 800 haploid genomes or 160,000,000 bases of DNA sequence. Using the Applied Biosystems SOLID system, we generated >500,000,000 bases of sequence. We are currently analyzing these data using bioinformatics tools to discover novel common and rare variants in the EpHB2 gene, with a focus on regions encompassing the ten SNPs that show association to prostate cancer. We will present data on validation of variants detected by SNP prediction algorithms from pooled data. We hope to further validate SNPs showing association to prostate cancer and any nearby novel SNPs identified by our sequencing efforts in an independent cohort of men of West African ancestry. These data form the basis of an extremely thorough analysis of EpHB2 as a genetic risk factor for prostate cancer among men of recent African descent.

## 1078/W/Poster Board #736

**Zoom-in CGH-array on 10 genes implied in breast cancer susceptibilities in a cohort of 413 *BRCA1*/*BRCA2*-negative patients : 4 new large rearrangements validated.** *E. ROULEAU[1], A. BRIAUX[1], V. CHABAUD[2], S. TOZLU-KARA[1], B. JESSON[2], C. ANDRIEU[1], V. SYLVAIN-VIDAL[2], V. VIDAL[2], L. DEMANGE[1], C. NOGUES[1], I. BIECHE[1], R. LIDEREAU[1]*. 1) Oncogenetic Lab, Centre René Huguenin, St Cloud, France; 2) IMAXIO Division Diagnogene, Saint-Beauzire, France.

Hereditary breast cancer accounts for up to 5-10% of all breast carcinomas. *BRCA1* and *BRCA2* remains the main pathway to explain high penetrance of breast cancer (BC). However, in the diagnostic process, biologists are often faced the major challenge in typical families without any deleterious mutations. Several hypothesis may explain these hereditary breast cancers; 1) *BRCA1*/*BRCA2* are affected but the mutations have not been characterized by conventional screening techniques, 2) a series of other susceptibility genes are implied in the DNA repair system and can confer a moderate risk to breast cancer. Many studies had already explored the presence of deleterious mutations in those genes (heterozygous *ATM*, *RAD50*, *RAD51*, *CHEK2*, *PALPB2*, *RAP80*, *BRIP1*, *BARD1*). Very few point mutations were found and no large rearrangement were reported in breast cancer susceptibility, except for *CHEK2*. We then explored the large rearrangements in 413 patients with typical families phenotype but negative *BRCA1*/*BRCA2* by routine techniques (sequencing, MLPA, QMPSF). We used a dedicated zoom-in CGH-array covering the whole gene (introns and exons) and the 5' and 3' flanking regions (100kb on average). A dedicated zoom-array (8x15k Agilent Technologies) was home designed : *BRCA1* (2427 oligonucleotides), *BRCA2* (2810), *ATM* (821), *BARD1* (493), *CHEK2* (358), *BRIP1* (674), *RAD50* (520), *RAD51* (301), *RAP80* (536), *PALPB2*(370) and covering the whole genome (4722). A dedicated software was programmed. The arrays were shared and hybridized in two differents platform with 5 common positive and 4 common negative control DNA. The rearrangement identified were confirmed with qPCR (LightCycler480, Roche Diagnostic) and specific classic PCR. No large rearrangements were found for the following genes : *ATM*, *RAD50*, *RAD51* and *PALB2*. 13 large rearrangements (4 duplications - 9 deletions) were identified in which 6 were fully confirmed at that time. Two large rearrangements already described in the litterature involved exons 9 to 10 in the CHEK2 gene. Most of the other rearrangements were identified in non-coding region and the deleterious impact should be further assessed. Several interesting conclusions were drawn from the quality data of arrays. This study confirmed that MLPA and QMPSF covered all possible coding large rearrangements in the *BRCA1* and *BRCA2* genes. The deleterious impact of events found could have some important consequences in the routine testing in breast cancer susceptibility.

## 1079/W/Poster Board #737

**Genetic Variation in Apoptosis Genes and Susceptibility to non-Hodgkin Lymphoma.** *J. Schuetz[1], J.J. Spinelli[2], D. Zamar[3], J.M. Connors[4], R. Gascoyne[5], D. Daley[3], A. Brooks-Wilson[1,6]*. 1) Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, BC, Canada; 2) Cancer Control Research, British Columbia Cancer Research Centre, Vancouver, BC, Canada; 3) James Hogg iCAPTURE Centre, University of British Columbia, Vancouver, BC, Canada; 4) Medical Oncology, British Columbia Cancer Agency, Vancouver, BC, Canada; 5) Pathology and Laboratory Medicine, British Columbia Cancer Agency, Vancouver, BC, Canada; 6) Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, Canada.

**Background**. Non-Hodgkin Lymphoma (NHL) is the 5th most common cancer in the United States and Canada. NHL is a complex disease, with both environmental and genetic contributions. Most cases are sporadic. This study aims to identify genetic factors contributing to NHL susceptibility in the general population by means of a population-based case/control study in Vancouver, Canada. Lymphocytes have properties that would be advantageous to a cancer, such as a long lifespan and the ability to divide rapidly when stimulated. If such a cell escapes apoptosis, it creates an ideal environment for further mutations. Low apoptosis levels could contribute to increased survival of pre-cancerous lymphoid cells, enlarging the pool of available cells in which mutations leading to cancer can occur. We are examining whether inherited genetic variation in 16 candidate genes with key roles in apoptosis influences susceptibility to NHL.

**Methods**. We are using constitutional DNA samples from 800 NHL patients and 800 controls previously collected.Targeted re-sequencing was used to supplement the choice of tagSNPs from public databases, for a subset of genes for which genetic variation information was limited. Re-sequencing was done in DNA samples from 47 NHL. TagSNPs and novel variants were genotyped using an Illumina custom GoldenGate multiplex assay. The contribution of the genotyped variants to NHL overall and major NHL subtypes is being assessed by logistic regression. Analysis is presently ongoing and results will be presented.

**Significance**. The identification of markers of genetic susceptibility to lymphoma will lead to a better understanding of this cancer. It will also contribute to the prediction of NHL risk and the development of surveillance programs for at-risk individuals, and potentially insight into treatment options.

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## 1080/W/Poster Board #738

**Capture Sequencing of Chromosome 4q21.3-25 Region Frequently Lost in the Hepatocellular Carcinorma.** *J. Su[1,12], K. Wu[1,2,12], Y. Chang[1,12], J. Li[1], Y. Lin[3], Y. Liu[1], C. Lin[1], H. Weng[4], T. Liu[4], A. Lin[1], H. Chen[1], C. Yuh[1,5,6], S. Huang[1,7], M. Tsou[8], S. Yeh[9,10], P. Chen[10,11], S. Wang[1], S. Tsai[1,2,3,4].* 1) Division of Molecular and Genomic Medicine, National Health Research Institutes, Zhunan, Taiwan; 2) Institute of Biomedical Informatics, National Yang-Ming University, Taipei, Taiwan; 3) Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan; 4) Genome Research Center, National Yang-Ming University, Taipei, Taiwan; 5) College of Life Science and Institute of Bioinformatics and Structural Biology, National Tsing-Hua University, Hsin-Chu, Taiwan; 6) Department of Biological Science & Technology, National Chiao Tung University, Hsin-Chu, Taiwan; 7) Department of Pathology, Chang Gung Memorial Hospital, Taipei, Taiwan; 8) Department of Pathology, Koo Foundation Sun Yat-Sen Cancer Center, Taipei, Taiwan; 9) Department of Microbiology, National Taiwan University College of Medicine, Taipei,Taiwan; 10) Hepatitis Research Center and Division of Gastroenterology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; 11) Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan; 12) These authors contribute equally to this work.

Multiple genomic lesions occur in tumor tissues of hepatocellular carcinoma (HCC)have been studied by loss of heterozygosity mapping and comparative genomic hybridization (CGH). However, the exact position and nature of the genetic changes often cannot be determined because of individual variation and resolution limit. We developed a procedure for enriching genomic sequences from chromosome 4q13.3-q25, a region well characterized for DNA copy loss in HCC. After hybridization with a high-density oligonucleotide array designed for the 4q region (76-115 Mb), the captured DNA was eluted and processed for parallel sequencing, using both Roche/454 and Illumina/Solexa systems. We found that the returned sequences were significantly enriched for the target region, allowing us to perform nucleotide-level sequence comparison between the tumor and control tissues for the region. Taking paired samples from one HCC case, we identified 15,486 variations by comparing the captured sequences with the reference genome, and validated allelic changes involving the coding sequences, including DAPP1 (Arg143Gln) and MANBA (Leu749His). Additionally, 13 somatic mutations in the non-coding sequences were confirmed by Sanger sequencing. Our protocol connects capture sequencing with array CGH and enables high-resolution analysis of a selected target in the HCC genome. The experimental procedures reported here can facilitate future discovery of HCC-related genes and are generally applicable to mutation detection for cancers or genetic disorders.

## 1081/W/Poster Board #739

**Abnormal expression of candidate genes and miRNAs and their association with lung carcinoma.** *J. Wang[1], X. Wang[1], J. Xu[2], Y. Bao[2], H. Dong[1], Y. Wang[1], Z. Zhao[1], Y. Ma[1], Z. Xu[2], M. Xiong[1,3], L. Jin[1].* 1) State Key Laboratory of Genetic Engineering, MOE Key Laboratory of Comtemporary Anthropology, Fudan University, Shanghai, China; 2) Department of Cardiac and Thoracic Surgery, Changhai Hospital, Shanghai, China; 3) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77030, USA.

TGF-β is a multifunctional cytokine and plays a central role in the signaling networks. According to our and other's research, the disruption of TGF-βpathway, such as the defective expression of the components downstream of TGF-β, contributed a lot in carcinogenesis of lung cancer. In this study, microarray analysis of gene expression and miRNA expression of 7 pairs of pulmonary squamous cell carcinoma tissues was conducted, and a robust statistical method was developed to determined the differentiated expressed genes and miRNAs between tumor and normal samples. Differentially expressed genes downstream of TGF-β were selected as candidate genes and examined in a larger sample size consisting of 92 pairs of lung tumor tissues and 10 strains of lung cancer cells. Differentially expressed miRNAs which might contribute to the changes of the candidate genes and other genes associated with carcinogenesis were also examined in these tissues and cells. Statistical analyses were performed to find the association among gene expression, miRNA expression and lung carcinogenesis. As results, nine genes (8 were down-regulated and 1 was up-regulated in cancer) in TGF-β pathway and 51 miRNAs (30 down-regulated and 21 up-regulated) were found significantly differentially expressed between the tumor and normal samples. Pathway-based LCT analysis suggested that eight sets of gene expression including 2 to 9 genes were associated with lung tumorigenesis. Chi square analysis indicated DACH1 expression was associated both with patient gender and subtype. The expression of 8 genes was associated with metastasis. According to miRNA database and our expression data, the irregular expression of COL1A2 mRNA in tumors might be caused by the changes in miR-29c, 196a, or 224, which had opposite expression with COL1A2. The aberration of LPHN2, COL11A1, and NRP1 expression might associated with the expession changes in miR-9, 224, 521, and/or 767-5a. Other differentially expressed miRNAs, such as miR-133a, 133b, and 375, might contribute to API5 and JAK2 expression, which were not included in the above 9 genes but associated with carcinogenesis. Other 5 genes in TGF-β pathway have not shown correlation with miRNA expression accoding to gene-miRNA-regression analysis. Their differentiated expression might come from differentiated methylation status or gene mutation or other factors. The association between genes and miRNAs will be further validated by functional analysis.

## 1082/W/Poster Board #740

**Identification of a Novel Lung Tumor Suppressor Candidate at the Lung Cancer Susceptibility Locus 6q23-25.** *I.M. Wilson, R.C. Chari, T.P.H. Buys, S.L. Lam, W.L. Lam.* British Columbia Cancer Research Centre, Vancouver, British Columbia, Canada.

Background: Familial heritance of lung cancer risk has long been suspected. Indeed, recent work examining familial lung cancers has identified a region associated with lung cancer susceptibility located at 6q23-25. Although gene candidates from this region have been pursued, none have yet been confirmed as the focal point of the region. The identification of novel tumor suppressor genes (TSGs) within this region would provide targets for therapeutics and screening, as well as insights into the germ line and somatic alterations responsible for lung cancer susceptibility.

Objective: Our primary goal is to identify novel TSGs in the lung cancer susceptibility locus using a two-hit integrated screen that highlights genes somatically silenced by both DNA hypermethylation and deletion.

Methods: 30 lung adenocarcinomas and histologically normal parenchyma were obtained during surgery. After pathology review, tumor cells were isolated by microdissection and DNA/RNA were extracted from the tumor and parenchyma. Copy number status was determined by aCGH using the BCCRC whole-genome tiling path array. DNA deletions were defined using a aCGH Smooth. DNA methylation status was determined for tumor and parenchyma DNA using the Illumina Infinium assay. Hypermethylated genes were identified where the difference between tumor and normal β-values was ≥0.25. Gene expression data were generated using the Agilent 44k expression microarray. Combined analysis of DNA copy number and methylation data in each tumor detected two-hit regions of alteration. The frequency of two-hit alteration was calculated, and those probes altered in ≥15% of samples were investigated further. This included analysis of mRNA expression, validation of DNA hypermethylation (MSPCR), and evaluation of DNA methylation control by 5'-aza treatment of cancer cells.

Results: Importantly, our analysis identified frequent somatic hypermethylation and deletion of two genes located within the susceptibility locus (6q23-25). One of these genes was also significantly under-expressed in tumors relative to normal lung ($p \leq 0.05$). DNA hypermethylation of this gene was validated by MSPCR in lung cancer samples as well as in lung cancer cell lines. Cell lines with methylated alleles were treated with 5'-aza, which restored expression of the gene.

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## 1083/W/Poster Board #741

**Replication study of genetic variants associated with prostate cancer in African American men: The Flint Men's Health Study.** *K. Zuhlke[1], A. Ray[1], Y. Wang[2], T. Rebbick[3], K.A. Cooney[1], E.M. Lange[2].* 1) Dept Internal Med, Univ Michigan, Ann Arbor, MI; 2) Depts. Genetics and Biostatistics, Univ North Carolina, Chapel Hill, NC; 3) Dept. Epidemiology and Biostatistics, Univ. Pennsylvania, Philadelphia, PA.

Prostate cancer is the most common cancer among men in the United States with 186,320 new cases diagnosed and 28,660 deaths estimated in 2008. In addition to increasing age, race is one of the most important recognized risk factors for the disease. African-American men have an approximately 1.6 fold greater chance of being diagnosed with prostate cancer compared to European-American men and a 2.4 fold greater chance of dying from the disease. Genome-wide association studies (GWASs) have been successful in identifying a number of single nucleotide genetic polymorphisms (SNPs) that are associated with prostate cancer in men of European descent. Many of these association findings have been replicated successfully across multiple studies. Unfortunately, despite their increased risk for developing and dying from the disease, African-Americans are typically under-represented in genetic association studies of prostate cancer. We genotyped 24 SNPs previously reported to be associated with prostate cancer in men of European descent in a population-based case-control sample (n = 127 cases and 345 screened controls) of African-American men from the Flint Men's Health Study (FMHS). We found significant evidence for association ($p < 0.05$) between 5 SNPs and prostate cancer in unadjusted logistic regression models with SNP analyzed assuming a multiplicative risk model: rs6983561 (p=0.004; OR=1.55), rs7000448 (p=0.03; OR=0.71), and rs16901979 (p=0.003; OR=1.60) at 8q24; rs7904463 (p=0.03; OR=0.70) and rs10740051 (p=0.01; OR=0.51) at 10q11 in MSMB. One additional SNP, rs6983267 (p=0.06; OR=0.61) at 8q24 had suggested evidence for an association. ORs > 1.0 reflect increased frequency of minor allele in cases. Results were similar when covariate adjustment was made for age and estimated proportion of African ancestry. These results, combined with two other recent reports, show that a subset of SNPs that have been identified to be associated with prostate cancer in men of European descent are important risk factors for prostate cancer in African-Americans.

## 1084/W/Poster Board #742

**Chromosomal and genetic alterations in human hepatocellular adenomas associated with type Ia glycogen storage disease.** *L. Li[1], P. Kishnani[2], T. Chuang[3], D. Bali[2], D. Koeberl[2], S. Austin[2], D. Weinstein[4], E. Murphy[5], Y. Chen[3], K. Boyette[2], C. Liu[3], Y. Chen[1,2].* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Division of Medical Genetics, Duke University Medical Center, Durham, North Carolina, USA; 3) National Genotyping Center, Academia Sinica, Taipei, Taiwan; 4) Glycogen Storage Disease Program, University of Florida College of Medicine, Gainesville, FL, USA; 5) Charles Dent Metabolic Unit, National Hospital for Neurology and Neurosurgery, London, UK.

Hepatocellular adenoma (HCA) is a frequent long-term complication of type I glycogen storage disease (GSD I) and malignant transformation to hepatocellular carcinoma (HCC) is known to occur in some cases. There has been no systematic study of chromosomal and genetic alterations in HCA associated with GSD I. We performed genome-wide SNP analysis and mutation detection of target genes in ten GSD Ia associated HCA and compared the results to seven general population HCA cases. Chromosomal aberrations were detected in 60% of the GSD Ia HCA and 57% of general population HCA. Intriguingly, simultaneous gain of chromosome 6p and loss of 6q were only seen in GSD Ia HCA (3 cases) with one additional GSD I patient showing submicroscopic 6q14.1 deletion. The sizes of GSD Ia adenomas with chromosome 6 aberrations were larger than the sizes of adenomas without the changes (10.9 +/- 1.8 cm versus 5.7 +/- 2.2 cm, p= 0.012). Expression of two selected candidate tumor suppressor genes at 6q was reduced in more than 50% of GSD Ia HCA that were examined (n= 7). None of the GSD Ia HCA had biallelic mutations in the HNF1A gene. Conclusions: These findings strongly suggest that chromosome 6 alterations could be an early event in the liver tumorigenesis in GSD I. Genetic alterations in GSD I HCA were different from that in the general population HCA, suggesting that molecular pathways responsible for the pathogenesis of GSD Ia HCA could be distinct. These results also suggest an interesting relationship between GSD Ia HCA and steps to HCC transformation.

## 1085/W/Poster Board #743

**The Application Of SNP-based Array CGH For The Detection Of Novel Cancer Susceptibility Genes In Multi-cancer Families.** *G. Chenevix-Trench[1], N. Wayte[1, 2], P. Simpson[2], L. DaSilva[2], S. Lakhani[2], N. Hayward[1], D. Duffy[1], kConFab.* 1) Queensland Inst Medical Res, Brisbane, Australia; 2) University of Queensland, Brisbane, Australia.

Most heritable cancer syndromes have a restricted range of tumour types associated with them, even though most of the responsible tumour suppressor genes are ubiquitously expressed. We hypothesise that there are general cancer susceptibility syndromes, caused by rare germline mutations in other tumour suppressor genes, and that loss of heterozygosity (LOH) will constitute the 'second hit' in these tumours. There are seldom enough living affected individuals in these 'general cancer families' for linkage analysis because of the high mortality rates of many cancers, but we propose that the genes responsible can still be located by SNP-based array CGH analysis, because all the tumours in the family will share retention of the risk haplotype but lose the other allele. Based on population age-, sex- birth cohort- and cancer rates, we have identified 6 non-BRCA1/2 families ascertained by kConFab with a significant excess of multiple cancer types (at least 3), as well as 2 non-CDKN2A families with multiple cases of melanoma and other cancer types. Because the tumours in these families are often small and the DNA rate-limiting, we have assessed the performance of formalin-fixed, paraffin embedded (FFPE) tumour samples on the GoldenGate Assay (Illumina) which is suited to the use of fragmented DNA, using small amounts of template DNA. We found that lower amounts of DNA input (down to 75ng) perform adequately in the GoldenGate assay, and that loss of information and miscalling was minimal when SNP calls for the FFPE samples were compared to matched fresh frozen pairs. We also assessed the performance of a panel of 30 FFPE tumours from the multiple cancer families we had identified, that ranged in tumour site, age of pathology block (2-20 years) and processing pathology lab. Included in this panel were tumours of the breast, ovary, colon, testis, pancreas, brain, stomach, thyroid and melanoma. We successfully obtained SNP genotyping data from 28/30 tumours and used the R package beadarraySNP to perform concurrent analysis of DNA copy number and LOH on these tumours, comparing tumours within each family to identify common regions of loss or gain. This approach will be combined with genetic linkage analysis in families where germline DNA samples are available to identify candidate cancer predisposition genes in families with apparent inherited cancer susceptibility but for which no known cancer syndrome is apparently responsible.

## 1086/W/Poster Board #744

**Contribution of MTAP variation to disease phenotype in melanoma-prone families with CDKN2A mutations.** *A.M. Goldstein, X.R. Yang, M.A. Tucker, Core Genotyping Facility/DCEG/NCI.* Genetic Epidemiology Branch, DCEG, National Cancer Inst, NIH, Bethesda, MD.

CDKN2A, the major susceptibility gene for cutaneous melanoma (CM), is located on chromosome 9p21. A subset of CM-prone families with CDKN2A mutations has an increased risk for pancreatic cancer (PC) but the reasons for this association are not fully understood. One gene in the 9p21 region, MTAP (that encodes methylthioadenosine phosphorylase), is often co-deleted with CDKN2A in CM and PC tumors. The goals of this study were to determine whether variation in MTAP might influence disease risk in families with CDKN2A mutations. 23 American CM-prone families with CDKN2A mutations (96 CM patients, 143 related controls, 75 spouse controls) were genotyped for variation in MTAP. MTAP tagging based on minor allele frequency >5% and $r^2<0.8$ yielded 17 SNPs. Conditional logistic regression, matched on families to account for family ascertainment and differences in disease prevalence, was used to estimate the odds ratio and 95% confidence interval between CM risk and each SNP, using co-dominant coding for genotypes (0, 1, 2) with the homozygote of the common allele as the referent group. Examination of all families revealed 7 MTAP SNPs that showed significant associations with CM risk. After adjustment for CDKN2A mutation status, no SNPs were significant. Evaluation of families stratified by type of CDKN2A mutation (PC-related vs not PC-related) also showed several different SNPs significantly associated with CM risk. Again, however, after adjustment for CDKN2A mutation status and total nevi, no SNPs remained significant. To further evaluate variation in MTAP, we examined the association between MTAP and several well-known melanoma risk factors including dysplastic nevi (DN), total number of nevi, freckles, complexion, and solar injury. No significant associations were seen between DN, total nevi, complexion or freckles and any MTAP SNPs after adjustment for confounding factors. In contrast, increased solar injury was significantly associated with multiple MTAP SNPs in controls and all subjects together even after adjustment for confounding factors. Given the small sample size, additional CM-prone families with CDKN2A mutations are needed to further evaluate whether MTAP variation might lead to increased solar injury in susceptible individuals. Larger studies are also needed to further examine whether variation in MTAP may contribute to some of the increased risk for CM or pigmentary or nevus-related factors in CM-prone families with CDKN2A mutations.

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## 1087/W/Poster Board #745

**A novel family with tylosis with oesophageal cancer: haplotype and expression analysis of the TOC minimal region.** *S. Saarinen[1], P. Vahteristo[1], R. Lehtonen[1], T. Kiviluoto[2], V. Launonen[1], L. Aaltonen[1].* 1) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Department of Surgery, Helsinki University Central Hospital.

Tylosis is an autosomal, dominantly inherited disorder with palmar and plantar skin keratosis. So far, three families have been reported in which tylosis segregates together with oesophageal cancer. The tylosis with oesophageal cancer (*TOC*) locus has been mapped to chromosome 17q25 in a 42.5 kb region. The *TOC* minimal region has been fully sequenced, but the causative gene defect has not been identified. Only two known genes are located within the *TOC* minimal region, Cytoglobin (*CYGB*) and Rhomboid 5 homolog 2 (*RHBDF2*).Here we introduce a novel Finnish family with tylosis and oesophageal cancer. Three family members have been diagnosed with tylotic keratoderma and three other members with oesophageal cancer. In addition, one family member has breast cancer, one has died of colon cancer and one of cardia cancer. To evaluate whether the Finnish family is linked to the *TOC* minimal region, we performed a haplotype analysis of chromosome 17q25 using microsatellite markers and SNPs. We also sequenced all the known and predicted genes and their promoter regions located in the *TOC* minimal region. In addition, the expression profiles of *CYGB* and *RHBDF2* were obtained from blood derived RNA of three affected family members. The haplotype analysis revealed a common haplotype in both tylosis and the breast cancer patients. This common region was larger than the previously reported *TOC* minimal region of 42.5 kb. Mutation screening of the known and predicted genes did not reveal a disease specific alteration. Expression analysis of *CYGB* and *RHBDF2* revealed that the relative expression of *CYGB* was highly reduced in the tylosis patients compared to the controls. Although it has been possible to refine the *TOC* minimal region to 42.5 kb, disease causing mutations have not been found. Here we have characterized a novel family with *TOC* and shown that it is compatible with linkage to *TOC* minimal region. The expression of *CYGB* was highly reduced in blood of three members of the Finnish tylosis family. It was recently reported that in eosophageal biopsies from tylotic patients the expression of *CYGB* is reduced by 70% and *CYGB* was supposed to be a strong candidate for contributing the *TOC* phenotype. Our study also suggests a possible role for *CYGB* in the pathogenesis of *TOC*. However, more studies are needed to understand the underlying alteration in the *TOC* minimal region causing both tylosis an oesophageal cancer.

## 1088/W/Poster Board #746

**Variation at 8q24 and 9p24 and Risk of Epithelial Ovarian Cancer.** *K.L. White[1], R.A. Vierkant[1], C.M. Phelan[2], B.L. Fridley[1], J.M. Schildkraut[3], Y. Tsai[2], H. Song[4], D.J. Duggan[5], P.P.D. Pharoah[4], F.J. Couch[1], J.M. Cunningham[1], T.A. Sellers[2], E.L. Goode[1].* 1) Mayo Clinic College of Medicine, Rochester, MN; 2) H. Lee Moffitt Cancer Research Institute, Tampa, FL; 3) Duke University, Durham, NC; 4) University of Cambridge, Cambridge, UK; 5) The Translational Genomics Research Institute, Phoenix, AZ.

The chromosome 8q24 region is known to harbor variants associated with risk of breast, colorectal, and prostate cancer, and functional studies have started to emerge shedding light on underlying mechanisms. One recent association study of 1,975 invasive ovarian cancer cases and 3,411 controls found evidence of the 8q24 ovarian cancer susceptibility loci rs10505477 (per-allele OR 1.14, 95% CI 1.04-1.23, p-trend 0.002) and rs6983267 (per-allele OR 1.11, 95% CI 1.03-1.20, p-trend 0.01) [Ghoussaini et al., 2008]. Here we used a multi-site case-control study of 749 women with invasive ovarian cancer and 1,041 controls to evaluate associations between SNPs in 8q24, as well as in the 9p24 colorectal cancer associated-region [Poynter et al., 2007], and risk of ovarian cancer. A total of 35 tagging SNPs and SNPs associated with risk of other cancers were assessed using an Illumina GoldenGate array; logistic regression was used to estimate odds ratios (ORs) and adjust for population structure and other potential confounders. No association between genotypes and risk of ovarian cancer was observed considering all invasive cases or invasive serous cases only. Although 8q24's rs10505477 and rs6983267 were associated with ovarian cancer risk in a previous report, analyses here yielded per-allele ORs of 0.95 (95% CI 0.82-1.09, p-trend 0.46) and 0.97 (95% CI 0.84-1.12, p-trend 0.69), respectively. Risk estimates for the breast cancer risk SNP 8q24 rs13281615 were also close to 1.0 (OR 0.97, 95% CI 0.85-1.12, p-trend 0.72), consistent with data from the Ovarian Cancer Association Consortium [Song et al., 2009]. These null results indicate that despite intriguing initial ovarian cancer associations, confirmed associations with risk of other cancers in 8q24, and our hypothesis that 9p24 variants may associate with ovarian cancer risk, the 35 informative SNPs studied here are not related to etiology of this gynecologic malignancy. Thus, much remains to be learned about the site-specific role that variants in these regions play in carcinogenic processes.

## 1089/W/Poster Board #747

**Causative mutations in Lynch syndrome: what are we missing?** *M. Clendenning[1], D.D. Buchanan[1], M.D. Walsh[1], M.A. Jenkins[2], J.L. Hopper[2], J.P. Young[1].* 1) Queensland Institute of Medical Research, Herston, QLD, Australia; 2) Centre for MEGA, School of Population Health, University of Melbourne, Carlton, VIC, Australia.

The current practice for identifying individuals with Lynch syndrome (defined as carriers of a deleterious mutation within one of the four primary DNA mismatch repair (MMR) genes, *MLH1*, *MSH2*, *PMS2* or *MSH6*) involves obtaining an extensive family history and assessing Lynch syndrome associated tumours for microsatellite instability and/or immunohistochemical (IHC) loss of MMR proteins, followed by a targeted approach, guided by the IHC findings, of large deletion screening via MLPA and exonic sequencing. Serrated neoplasia families are separated from Lynch syndrome via somatic *BRAF* mutation testing in MLH1 deficient tumours. This approach has proven both logical and efficient for the identification of mutations in the MMR genes, however there are still many families who fail to have a mutation identified, despite multiple affected individuals displaying loss of staining for one or more of the MMR proteins. The inability to identify a mutation in these families is of significant clinical importance as individuals, who have an increased risk of developing CRC, cannot be identified. In an attempt to address these issues we performed basic linkage analysis, across three regions that contain the four main MMR genes (2p21, 3p22.2 and 7p22.1), in families where a mutation had not been identified despite immunohistochemical indications of gene inactivation in these regions. Three families containing a known mutation were used as a proof of principle, and in each case a common, mutation associated, haplotype was identified that was not present in any non-mutation carriers. Within each family where no mutations had been detected, we were able to identify a common haplotype which correlated with the IHC findings of affected individuals. These haplotypes suggest that any causative mutation in these families lies in *cis* with the IHC associated gene and as such, current screening procedures must be failing to detect them. Regulatory regions such as promoters and intronic enhancer elements may prove to be a source of mutations that affect transcription and/or translation, and the recent identification of germline methylation of *MSH2* and mutations in the *TACSTD1* gene also supports a role for mutation screening beyond current practices. Such common haplotypes may also prove to be very useful from a clinical point of view, as they could be used as an alternative way to identify family members who are at an increased risk of developing Lynch syndrome.

## 1090/W/Poster Board #748

**Genetic associations with mean telomere length and risk of breast cancer.** *K.A. Pooley[1], J. Tyrer[2], M. Shah[2], K.E. Driver[2], B.A.J. Ponder[3], P.D.P. Pharoah[2], D.F. Easton[1], A.M. Dunning[2].* 1) Cancer Research UK Genetic Epidemiology Unit, Dept. of Public Health, Strangeways Research Lab, Worts Causeway, Cambridge, United Kingdom; 2) Dept. of Oncology, Strangeways Research Lab, Worts Causeway, Cambridge, United Kingdom; 3) Cancer Research UK Cambridge Research Institute, Li Ka Shing Centre, Robinson Way, Cambridge, United Kingdom.

Previous studies have indicated that telomere length is heritable and that shorter mean telomere length in lymphocytes is associated with an increased risk of common cancers, including breast cancer. A recent report has suggested that a SNP (rs401681) in the TERT gene region is associated with reduced mean telomere length and increased risk of certain cancers (Rafnar et al. Nat Genet 41.2 (2009): 221-27). We measured mean telomere length, in blood-extracted DNA, using a high throughput quantitative real time PCR assay (McGrath et al. CEBP 16.4 (2007): 815-19). Estimates of mean telomere length obtained with this assay are reproducible, they display the established reduction of length with increasing age and they demonstrate the heritability of mean telomere length - with a broad-sense heritability estimate of 0.48 among 2000 siblings. In 7000 subjects, assayed for mean telomere length and genotyped for the TERT rs401681 SNP, we see no association between genotype at this locus and age-adjusted telomere length (p-trend=0.77). In a sample of 6600 cases and 6800 controls from the East Anglian SEARCH study, there was no association between the TERT rs401681 SNP and breast cancer risk. However, in this study, mean telomere length was approximately 3 percent shorter in cases than controls. Subjects with a mean, age-adjusted telomere length in the lowest quartile group had an Odds Ratio of being a breast cancer case of 15.51 (11.57 - 20.80), p-het=$5.7 \times 10^{-75}$, relative to those in the highest quartile. Since SEARCH is a retrospective case-control study i.e. the blood samples from SEARCH cases are obtained after diagnosis, it is not clear if this association is indicative of a genuine aetiological relationship, or it occurs after tumour development or treatment. Ongoing studies to further understand the relationship between cancer status and mean telomere length are underway. In conclusion, we see no association of rs401681 with either mean telomere length or increased risk of breast cancer. However, we are currently conducting genome-wide studies to identify loci associated with both mean telomere length and cancer susceptibility.

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## 1091/W/Poster Board #749

**The relationship between single nucleotide polymorphisms in inflammation genes and serum androgen levels.** *T. Meyer[1], L. Chu[1], Q. Li[1], K. Yu[1], P. Rosenberg[1], I. Menashe[1], W. Huang[1], J. Weiss[1], R. Kaaks[2], R. Hayes[1], S. Chanock[1], A. Hsing[1].* 1) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA; 2) German National Cancer Center, Division of Cancer Epidemiology, Heidelberg, Germany.

It has been shown that serum androgens correlate with inflammation and expression of inflammation-related genes. Much less is known about the relationship between variants in inflammation genes and circulating androgen levels. Therefore, we evaluated the correlation between 10,867 single nucleotide polymorphisms (SNPs) in 778 inflammation-related genes and four serum androgen measures (total testosterone [T], 3$\alpha$-androstanediol glucuronide [3$\alpha$diol G], androstenedione, and the calculated ratio of T to its binding protein, sex hormone binding globulin [T:SHBG]), in 570 healthy male subjects (median age 64) from the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. SNP-hormone correlations were evaluated using linear regression of log-transformed hormone variables adjusted for age. SNPs were coded in additive genetic form. Preliminary results show that several SNPs from inflammation-related genes were associated with circulating androgen levels. One SNP in *CD14*, which encodes a surface protein involved in pathogen recognition, was associated with total T (rs3822356 $P$=2.4x10$^{-5}$). Two SNPs in the *MMP2* gene, which is involved in remodeling of injured tissue, were associated with total T (rs893226 $P$= 1.4x10$^{-5}$ and rs1816595 $P$=6.1x10$^{-5}$). Two SNPs in *SMAD7* and *LTBP1*, genes thought to interact with transforming growth factor $\beta$ to help regulate pro-inflammatory and fibrotic signaling cascades, were associated with T:SHBG (rs884013 $P$=3.4x10$^{-5}$ and rs11885904 $P$=7.7x10$^{-5}$, respectively). Finally, variants in genes involved in the T-cell immune response, including rs348373 in *TNFSF9* and rs4240205 in *CD8B* were associated with androstenedione ($P$=5.3x10$^{-6}$) and 3$\alpha$diol G ($P$=8.4x10$^{-5}$), respectively. Further adjustment for body mass index attenuated effects only slightly. Our results show that SNPs in genes involved in different stages/mechanisms of inflammation are associated with serum androgens, suggesting a complex interplay between androgens and inflammatory processes, which together may play a role in certain hormone-related cancers. Future studies are needed to confirm our findings and to clarify the interrelationship between inflammation and androgens and their effects on cancer.

## 1092/W/Poster Board #750

**Familial Haematological Malignancies: The search for a causative gene continues.** *E.M. Tegg[1,2], R. Thomson[1], J. Stankovich[1], D. Perera[1], K.A. Marsden[2], J. Charlesworth[1,5], Y. Hu[3], P. Danoy[4], M. Brown[4], M. Bahlo[3], G.K. Smyth[3], R.M. Lowenthal[2], S. Foote[1], J.L. Dickinson[1].* 1) Menzies Research Institute, Hobart, Tasmania, Australia; 2) Royal Hobart hospital, Hobart, Tasmania, Australia; 3) The Walter and Eliza Hall Institute, Melbourne, Victoria, Australia; 4) UQ, Diamantina Institute, University of Queensland, Queensland; 5) Southwest Foundation for Biomedical Research, Texas, USA.

**Aim:** To identify genes that result in an increased susceptibility to develop a haematological malignancy (HM) **Background:**The Tasmanian Familial Haematological Malignancy Study was commenced in 2006, to the investigate the genetics of HMs in a dataset comprising families ascertained from a population based study conducted between 1972-1980 in Tasmania, Australia, which recruited all cases of HMs diagnosed during that period. **Methods:** Extensive genealogical research and use of the Tasmanian Cancer Registry (TCR), has permitted the identification of 13 priority families demonstrating a marked dense aggregation of multiple HM disease types. Of 133 reported HM cases in these families, 105 have been confirmed by a pathologist and reclassified according to the current WHO classification of HM. Pathology specimens (bone marrow smears, formalin fixed paraffin embedded tissues) for confirmed cases have been collected, and used as a source of DNA for deceased cases. Consenting living affected people in the 13 priority families and children of deceased affected individuals have been genotyped using the Illumina 610 array (124 people). **Results and Discussion:** Familial genetic linkage analysis of the SNP genome wide scan data is underway. One rare family, LK16 family contains an unusual cluster of 5 siblings, 4 with CLL and one with diffuse large B cell lymphoma, whose parents were first cousins. Initial analysis of the SNP data arising from this family using sub-pedigree analysis of IBD sharing by Merlin, and subsequent microsatellite genotyping of pathology specimens from deceased individuals identified a 8MB region on chromosome 15. In addition, RNA has been obtained from EBV transformed cell lines generated from selected individuals from this family to provide differential gene expression data. Analysis of these samples utilizing the Affymetrix Human Exon 1.0 ST expression array identified three significant differentially expressed genes. Closer examination of one of these genes has identified a known SNP of interest. This SNP results in a non conservative amino acid change, and the frequency of this SNP is rare in populations were CLL is rare (Asian populations). Gene expression array data has been examined in the context of the 8MB region of interest and genetic analyses are continuing.

## 1093/W/Poster Board #751

**CFTR, SPINK1, PRSS1 and CTRC mutations are not associated with pancreatic cancer in German patients.** *M. Stuhrmann[1], K. Brakensiek[1], F. Traub[2], E. Schreiber[3], J. Gaedcke[2], M. Maelzer[1], A. Hein[1], B. Marohn[1], K. von Kopylow[1], H. Kreipe[2], S. Schubert[1].* 1) Inst Human Gen, Med Hochschule, Hannover, Hannover, Germany; 2) Dep Pathology, Med Hochschule, Hannover, Hannover, Germany; 3) Celera Diagnostics, Foster City, USA.

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, the serine protease inhibitor Kazal type 1 (SPINK1) gene, the cationic trypsinogen (PRSS1) gene and the chymotrypsinogen C (CTRC) gene are associated with an elevated risk for chronic pancreatitis. Chronic pancreatitis is a known risk factor for pancreatic cancer. Therefore, we hypothesised that mutations in the above mentioned genes may be associated with pancreatic cancer (PC). DNA was extracted from paraffin embedded tissue samples from 124 PC patients, 54 of which were classified as having had chronic pancreatitis. 33 of the most frequent CFTR mutations were analysed by an oligonucleotide ligation assay (OLA), polycrylamide gel electrophoresis (PAGE) and direct sequencing, covering 90% of cystic fibrosis (CF) alleles in the German population. Additionally, we sequenced the relevant exons and flanking intronic regions of the SPINK1, PRSS1 and CTRC genes for the detection of the most common mutations. Of 124 PC samples tested, one was compound heterozygous for F508del and I148T, one patient was heterozygous for the 5T (12TG) allele in CFTR intron 8, and seven patients were heterozygous for 5T (11TG). The distribution of CFTR mutations was not significantly different between PC patients with or without pancreatitis. The CFTR mutation/variation frequency in PC patients was even lower, albeit statistically not significant, in comparison to 136 healthy controls, mostly partners of CF patients or carriers. In the SPINK1 gene, heterozygosity for IVS3+2T>C was observed in one PC patient with pancreatitis, only. Compared to the expected SPINK1 mutation prevalence in the German population, no elevated frequency was observed. No mutation was detected in the PRSS1 and CTRC genes. We conclude that CFTR, SPINK1, PRSS1 and CTRC mutations do not play a major role in the aetiology of pancreatic cancer.

## 1094/W/Poster Board #752

**An unusual case of Fanconi Anemia with adult onset, mosaicism in an asymptomatic sibling, and a possible molecular explanation.** *M.A. Johnson[1], S. Olson[1], B.P. Alter[2], N. Giri[2], W.J. Hogan[3], C.S. Richards[1].* 1) Dept Molec & Med Gen, MP350, Oregon Health & Sci Univ, Portland, OR; 2) Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD; 3) Division of Hematology, Mayo Medical Center, Rochester, MN.

The proband is a 42 year old female with aplastic anemia (AA) who died following a bone marrow transplant. Fanconi Anemia (FA), the most common genetic cause of AA, should be considered in patients with no apparent cause for AA. Lymphocyte chromosome breakage analysis confirmed the diagnosis of FA in the proband, complementation analysis indicated complementation group A (FA-A), and molecular analysis identified two mutations in the FANCA gene. One mutation, p.S1208S (c.3624C>T) was a splice site mutation occurring in exon 36 and was previously described. The second mutation was a novel nonsense mutation in exon 23, p.S674X (c.2021C>A). This individual's six adult siblings were tested by breakage analysis of lymphocytes and fibroblasts prior to selection of a donor for the transplant. Five siblings had normal breakage results; four were heterozygous for the nonsense mutation and one was negative for both mutations. The sixth sibling, an asymptomatic 55 year old female, had increased chromosome breakage in fibroblasts, diagnostic of FA, while breakage within the blood was within normal limits. This individual with mosaicism for FA had the familial splice mutation in both blood and fibroblasts, and the familial nonsense mutation in fibroblasts, but was skewed heavily toward wild-type in blood. cDNA studies confirmed that the predicted splice mutation created an alternate splice site resulting in multiple transcripts, including exon skipping, which varied in different tissues. Mosaicism is common in FA patients, estimated at 20-30%, and multiple molecular explanations have been demonstrated, including mitotic recombination, a second mutation in cis, and a reversion to wild type. While the molecular mechanism is not completely clear, it is most likely the result of a back mutation due to a hot spot. In this case, reversion of one FANCA mutation probably occurred in a hematopoietic stem cell which was selected for and repopulated the blood. In addition, a plausible explanation for the lack of FA clinical features in this individual is the leaky splice mutation which may provide sufficient levels of protein for normal function in DNA repair. In families with diagnosed FA, family members should be tested by breakage analysis of fibroblasts in order to identify those who may be mosaics, at risk for solid tumors which occur in FA, as well as AA, myelodysplastic syndrome, and leukemia, if a non-gene corrected hematopoietic stem cell were to emerge.

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## 1095/W/Poster Board #753

**Identification of men with a genetic predisposition to prostate cancer: targeted screening of *BRCA1/2* mutation carriers and controls: The IMPACT study.** *E.K. Bancroft[1, 2], E.C. Page[1], A.V. Mitra[1], E. Castro[1], R.A. Eeles[1, 2], IMPACT Steering Committee and Collaborators.* 1) Institute of Cancer Research, London, United Kingdom; 2) Royal Marsden NHS Foundation Trust, London, United Kingdom.

**Introduction:** IMPACT, an international collaborative study, is a targeted prostate cancer screening study of men with a known germline mutation which is thought to predispose to the disease. The study is recruiting male *BRCA1/2* mutation carriers and a control group who have tested negative for a mutation known to be present in their family.

**Method:** Eligible men aged between 40 and 69 years are being recruited through 33 collaborating genetics centres in 10 countries, and offered annual serum PSA testing for five years. The PSA threshold used to determine prostate biopsy is >3ng/ml. To date (May 2009) 655/1700 men have been recruited. The pilot data of 300 men have been analysed to determine the feasibility and acceptability of the study approach.

**Results:** The latest recruitment data will be presented together with the results from this pilot analysis. Analysis of the pilot cohort demonstrated that 28/300 men had a raised PSA, which is a threshold rate for biopsy of 9.3%, of which 11 had a prostate cancer diagnosed, 9 in mutation carriers and 2 in controls. A larger proportion of gene carriers developed clinically significant prostate cancers (those that would need radical treatment on UK treatment guidelines) compared with the control group.

**Summary:** Targeted screening based on *BRCA1/2* germline mutation is an acceptable and feasible approach. Biopsy rates are lower and a larger proportion of men developed clinically significant prostate cancer than previously reported in population screening studies. The positive predictive value of PSA is higher than has been reported in population screening studies but the confidence intervals are wide. The study is on target to complete recruitment by the end of 2011 and further work looking at the psychosocial impact of targeted screening is being undertaken.

## 1096/W/Poster Board #754

**Using Action Research to Evaluate and Innovate Personalized Cancer Genetics Training for Personalized Medicine.** *K. Blazer[1], S. Sand[1], D. MacDonald[1], J. Culver[1], C. Huizenga[1], L. Rose[2], J. Weitzel[1].* 1) City of Hope Medical Center, Department of Population Sciences, Division of Clinical Cancer Genetics, 1500 E. Duarte Road, Duarte, California 91010; 2) University of California Los Angeles, Graduate School of Education and Information Studies, Educational Leadership Program, 1029 Moore Hall,Los Angeles, California 90095.

Action research is a disciplined process of reflection, progressive inquiry and informed action, frequently employed within educational communities of practice to generate program improvements and enhance practitioner professionalism. The City of Hope conducts an annual R25E-funded intensive course to address the national need for clinicians trained to provide genetic cancer risk assessment services. As designed, the course cannot meet the demand for interdisciplinary cancer genetics training. An action research project was conducted from January to June of 2008 to evaluate course effectiveness and generate ideas to make the course accessible to more practitioners with diverse training needs. METHOD: A mixed-methods approach was employed, combining quantitative outcomes assessment and evaluation data with interviews, surveys and roundtable sessions with key course stakeholders (participants, alumni and faculty). Qualitative data were triangulated with quantitative outcomes data, summarized and reviewed by course leadership to generate action items for course improvements and innovations. RESULTS: Findings demonstrate that the course is effective in training clinicians from diverse professional disciplines and practice settings. Interdisciplinary case-based training and collegial interactions were identified as the most efficacious learning experiences. The action research process stimulated immediate improvements in the current course and generated a project to incorporate case conferencing into distance-mediated training, as well as the development of specialized training and leadership tracks, as part of a new course design that is more flexible, personalized and accessible to clinicians across the globe. These developments demonstrate the practical utility of using action research to evaluate and improve education and training initiatives for medical professionals.

## 1097/W/Poster Board #755

**Clinical validation of a buccal mouthwash collection and extraction protocol increases accessibility to *BRCA1* and *BRCA2* genetic testing.** *K. Bowles, S. Rajamani, S. Chen, R. Henderson, J. Trost, R. Wenstrup, B. Leclair, B. Roa.* Myriad Genetic Laboratories, Myriad Genetics, Inc, Salt Lake City, UT.

**Background:** Early identification of individuals carrying *BRCA1* and *BRCA2* genetic mutations by full sequencing DNA analysis strongly impacts clinical management, significantly reducing morbidity and mortality from hereditary breast and ovarian cancer. As healthcare providers continue to recognize the importance of genetic testing, testing must be more accessible. Analysis of DNA extracted from blood is the gold standard of molecular diagnostic testing. However, a blood venipuncture can pose a challenge due to lack of on-site phlebotomy services, patient non-compliance with outsourced testing, and the inability of some patients to provide a blood sample due to poor venous access or needle anxiety. We have developed and validated a buccal mouthwash collection and DNA extraction method for full sequencing DNA analysis which provides results identical to blood-based clinical testing. **Methods:** Patient samples were obtained with informed consent through healthcare providers, who collected blood and buccal samples from approximately 450 patients ordering clinical full sequencing DNA analysis. Our laboratory extracted DNA and performed full gene sequencing and large rearrangement analysis of the *BRCA1* and *BRCA2* genes on both sample types. **Results:** Full sequencing DNA analysis results obtained from blood and buccal samples were compared. Sequencing results based on analysis of 2090 sequence variants found among 450 patients were 100% concordant for blood and buccal samples. While equivalent in test performance for detecting presence/absence of mutations, statistical analysis indicated that sequencing data quality of buccal samples was enhanced over that of blood. Results of large rearrangement analyses showed 100% concordance between buccal and blood samples, based on 468 paired samples for the large rearrangement panel test of 5 *BRCA1* large rearrangements, and 437 paired samples for the large genomic rearrangements covering the entire *BRCA1* and *BRCA2* coding regions. **Conclusions:** Clinical validation studies demonstrated that buccal wash samples provide DNA of sufficient quality and quantity to perform accurate full sequencing DNA analysis. Genetic analyses of DNA extracted from paired blood and buccal wash samples were 100% concordant. Development of a buccal wash protocol should make genetic analysis for hereditary breast and ovarian cancer more accessible to healthcare providers and their patients, especially when a blood venipuncture is a challenge.

## 1098/W/Poster Board #756

**Tumour Pathology Predicts Microsatellite Instability in a Population-Based Cohort of Colorectal Cancer Cases.** *A. Hyde[1], D. Fontaine[2], S. Stuckless[3], R. Green[1], A. Pollett[4], M. Simms[1], P. Parfrey[3], H.B. Younghusband[1].* 1) Discipline of Genetics, Memorial University of Newfoundland, St John's, NL, Canada; 2) Discipline of Pathology, Memorial University of Newfoundland, St John's, NL, Canada; 3) Discipline of Medicine, Memorial University of Newfoundland, St John's, NL, Canada; 4) Department of Pathology, Mount Sinai Hospital, Toronto, ON, Canada.

Background: Lynch Syndrome is an autosomal dominant trait that accounts for 2% to 5% of all cases of colorectal cancer (CRC). It is caused by mutations in DNA mismatch repair (MMR) genes, causing high levels of microsatellite instability (MSI-H) in the tumours. Approximately 15% of CRCs are MSI-H, and of these about one quarter are due to Lynch Syndrome. MSI testing of all CRCs to identify potential Lynch Syndrome cases is not practical. Purpose: to create and validate a model for predicting MSI-H tumours, using clinical and pathological features to compare the accuracy of our model with existing models of predicting MSI-H tumours, Methods: We collected a population-based cohort of 716 CRC cases diagnosed before age 75 years in Newfoundland. For each of these cases we collected family history, performed MSI analysis, and scored a number of pathological features. Univariate and multiple linear logistic regression analyses were performed to determine the value of the clinical and pathological features at predicting MSI-H tumours. The most powerful predictors were used to create a new model. We compared our model with the Revised Bethesda Guidelines (Umar et. al. 2004), which is based on clinical features and family history, and the MsPath model (Jenkins et. al. 2007) for predicting MSI-H tumours. The MsPath model is based on the histological features described in the rarely used 3rd Bethesda criterion. Results: Our model has a sensitivity of 96% and a specificity of 55% for identifying MSI-H tumours. This is an improvement over the existing models. In our population the MsPath model has a sensitivity of 95% and a specificity of 39%, while the Bethesda Guidelines have a sensitivity of just 67% and a specificity of 51%. Our model has been validated in a different cohort, by an independent pathologist. Conclusions: Predictive models of MSI-H are more accurate, cheaper, and faster when dependent upon histology than when dependent upon family history. Our model demonstrates that histological features other than those used in the Bethesda Guidelines can also be predictive of MSI-H tumours. Although both the MsPath and Bethesda Guidelines limit the use of their histology-related criteria to patients diagnosed less than age 60 years, we have found that this limitation can be removed, and the criteria applied to at least those diagnosed up to age 75 years.

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## 1099/W/Poster Board #757

**Gastrointestinal Polyposis and *PTEN* Mutations: An Under-Acknowledged Diagnostic Criterion.** *B. Leach, J. Mester, C. Eng.* Genomic Med Inst, Cleveland Clinic, Cleveland, OH.

The International Cowden Consortium (ICC) created operational diagnostic criteria that specify gastrointestinal (GI) hamartomas as a minor criterion. Previous review of reported case studies found that 35-85% of Cowden syndrome (CS) patients had GI hamartomas. Our goal is to describe the GI phenotype of our PTEN mutation positive(+) series. Blood was collected for PTEN mutation analysis and medical records were requested to document diagnoses. Patients who are PTEN+ with ≥5 GI polyps, ≥1 of which is hyperplastic (hyp) or hamartomatous (ham, n=4) or who met relaxed ICC criteria (n=118) were analyzed. Upper and lower GI endoscopy and pathology reports were reviewed and findings are reported descriptively. Fisher's 2-tailed exact test and unpaired T-tests were utilized for comparison of PTEN+ patients with and without polyps. Out of 122 PTEN+ patients, 64 underwent ≥1 endoscopy, and 60(50%) had polyps or colorectal cancer (CRC). Average age at first colonoscopy and upper endoscopy was 37yrs(range: 2-73) and 40(2-73) respectively. Number of polyps ranged from 1-innumerable. Polyps were found in the colorectum, ileum, duodenum, stomach, and esophagus. Pathology includes serrated adenomas; ham, hyp, adenomatous (ade), and inflammatory polyps; lymphoid aggregates; neuromas; lipomas; and ganglioneuromas. 16 patients had a hyp or ham polyposis mixed with other types of polyps, 13 had purely hyp or ham polyposis, and 6 had ganglioneuromatosis. 8 patients (6.6%) had CRC, 1 of whom did not have colorectal polyposis. One patient had gastric signet ring cell carcinoma in the setting of diffuse mixed hyp and ade polyposis. Polyposis patients were older at the time of study enrollment (mean=41.6yrs) compared to non-polyposis patients (26.7yrs, p=0.0001). The most common CS feature in polyposis patients was macrocephaly (70%). When compared to patients without polyps, those with polyps were more likely to have goiter/thyroid nodules (p=0.0001), trichilemmomas (p=0.0018), and papillomatous papules (p=0.0001) but less likely to have breast cancer (p=0.0412) and mental retardation/developmental delay (p=0.0062). GI polyposis is the second most common CS feature in our series. Inclusion of this manifestation as a major criterion would result in an additional 19 patients (16%) meeting ICC criteria. We propose that the ICC revise their guidelines to include GI polyposis (defined as ganglioneuromatosis, mixed hyp or ham polyposis, ham or hyp polyposis) as a major criterion.

## 1100/W/Poster Board #758

**Clinical and histopathological characteristics of Finnish familial prostate cancer cases.** *S. Pakkanen[1], M.P Matikainen[2], N. Ha[1], P. Kujala[3], P. Koivisto[4], J. Schleutker[1], T.L.J Tammela[2].* 1) Lab Cancer Genetics, Inst Medical Technology, Tampere, Finland; 2) Department of Urology,Tampere University Hospital and Medical School, University of Tampere, Finland; 3) Department of Pathology, Tampere University Hospital and Medical School, University of Tampere, Finland; 4) Laboratorio of Molecular Genetics, Tampere University Hospital, Finland.

Background: Prostate cancer (PrCa) is the most common malignancy in men in many industrialized countries and positive family history of the disease is one of the known risk factors. However, clinical features of familial PrCa are still poorly known. Families with prostate cancer have been collected in Finland since 1995. Here the aim was to describe clinical characteristics of the Finnish PrCa families through detailed analysis of the cases in the families. Materials and methods: 202 Finnish families with 617 PrCa cases with confirmed genealogy was collected. Complete clinical data including age and PSA at diagnosis, stage, grade and primary treatment. All the diagnostic biopsy samples (n=323) were gathered and re-graded by the same experienced uropathologist. The mean number of affected men per family was 3.1. Results: The mean year of diagnosis was 1993 (range 1962-2006) and the mean age at diagnosis was 68 (range 43 -98). The mean value of primary PSA was 133 (median 16.0, range 0.8-11000). 17% of the affected had metastases at the time of diagnosis. The most common primary treatments were surgical castration (27%), followed by radical prostatectomy (24%), chemical castration (11%) and radiation therapy (10%.) 11% of the males had WHO III, 56% had WHO II and 33% WHO I, which changed after re-gradering to 22% WHO III, 65% WHO II and 13% WHO I, respectively. In Gleason grading the changes were even larger from 42% of Gleason score 2-5, 48% of Gleason score 6-7 and 10 % of Gleason score 8-10 to 3.7 % of Gleason score 2-5 71% of Gleason score 6-7 and 25% of Gleason score 8-10, respectively. The changes were statistically significant (p=0.0015 in WHO grading and p= $6.9 \times 10^{-8}$ in Gleason grading). Conclusions: Familial PrCa has an earlier age of onset than sporadic PrCa. The criteria for Gleason grading has changed remarkably during the 14 years of collection. Therefore, when comparing pathological grading from a long time period the tumours should be carefully regarded by the same experienced uropathologist.

## 1101/W/Poster Board #759

**Uptake of genetic testing and long-term tumor surveillance in von Hippel-Lindau disease.** *A. Rasmussen[1,2], E. Alonso[2], A. Ochoa[2], I. De Biase[1], I. Familiar[3], M. Lopez-Lopez[4], S.I. Bidichandani[1].* 1) Dept Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2) Dept Neurogenetics and Molecular Biology, Instituto Nacional de Neurologia y Neurocirugia, Mexico City, Mexico; 3) Dept of Mental Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 4) Dept Biological Systems, Division of biological and Health Sciences, Universidad Autonoma Metropolitana-Xochimilco, Mexico City, Mexico.

von Hippel-Lindau (VHL) disease is a hereditary cancer syndrome caused by germline mutations in the *VHL* gene. Patients have significant morbidity and mortality secondary to vascular tumors. Disease management is centered on tumor surveillance that allows early detection and treatment. Presymptomatic genetic testing is therefore recommended, including in at-risk children. We tested 17 families (n = 109 individuals) for *VHL* mutations including 43 children under the age of 18. Personalized genetic counseling was provided pre- and post-test and the individuals undergoing predictive testing filled out questionnaires gathering socio-demographic, psychologic and psychiatric data. Mutations were identified in 36 patients, 17 of whom were asymptomatic. In the initial screening, we identified at least one tumor in five of 17 previously asymptomatic individuals. All mutation-carriers were offered follow-up annual examinations. At the end of five years, only 38.9% of the mutation-carriers continued participating in our tumor surveillance program. During this time, 14 mutation carriers developed a total of 32 new tumors, three of whom died of complications. Gender, education, income, marital status and religiosity were not found to be associated with adherence to the surveillance protocol. Follow-up adherence was also independent of pre-test depression, severity of disease, or number of affected family members. The only statistically significant predictor of adherence was being symptomatic at the time of testing (OR=5; 95% CI 1.2 - 20.3; p = 0.02). Pre-test anxiety was more commonly observed in patients that discontinued follow-up (64.7% vs. 35.3%; p = 0.01). The high initial uptake rate of genetic testing for VHL disease, including in minors, allowed the discontinuation of unnecessary screening procedures in non mutation-carriers. However, mutation-carriers showed poor adherence to long-term tumor surveillance. Therefore, many of them did not obtain the full benefit of early detection and treatment, which is central to the reduction of morbidity and mortality in VHL disease. Studies designed to identify the determinants of adherence to vigilance protocols will be necessary to improve treatment and quality in life of patients with hereditary cancer syndromes.

## 1102/W/Poster Board #760

**Clinical Description of Hereditary Paraganglioma Syndrome identified through a High-Risk Cancer Genetics Clinic.** *V. Raymond[1], M. Marvin[1,3], J. Everett[1], C. Bradford[2], S. Gruber[1, 3, 4].* 1) Internal Medicine, University of Michigan, Ann Arbor, MI; 2) Otorhinolaryngology, University of Michigan, Ann Arbor, MI; 3) Human Genetics, University of Michigan, Ann Arbor, MI; 4) Epidemiology, University of Michigan, Ann Arbor, MI.

Hereditary Paraganglioma Syndrome (HPGL) due to *SDHB*, *SDHC*, *SDHD* (collectively termed *SDH*x) mutations has been described as having defined genotype-phenotype correlations, with *SDHD* mutations more frequently presenting with head and neck(H&N) paraganglioma(PGL) and a parent of origin inheritance pattern, and *SDHB* mutations more frequently presenting with abdominal PGL/pheochromocytoma and a higher probability of malignant tumors. *SDHC* mutations have less well described genotype-phenotype correlations due to the small number of identified families. In 17 mutation positive families identified through the University of Michigan Cancer Genetics Clinic we demonstrate that these genotype-phenotype correlations are imperfect and can be used to guide, but not restrict a sequential genetic workup. *SDHC* should be included in the diagnostic workup of HPGL regardless of clinical presentation. Among 34 unrelated probands presenting with features suggestive of HPGL, 17 (50%) were positive for an SDHx mutation (8-*SDHB*, 2-*SDHC*, 7-*SDHD*). Eleven mutation-positive probands (65%) had a family history of PGL. Six of 23 (26%) cases of isolated PGL were positive for an *SDH*x mutation (2-*SDHB*, 2-*SDHC*, 2-*SDHD*). Eleven mutation-positive probands presented with H&N PGL (2-*SDHB*, 2- *SDHC*, 7-*SDHD*). Six mutation-positive probands presented with abdominal PGL/pheochromocytoma (6-*SDHB*). Four mutation-positive probands presented with malignant tumors (3-*SDHB*, 1-*SDHD*), illustrating that the malignant phenotype is not restricted to *SDHB*. Five of 7 *SDHD* families carried the recurrent P81L mutation. All 5 families with *SDHD* P81L presented with H&N PGL between ages 16-48, 4 of 5 probands had a positive family history, and 1 proband had metastatic PGL. Two families that presented with H&N PGL were found to have *SDHB* mutations rather than the more commonly reported *SDHD* mutations associated with H&N PGL. Among all families, an average of 2.94 relatives had testing for the family mutation. Family history is reasonably sensitive (65%) and specific (100%) for *SDH*x mutations, with acceptable positive predictive value (100%) and negative predictive value (73.9%) in a high risk population. Genotype-phenotype correlations can be used to prioritize genetic testing sequentially, but phenotype information should not be used to exclude a mutation given the varying clinical presentation of HPGL families. Screening recommendations should consider the full phenotypic spectrum of *SDH*x mutations.

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## 1103/W/Poster Board #761

**Glioblastoma multiforme as an increasingly important manifestation of NF1.** *V. Riccardi[1], R. Anderson[2].* 1) Neurofibromatosis Inst, La Crescenta, CA; 2) NF Support Group of Western Michigan, Grand Rapids, MI.

The purpose of this presentation is to enhance appreciation of the substantial risk for glioblastoma multiforme (GBM) among NF1 patients and to reconsider the utility and efficacy of cranial MRI screening of NF1 patients. The data derive from two sources: 1. the long-term follow-up of NF1 patients accrued through the Baylor NF Program (1978-1990) and Neurofibromatosis Institute (1990-2009); 2. the accumulation of inquiries of the NF Support Group of Western Michigan (1990-2009). In all instances there was special emphasis on particularly compromising manifestations of NF1. In contrast with data from surveys dealing with the frequency and nature of intracranial gliomas in NF1 and published from 1951 through 1990 (including those from the Baylor NF Program), there appears to be a clear excess of GBM in recent years. Moreover, NF1 GBM appears to develop in all four possible predisposition scenarios: 1. no neuroimaging screen; 2. no suspicious lesion on screening cranial neuroimaging (no documented mass); 3. a suspicious or asymptomatic static lesion; or 4. a progressive lesion, with or without therapeutic intervention. Given recent documentation of the NF1 locus undergoing somatic mutation in a significant proportion (15%) of non-NF1 GBM, we might expect a larger number/percentage of patients with NF1 to manifest this tumor, compared to what has been the published experience up through the early 21st Century. With the apparent increase indicated here, there is then the question of whether, on the one hand, the GBM frequency in NF1 was simply underappreciated early on or, on the other hand, there has been a recent increase in the risk among NF1 patients. The latter possibility is important given the increasing frequency of GBM in the adult population as a whole. In conclusion, we suggest, first, that the present data indicate a special need for alerting NF1 patients and families to the significant risk of GBM as part of the natural history of the disorder. Second, patients with suspected or known intracranial lesions, including previously-treated ones, should be followed with special diligence and forewarning. Third, the development of GBM in previously asymptomatic patients makes a strong argument for more aggressive - even uniform - cranial MRI screening of all patients with NF1. Fourth, NF1 patients may be a bellwether for identifying factors that contribute to the pathogenesis of GBM in the general population.

## 1104/W/Poster Board #762

**Non-synonymous germline *CDH1* variants in women with lobular breast cancer.** *K.A. Schrader[1,2], S. Masciari[3], N. Boyd[1], J. Senz[1], P. Kaurah[2], M.B. Terry[4], E.M. John[4], I. Andrulis[4], J. Knight[4], F.P. O'Malley[4], M. Daly[4], P. Bender[4], M.C. Southey[4,5], J.L. Hopper[4,5], N. Tung[6], J. Balmana[7], J. Garber[3], D.G. Huntsman[1,2], kConFab, Breast CFR, BCRF Breast Cancer Genetics Consortium.* 1) Department of Pathology and Laboratory Medicine, UBC, Vancouver, BC; 2) Hereditary Cancer Program, BCCA, Vancouver, BC; 3) Department of Medical Oncology, DFCI, Boston, MA; 4) Breast Cancer Family Registry; 5) Kathleen Cuningham Foundation Consortium for research into Familial Breast Cancer; 6) Beth Israel-Deaconess Medical Center, Boston, MA; 7) Hospital Vall d'Hebron, Barcelona, Spain.

Background: *CDH1* encodes the cell-cell adhesion molecule, E-cadherin, for which loss of expression facilitates the infiltrative and metastatic potential of cancers. Germline mutations in *CDH1* are associated with risk of hereditary diffuse gastric cancer (HDGC), and in this setting female carriers have been estimated to have a 39-50% risk of lobular breast cancer (LBC) by age 80 years. Aim: To determine the frequency of *CDH1* germline mutations in women with multiple or early-onset LBC unselected for family history of gastric cancer. Methods: Through a multicentre collaboration, germline DNA analysis of *CDH1* was undertaken in 384 women recruited through two different approaches; population-based cancer registries (n = 235) and those ascertained from hereditary cancer clinics (n=149). Criteria for selection were a personal history of LBC in women without germline *BRCA1* and *BRCA2* mutations, who were: (1) diagnosed before the age of 45 years or (2) diagnosed after the age of 45 years and had at least two 1st or 2nd degree relatives with breast cancer. Results: Of 369 LBC cases, six had novel non-synonymous variants and one had a previously characterized missense mutation that has been associated with HDGC. One novel variant was detected in two cases, making the proportion of cases with novel changes or a missense mutation, 1.9%. Haplotype analysis indicated that the recurrent change identified in two women could represent an ancient non-synonymous variant. The likely pathogenicities of the novel non-synonymous variants are currently being assessed via biological (functional) analyses. Unreported common silent changes have also been identified and will be measured in control samples to determine if they are related to LBC risk. None of the family cancer histories of the cases with non-synonymous variants and the missense mutation involved gastric cancer. Conclusion: Based on our selection criteria, the proportion of LBC associated with non-synonymous germline *CDH1* variants is small. Without having a family history of gastric cancer or multiple early-onset LBC, the likelihood of a woman with LBC carrying a germline *CDH1* mutation appears to be minimal.

## 1105/W/Poster Board #763

**TIME-trial: timing of genetic counseling and testing in breast cancer. Behavioral and psychosocial effects of rapid genetic counseling and testing in newly diagnosed breast cancer patients: a multicenter study.** *M.R. Wevers[1,2], M.G.E.M. Ausems[2], S. Verhoef[3], E.M.A. Bleiker[1], D.E.E. Hahn[3], E.J.Th Rutgers[4], R. van Hillegersberg[5], F.B.L. Hogervorst[6], R.B. van der Luijt[2], H. Valdimarsdottir[7], N.K. Aaronson[1].* 1) Psychosocial Research and Epidemiology, Netherlands Cancer Institute, Amsterdam, Netherlands; 2) Department of Medical Genetics, University Medical Center Utrecht, Netherlands; 3) Familial Cancer Dept, Netherlands Cancer Institute, Amsterdam, Netherlands; 4) Surgical Oncology Dept, Netherlands Cancer Institute, Amsterdam, Netherlands; 5) Surgery Dept, University Medical Center, Utrecht; 6) Diagnostic Oncology, Netherlands Cancer Institute, Amsterdam, Netherlands; 7) Oncological Sciences, Mount Sinai School of Medicine, New York, USA.

**Background** It is estimated that 5-10% of breast cancer patients carry a BRCA1/2 gene mutation. These women are at an increased risk of second tumors, such as contralateral breast cancer, and therefore may opt for preventive surgery. Usually, genetic counseling and DNA-testing are offered to eligible patients after their primary treatment, and take 4-6 months to complete. However, some laboratories can generate test results within 3 to 6 weeks. Little is known about the effect of such rapid procedures on treatment decisions and psychosocial health. **Methods** The TIME-trial is a randomized controlled trial. In total, 255 newly diagnosed breast cancer patients with at least a 10% risk of having a BRCA mutation, recruited from 12 hospitals in the Netherlands, are randomized in a 2:1 ratio to either rapid genetic counseling (referral for genetic counseling within a week after diagnosis), or to a usual care control group. If indicated, DNA-test results are made available within 3-6 weeks. Primary study outcomes are choice of primary treatment including the uptake of direct bilateral mastectomy and of delayed preventive contralateral mastectomy, cancer risk perception, cancer-related worry and distress, health-related quality of life and decisional satisfaction. Psychosocial assessments take place at study entry, and at 6 and 12 months. **Results** The trial recently opened for patient recruitment. To date, 45 women have been invited to participate in the trial, of whom 36 have agreed and have been randomized (response of 80%). **Conclusion and implications** This study will provide essential information about the impact of rapid genetic counseling and testing on the choice of primary surgical treatment among women with breast cancer whose cancer is likely to have a hereditary basis. Additionally, the study will provide data on the psychosocial consequences of genetic testing and risk-reducing behavior in this high-risk population.

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## 1106/W/Poster Board #764

**The difference of HPV (human papillomavirus) DNA genotypes may influence the speed of carcinogenesis in cervical squamous intraepithelial lesions among Japanese patients.** *K. Yamasaki[1], K. Miura[1], S. Miura[1], T. Shimada[1], A. Fujishita[3], T. Sameshima[4], M. Murakami[5], K. Yoshiura[2], H. Masuzaki[1].* 1) Department of Obstetrics and Gynecology, School of Medicine, Nagasaki University, Nagasaki, Japan; 2) Department of Human Genetics, School of Medicine, Nagasaki University, Nagasaki, Japan; 3) Department of Obstetrics and Gynecology, Nagasaki Prefectural Saiseikai Hospital, Nagasaki, Japan; 4) Department of Obstetrics and Gynecology, Nagasaki Atomic Bomb Hospital, The Japanese Red Cross Nagasaki, Nagasaki, Japan; 5) Department of Obstetrics and Gynecology, Sasebo Municipal Hospital, Nagasaki, Japan.

Objectives. Continuous HPV infections are associated with the progression from low-grade squamous intraepithelial lesions (LSIL) to high-grade cervical intraepithelial neoplasia (HSIL). In this study, we determined individual HPV-DNA genotypes in Japanese women and ascertained the temporal changes of HPV infections correlate with the histological results. Methods. Between August 2007 and December 2008, 804 cervical smears on liquid medium were obtained. All cervical smears were evaluated by one particular cytoscreener in the distinct laboratory. The HPV-DNA tests were performed by Linear Array HPV Genotyping Test kit (Roche, Inc., USA). The temporal change of Pap test diagnosis meant the change of category in the Bethesda system, between four groups, “NLM (no intraepithelial lesion or malignancy)”, “LSIL or ASC-US (atypical squamous cells of undetermined significance)”, “HSIL or ASC-H (atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion)” ,and “SCC”. And we defined “progressive change” as histological diagnosis changing more progressive or cytological diagnosis which go to more progressive in atypical squamous cell findings, e.g.; the diagnosis changes from the group of “LSIL or ASC-US” to “HSIL or ASC-H”, and “regressive change” as histological diagnosis changing more regressive or cytological diagnosis which show less regressive in atypical squamous cell findings. Results. 178 cases which were obtained Pap smears and HPV-DNA tests multiple times in the time course manner were recruited to the study. 33 cases (18.5%) showed “progressive change” and forty-five cases (25.3%) showed “regressive change”. We could not observe the changes of pathological diagnosis in other a hundred cases (56.2%). 12 cases of thirty “progressive change” cases were infected with HPV 16 and 18 persistently, and eighteen cases were infected with the other types of HPV except HPV 16 and 18. Whereas only three cases of forty-five “regressive change” cases were infected with HPV 16 and 18 persistently. Other type of HPV were infected continuously in seventeen “regressive change” cases. In this one year the continuous infection of HPV 16 and18 were found significantly more in “progressive change” group ($p \div 0.04$, Fisher’s test). Conclusions. Persistent infection of HPV associated with the progressive changes of cytopathological findings. Especially HPV 16 and HPV 18 may affect those changes more rapidly than other type of HPV in Japanese women.

## 1107/W/Poster Board #765

**New Approaches to Identify and Characterize Splice Variants in *BRCA* genes.** *R.D. Brandao[1,2], K. van Roozendaal[1], D. Tserpelis[1], E.B. Gómez Garcia[1,2], M.J. Blok[1].* 1) Clinical Genetics, University Hospital of Maastricht, Maastricht, Netherlands; 2) GROW - School for Oncology and Developmental Biology, University Hospital of Maastricht, Maastricht, Netherlands.

Genetic screening of *BRCA* genes has been offered to families with breast and ovarian cancer syndrome. Most of the variants found in *BRCA1/2* genes are unclassified variants. A subset of UVs are in intronic sequences or close to exon-intron boundaries and may affect splicing. Putative *BRCA1* and *BRCA2* splice variants, as predicted *in silico*, were studied experimentally using traditional RT-PCR and a new approach based on MLPA technology. Short-term lymphocyte cultures from controls and UV carriers were established with PHA and IL-2 and treated with a nonsense-mediated decay inhibitor, 5h before harvesting. Amplification of cDNA was performed for specific fragments flanking the regions of interest and when possible allele-specific PCR was carried out. We first performed RT-PCR analysis for the following *BRCA2* putative splice variants: c.425G>T, c.6935A>T, c.6842-3T>C, c.6943A>G, c.7976+3del2, c.8350C>T, c.8662C>T, c.8754+3G>C. Variants c.425G>T and c.7976+3del2 give rise to transcripts with deletions of exon4 and exon17, respectively and c.8754+3G>C leads to 46bp of intron retention. Thus these variants were considered to be pathogenic. Additionally, new alternative splicing events were observed in controls, such as c.6842_6937del (exon12 skipping) and c.476_631del (exon 6 and 7 skipping). As these in-frame transcripts are also expressed in healthy controls and have an unknown function, their clinical relevance remains unclear. This has consequences for the classification of c.6935A>T, which leads to increased expression of c.6842_6937del compared to controls. We also evaluated the use of MLPA-technology for the detection of aberrant splice events on well characterized splice-variants, including *BRCA1* exon13 and exon22 deletions. Commercially available MLPA kits for *BRCA1* and *BRCA2* genes were used, in addition to home-made MLPA probe sets. Commercial probe sets include probes located in exons, therefore allowing detection of exon-deletion events in cDNA, whereas home made probes were designed to cross exon-exon boundaries which allow the detection of insertions and deletions at these borders. These approaches proved to be useful for the detection of alternative splice events. In conclusion, we characterized several new pathogenic BRCA2 splice variants and demonstrated that with MLPA technology it is possible to set-up a multiplex screen for semi-quantitative detection of alternative splice events which is widely applicable also for genes other than *BRCA1/2*.

## 1108/W/Poster Board #766

**Development and assessment of hereditary breast cancer genetic screening tools for the Hispanic population of Puerto Rico.** *J. Dutil[1], M. Echenique[2], J. Matta[1], R. Sutphen[3].* 1) Biochemistry, Ponce School of Medicine, Ponce, PR; 2) Hospital Auxilio Mutuo, San Juan, PR; 3) Moffitt Cancer Center, Tampa, FL.

Mutations in the BRCA1 and BRCA2 genes are responsible for the majority of hereditary breast cancers. Knowledge of the incidence and prevalence of BRCA mutations in a specific population or ethnic group is necessary to provide accurate genetic counseling for breast cancer patients and their families. However, these data have not been gathered in the population of Puerto Rico. Our long term objective is to provide the population of Puerto Rico with accurate and efficient tools for the clinical management of hereditary breast cancer. In this particular study, we are conducting a retrospective assessment of the breast cancer patients that have been clinically tested for the BRCA mutations in a high volume surgery practice from San Juan, Puerto Rico. Data collection includes 3-generation family cancer history and results from complete BRCA sequencing. The correlation between BRCA mutation and the model prediction is evaluated by the calculation of the C-statistic. We also hope to identify prevalent BRCA mutations in the population of Puerto Rico. To date, 29 individuals have been tested and 10 individuals found to have a mutation. This project looks for the first time at the characteristics of hereditary breast cancer in Puerto Rico, and assesses the accuracy of existing genetic risk assessment tools in that population. It is expected to have an immediate positive impact on the community as we will gain knowledge about clinical strategies related to hereditary breast cancer in Puerto Ricans.

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## 1109/W/Poster Board #767

**Role of rapid sequence whole-body MRI in *SDHB*-associated hereditary paragangliomas.** *K. Jasperson[1], W. Kohlmann[1], A. Gammon[1], A. Shaaban[2], H. Baskin[3], B. Bentz[4], J. Schiffman[1].* 1) High Risk Cancer Clinics, Huntsman Cancer Institute, Salt Lake City, UT; 2) Department of Radiology, University of Utah, Salt Lake City, UT; 3) Department of Radiology, Primary Children's Medical Center, Salt Lake City, UT; 4) Department of Surgery, University of Utah, Salt Lake City, UT.

**Background:** Germline mutations in *SDHB* cause hereditary paraganglioma syndrome. Penetrance for paragangliomas by 35 and 50 years of age is estimated to be 50% and 70%, respectively. Multiple tumors and malignant transformation is common and renal cell carcinomas, benign renal tumors, and thyroid cancers have also been reported. Limited data exists regarding the efficacy of tumor screening in mutation carriers. In addition, there are a number of barriers to screening including high associated costs, time burden, lack of insurance reimbursement, and inadequate sensitivities and specificities for certain screening techniques. **Objective:** To develop and implement an effective screening protocol for hereditary paraganglioma. **Methods:** Radiographic and biochemical screening were offered to members of a large kindred with an *SDHB* mutation. Whole-body MRI using axial ultra-fast T2 half Fourier acquisition single shot fast spin echo "HASTE" technique using a sliding table platform with an integrated phased-array surface coil was performed. The examination included the base of skull to the pelvis. A more comprehensive MRI examination was performed when a lesion was detected. Biochemical screening included plasma catecholamines, fractionated metanephrines, and chromogranin A. **Results:** Six asymptomatic *SDHB* mutation carriers were screened with MRI. Four (66.7%) were found to have lesions; one individual had a carotid body tumor, one had bilateral renal cysts, another had a carotid body tumor and a large 7 cm oncocytoma, and another was found to have a 4 cm thyroid nodule. Five of the six individuals also had biochemical screening, all were normal. **Conclusions:** Our current protocol has overcome a number of the limitations with screening. Whole-body rapid sequence MRI has reduced the costs and time of radiographic screening compared to individual MRIs of each potentially affected body system. Examination time was approximately 12-14 minutes and insurance has approved coverage of whole-body MRI in all individuals screened. In addition, two-thirds of the individuals screened thus far have been found to have SDHB-associated lesions. Expanding this protocol to other at risk individuals will be important to further delineate the impact and effectiveness of screening in paraganglioma syndromes.

## 1110/W/Poster Board #768

**Health Technology Assessment of Genetic Testing for Lynch Syndrome (Hereditary Nonpolyposis Colorectal Cancer, HNPCC).** *A.P. Lea, S.A. Levine, D.J. Allingham-Hawkins.* Hayes Inc., Lansdale, PA.

**Objectives:** Lynch syndrome is caused by sequence variants in the mismatch repair (*MMR*) genes (*MLH1*, *MSH2*, *MSH6* and *PMS2*) and is associated with an increased lifetime risk of cancer, especially colorectal cancer (CRC) and endometrial cancer. This health technology assessment analyzed the evidence for the use of genetic and molecular testing for the diagnosis of Lynch syndrome to evaluate the different diagnostic strategies and to define the patient population that should be screened. **Methods:** A literature search was performed to identify studies published between January 2003 and May 2009 that related to screening for and diagnosis of Lynch syndrome. The evidence provided by these studies was analyzed using the ACCE model, which was developed by the Centers for Disease Control and Prevention (CDC) specifically for assessment of genetic tests. **Results:** The literature search revealed that, in addition to tests for the presence of sequence variants in the *MMR* genes, other tests being researched for screening and diagnosis of Lynch syndrome include immunohistochemical (IHC) analysis to determine the expression of protein products of the *MMR* genes; microsatellite instability (MSI) testing; *BRAF* gene testing (for the variant c.1799T>A, p.V600E) to separate sporadic from familial CRC; and *MLH1* methylation analysis to separate germline from epigenetic hypermethylation of the *MLH1* promoter region in those lacking expression of *MLH1* protein products. Furthermore, several strategies (revised Amsterdam and Bethesda criteria) and computer models (Barnetson, Leiden, MMRpredict, MMRpro, and PREMM$_{1,2}$) that use family history to select patients for Lynch syndrome screening have been investigated. **Conclusions:** Currently available data are insufficient to justify expansion of screening for Lynch syndrome from those patients with a family history of CRC/Lynch syndrome to all patients with CRC. However, computer models have the potential to make better use of available information than the revised Bethesda or Amsterdam criteria in selecting patients for Lynch syndrome screening. As initial screening tests for Lynch syndrome, MSI and IHC analysis have similar sensitivities and specificities. For patients who are MSI and/or IHC positive, *BRAF* and *MLH1* methylation analysis both have the potential to identify those with sporadic CRC rather than Lynch syndrome. Testing for sequence variants in the 4 *MMR* genes is now the definitive diagnosis for Lynch syndrome.

## 1111/W/Poster Board #769

**The Familial Cancer Database online (www.facd.info).** *R.H. Sijmons.* Dept Genetics, Univ Med Ctr Groningen, Groningen, Netherlands.

To support clinicians and genetic counsellors in the genetic differential diagnosis of cancer patients and families we have developed a special online tool. This free tool, the Familial Cancer Database (FaCD), tries to match cancer types and typical non-tumor features observed in a family with those of the more than 450 disorders currently stored in its database. Search features are flexible and allow for filtering results for different levels of evidence that a particular cancer type is associated with a particular syndrome.

FaCD is a non-commercial, academic, project and complies with the international Health on the Net code standard (www.hon.ch). It was launched last year and is presently consulted by clinicians and genetic counsellors from 34 different countries. The contents of the database is updated on a weekly basis with the help of a growing panel of international experts. We invite colleagues to join this panel! The database can be found at **www.facd.info** .

## 1112/W/Poster Board #770

**Reliable Detection of PMS2 Germline Mutations: A Reference Laboratory Experience.** *C.P. Vaughn[1], J. Robles[2], W.S. Samowitz[1,3].* 1) ARUP Institute for Clincal and Experimental Pathology, Salt Lake City, Utah; 2) ARUP Laboratories, Salt Lake City, Utah; 3) Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, Utah.

Lynch syndrome patients harbor a mutation in one of the mismatch repair genes, MLH1, MSH2, MSH6, or PMS2. While screening for mutations in MLH1, MSH2, and MSH6 is fairly straightforward, the detection of mutations in PMS2 is greatly complicated by the presence of numerous pseudogenes. This has presented an obstacle to obtaining reliable data concerning the mutations present in patients whose tumors show isolated loss of PMS2 protein expression, the immunohistochemical (IHC) profile most commonly associated with a germline PMS2 mutation. We used a modification of a long-range PCR method described by Clendenning (Human Mutation, 2006) to evaluate the PMS2 gene in 102 patient samples submitted for clinical testing. Our modification avoids potential interference with the pseudogene PMS2CL by utilizing a long-range product spanning exons 11-15, with the forward primer anchored in exon 10. Long-range PCR was followed by nested PCR using exon-specific primers and Sanger sequencing. Large deletions were identified by MLPA. IHC results were provided by submitting clinicians. We identified deleterious PMS2 mutations in 18 of 50 patients whose tumors showed isolated loss of PMS2 by IHC. In 28 patients whose tumors showed absence of either another protein or combination of proteins, including 20 samples with concurrent MLH1/PMS2 loss, no deleterious mutations were identified. Two additional patients with deleterious mutations were identified from the 24 samples without IHC data. Two of the 18 patients with deleterious PMS2 mutations had biallelic mutations, one homozygous and one compound heterozygous. Nine of the 21 identified mutations were large deletions encompassing one or more exons. Three mutations were seen in more than one individual, all of whom appeared to be unrelated: c.137G>T (in three individuals), c.736_741del6ins11 (in two individuals), and an exon 10 deletion (in two individuals); these three mutations have been previously identified as founder or potential founder mutations. We conclude that long-range PCR can be used to preferentially amplify the PMS2 gene and avoid pseudogene interference, thus providing reliable clinical results. Deleterious mutations were identified in 36% of individuals whose tumors showed isolated loss of PMS2 protein expression. Deleterious mutations were not associated with any other IHC profile, including the concurrent loss of MLH1 and PMS2.

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## 1113/W/Poster Board #771

**Macrocephaly as an indicator of PTEN mutations in adults attending a Medical Genetics Clinic.** *R.L. Bennett, K. Hansen, W. Raskind.* Dept Med/Div Med Gen, Univ Washington, Seattle, WA.

Cowden syndrome (CS) is an autosomal dominant disorder affecting approximately 1 in 200,000 individuals. Mutations in the PTEN gene, which encodes for a known tumor suppressor with phosphatase activity, have been identified in 80% of CS patients. CS is characterized by hamartomatous overgrowths of tissues derived from all three germ cell layers as well as an increased risk of breast and thyroid cancers. Macrocephaly (OFC ≥ +2SD) is another clinical feature that has been reported in 38% of individuals with CS. This study was to determine if macrocephaly is a strong indicator of PTEN mutations, and whether or not head circumference can help determine when to order PTEN testing versus BRCA1/2 testing for breast cancer patients. A chart review of adult patients seen at the Medical Genetics Clinic at the University of Washington Medical Center was conducted. Charts were reviewed on 31 patient, all of whom had previous PTEN testing and head circumference measurements taken. Eight of the 31 patients had positive PTEN mutations identified, 22 were negative for PTEN mutations, and one patient had a PTEN variant of unknown significance. 100% of the PTEN positive patients had macrocephaly, with head circumferences ranging from +2.3 to +7.2 SD, while 23% of the PTEN negative patients had macrocephaly. Furthermore, when patients were grouped according to their head circumferences, it was found that the percentage of patients with positive PTEN mutations tended to increase with an increase in head circumference. These results indicate that macrocephaly is an important clinical feature that should be taken into account when considering PTEN testing. They also suggest that PTEN testing in macrocephalic breast cancer patients should be considered prior to BRCA1/2 testing. Because of the small sample size in this study, these results should not be used to exclude individuals from testing, but rather to argue for PTEN testing on the basis of macrocephaly.

## 1114/W/Poster Board #772

**Current BRCA Ordering Practices and Management Recommendations Among Obstetricians and Gynecologists.** *A. Trivedi[1], K. Swan[2], A. Gammon[3].* 1) Dept of Obstetrics and Gynecology, Northwestern University, Chicago, IL; 2) Graduate Program in Genetic Counseling, Northwestern University, Chicago, IL; 3) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT.

OBJECTIVE: This study aimed to investigate current BRCA ordering practices among ob/gyns, assess factors that influence these practices (including the Myriad marketing campaign), and describe ob/gyns' recommendations for cancer risk management based on BRCA results. METHODS: A written survey was mailed to 500 randomly selected ob/gyns who are members of ACOG. RESULTS: 107 (21%) questionnaires were returned. The majority of respondents (67.3%) had not been in contact with a Myriad representative and had not directly ordered BRCA tests (73.8%). Most participants (52.3%) reported feeling uncomfortable ordering BRCA tests. Participants who were in contact with Myriad ordered BRCA tests more frequently ($p<0.001$) and reported feeling more comfortable ($p=0.009$) ordering tests than those who were not in contact with Myriad. Perceived barriers to BRCA test ordering include time needed to order the test, lack of genetic knowledge, lack of confidence, lack of clinical utility, cost of test and possibility of insurance discrimination. Those who have been in contact with Myriad viewed both a lack of confidence interpreting genetic test results ($p=0.030$) and a lack of genetic knowledge ($p=0.048$) as less of a barrier to ordering BRCA tests than those who have not been in contact with Myriad. 85% of participants believe it is their role to discuss cancer risk management with their patients. When assessing participants' management recommendations for a BRCA mutation carrier, an average of 85% of recommendations were consistent with NCCN guidelines. When the scenario was less straightforward with an uninformative BRCA negative test result, there was greater variability among participants and management options were less congruent with NCCN guidelines. CONCLUSION: Our study found that 26% of our participants have directly ordered a BRCA test without referring to a genetics professional, which is much higher than reported in previous studies. Furthermore, this study suggests that Myriad's marketing campaign and perceived barriers to ordering BRCA tests may be influencing participants' BRCA ordering practices. Finally, this study suggests that participants make cancer risk management recommendations that are consistent with NCCN guidelines for a BRCA mutation carrier. However, there is much greater variability in recommendations for a high risk BRCA negative patient.

## 1115/W/Poster Board #773

***BRCA1/2* Mutation Prediction: PENN II Model Performs Well in Clinical Practice.** *K.J. Johnson[1], H. Harvey[2], V.S. Pankratz[3], K. Hunt[4], M.J. Wilson[5], N.M. Lindor[1].* 1) Medical Genetics, Mayo Clinic Rochester, Rochester, MN; 2) Seattle University, Seattle, WA; 3) Biostatistics and Informatics, Mayo Clinic, Rochester; 4) Hematology-Oncology, Mayo Clinic Scottsdale, AZ; 5) Laboratory Medicine and Pathology.

**Background**: Several models have been developed for probablilistic clinical prediction of an individual having a germline *BRCA1/2* mutation, primarily based upon input of cancer history and family history. The PENN II Risk Model, developed at the University of Pennsylvania, is a relatively new web-based model, found at https://www.afcri.upenn.edu:8022/itacc/penn2/index.asp. We sought to compare its performance characteristics with other risk prediction models in our clinical practice. **Methods**: Between 1996 and 2005, probands from 285 unique families from Mayo Clinic had collection of detailed family history, genetic counseling, and *BRCA1/2* testing at Myriad Genetics. Testing consisted of sequencing both genes for all patients, and testing for 5-site rearrangement panel in *BRCA1*, since August 2003. For this study, primary peritoneal cancer was considered equivalent to ovarian cancer and ductal carcinoma *in situ* was not counted as invasive breast cancer. **Results**: 277 of 285 probands were women (so men were not analyzed separately). 195 had breast cancer, with first diagnosis at mean age 45.0 years; 26 had bilateral breast cancer; 45 had ovarian cancer at mean age 51.7 years. Twenty seven probands were Ashkenazi Jewish (9.5%). A Receiver-Operating Characteristic (ROC) curve was constructed for subsets. The area under the curve (AUC) is a global measure of a model's predictive performance. For men plus women, the AUC for *BRCA1* and *BRCA2* combined was 0.787; for women only it was 0.791. For men and women combined, for *BRCA1* only, the AUC was 0.810 and for women only it was 0.808. For men and women combined, for *BRCA2* only, the AUC was 0.722 and for women only it was 0.725. In a prior study, we found AUCs for *BRCA1* and *BRCA2* combined for women only to be 0.73 for LAMBDA model, 0.76 for BRCAPRO, 0.72 for Couch 1.5, and 0.71 for Myriad II [Familial Cancer. 6(4):473-82, 2007]. **Conclusions**: The PENN II Risk Model is a convenient, free, web-based tool for *BRCA1/2* mutation prediction. In the clinic population studied, its performance characteristics, as assessed by area under the ROC curve, compared favorably with other commonly used prediction models.

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## 1116/W/Poster Board #774

**Candidate Gene Association Study of Esophageal Squamous Cell Carcinoma in a High-Risk Region in Iran.** *M.R. Akbari[1,2,3], R. Malekzadeh[1], R. Shakeri[1], D. Nasrollahzadeh[1], M. Foumani[2], Y. Sun[2], A. Pourshams[1], A. Sadjadi[1], E. Jafari[1], M. Sotoudeh[1], F. Kamangar[4], P. Boffetta[5], S.M. Dawsey[4], P. Ghadirian[6].* 1) Inst Med Sci, Univ Toronto, Toronto, ON, Canada; 2) Women's College Research Institute, University of Toronto, Toronto, Canada; 3) Institute of Medical Science, Faculty of Medicine, University of Toronto, Toronto, Canada; 4) Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland, USA; 5) International Agency for Research on Cancer, Lyon, France; 6) Epidemiology Research Unit Research Centre, CHUM- Hôtel-Dieu, University of Montreal, Montreal, Canada.

There is a region with a high risk for esophageal squamous cell carcinoma (ESCC) in northeast Iran. Previous studies suggest that hereditary factors play a role in the high incidence of cancer in the region. We selected 22 functional variants (and 130 related tagSNPs) from 15 genes which previously have been associated with risk of ESCC. We genotyped a primary set of samples from 451 Turkmens (197 cases and 254 controls). Seven of 152 variants were associated with ESCC at the P = 0.05 level; these SNPs were then studied in a validation set of 549 cases and 1119 controls, including both Turkmens and non-Turkmens. The association for a functional variant, in ADH1B, was confirmed and for a tagSNP, in MGMT, was borderline in the validation set after correcting the p-values for multiple testing. All other five variants which initially were associated with ESCC in the primary sample set were failed in the validation set. The histidine allele at codon 48 of ADH1B gene in the joint data set (primary and validation set) was associated with a significantly decreased risk of ESCC under a recessive model (OR = 0.41, 95% CI = 0.29 to 0.76; P = 4x10-4). The A allele of the rs7087131 variant of MGMT gene in the joint data set was associated with a decreased risk of ESCC under a dominant model (OR = 0.79, 95% CI = 0.64 to 0.96; P = 0.02,). In previous studies, the ADH1B Arg48His variant has been consistently reported in association with ESCC just among alcohol drinkers. Considering that only 4.5 % of our study subjects were alcohol drinkers, our observation is contrary to other studies which report no association between the Arg48His variant of the ADH1B gene and the risk of ESCC among non-drinkers. Our data suggests that, in Northern Iran, ESCC may be related to an environmental factor other than ethanol, but that is also metabolized by ADH1B. Polycyclic aromatic hydrocarbons (PAH) are known environmental factor in the pathogenesis of ESCC. 1-methylpyrene (1-MP) is a carcinogenic PAH which is metabolized to 1-hydroxymethylpyrene (1-HMP) by CYP450 enzymes. Animal studies showed that 1-HMP could be activated by sulphotransferases and forms DNA adducts, but most of 1-HMP is inactivated by ADH1B. It is possible that individuals with two slow-metabolizing Arg alleles at codon 48 of ADH1B gene do not inactivate 1-HMP as efficiently as those who carry one or more His alleles at this codon. In this case, more 1-HMP would be available for forming DNA-adducts.

## 1117/W/Poster Board #775

**A comprehensive Pathway-wide analysis of genetic variability of the mTOR, FAS and related genes and Prostate cancer risk.** *D. Campa, A. Husing, F. Canzian, R. Kaaks.* German Cancer Res Ctr (DKFZ), Heidelberg, Germany.

A Western lifestyle, characterized by low rates of energy expenditure and a high-energy diet rich in animal protein, saturated fats and refined carbohydrates, is associated with high incidence of prostate cancer in men. Recent studies have identified the mTOR (mammalian target of rapamycin) signal transduction pathway as the pathway that integrates various signals, regulating ribosome biogenesis and protein synthesis as a function of available energy and amino acids, and assuring an appropriate coupling of cellular proliferation with increases in cell size. Fatty acid synthesis, another pathway central to cellular proliferation, is regulated by insulin/IGF-I, essentially through the same signalling mechanisms that influence the mTOR pathway. We investigated the genetic variability of 96 key genes in the above mentioned pathways. We tested the association of 1412 tagging SNPs with prostate cancer risk in a study of 815 cases and 1269 controls nested within the European Prospective Investigation into Cancer and Nutrition (EPIC).To our knowledge this is the first report on polymorphisms of these genes and prostate cancer risk. We have found that several SNPs in the PI3K gene family have a statistically significant association with an altered risk of prostate cancer, the best being OR 0.62; p=0,000373.

## 1118/W/Poster Board #776

**Polymorphisms in genes related to Insulin-Like Growth Factor-I (IGF-I) and breast cancer risk in the NCI Breast and Prostate Cancer Cohort Consortium (BPC3).** *F. Canzian[1], D.G. Cox[2], D.O. Stram[3], R. Hoover[4], H. Blanché[5], G. Thomas[4], R. Kaaks[1], the NCI Breast and Prostate Cancer Cohort Consortium (BPC3).* 1) German Cancer Research Center (DKFZ), Heidelberg, Germany; 2) Harvard School of Public Health, Boston, MA USA; 3) University of Southern California, Los Angeles, CA USA; 4) Division of Cancer Epidemiology and Genetics, National Cancer Institute (NCI), Bethesda, MD USA; 5) Fondation Jean Dausset-CEPH - Biological Resource Centre, Paris, France.

Insulin and insulin-like growth factor-I (IGF-I) have been implicated in the development of breast cancer. We hypothesized that polymorphisms in IGF-I-related genes may influence breast cancer risk. We selected 550 tagging single nucleotide polymorphisms (SNPs) in 24 genes of the IGF-I pathway (GHRH, GHRHR, SST, SSTR1, SSTR2, SSTR3, SSTR4, SSTR5, POU1F1, GHR, IGF1, IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5, IGFBP6, IGFALS, IGF1R, IGF2, IGF2R, INSR, IRS1, IRS2), and tested them for association with breast cancer risk in a large collaboration of prospective studies, the NCI Breast and Prostate Cancer Cohort Consortium (BPC3), which includes the American Cancer Society Cancer Prevention Study II (CPS-II), the European Prospective Investigation into Cancer and Nutrition (EPIC), the Harvard Nurses' Health Study (NHS) and Women's Health Study (WHS), the Prostate, Lung, Colorectal, Ovarian Cancer Screening Trial (PLCO) and the Multiethnic Cohort (MEC). The BPC3 includes 6,292 cases of breast cancer and 8,135 controls. While these genes are strong a priori candidates to harbor breast cancer susceptibility alleles, none of the common polymorphisms tested was significantly associated ($p<0.0001$) with risk, after correction for multiple comparisons. This study had over 80% power to detect associations of relative risk RR=1.2 or greater for common alleles (frequency ≥10%) at $p<0.0001$. The results remained null when cases and controls were stratified by age at diagnosis/recruitment, advanced or non-advanced disease, BMI, with or without inclusion of in situ cases; or restricted to Caucasians. Data on estrogen receptor (ER) status were available for 4,193 cases. Of these, 3,423 (81.6%) were ER positive and 770 (18.4%) were ER negative. Among the ER- cases, a SNP located 3' of GHR was marginally associated with increased risk after correction for multiple testing (ptrend=1.5x10-4). While the IGF-I pathway may be involved in breast cancer development and progression, common variants in any of the individual genes we studied do not contribute substantially to breast cancer risk in Caucasians.

## 1119/W/Poster Board #777

**Cell cycle genes and ovarian cancer susceptibility: a tagSNP analysis.** *J. Cunningham[1], R. Vierkant[1], T. Sellers[2], C. Phelan[2], J. Schildkraut[3], F. Couch[1], X. Wang[1], B. Fridley[1], S. Gayther[4], H. Song[5], E. Hogdall[6], S. Kjaer[6,7], A. Whittemore[8], R. DiCioccio[9], S. Ramus[4], P. Pharoah[5], E. Goode[1].* 1) Dept Lab Med & Pathology, Mayo Clinic & Fndn, Rochester, MN; 2) H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL; 3) Duke University Medical Center, Durham, NC; 4) Translational Research Laboratories, University College, London, UK; 5) Cancer Research United Kingdom and Strangeways Research Laboratory, Cambridge University, Cambridge, UK; 6) Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark; 7) Gynecologic Clinic, Juliane Marie center, Righospitalet, University of Copenhagen, Copenhagen, Denmark; 8) Stanford University School of Medicine, Palo Alto, CA; 9) Roswell Park Cancer Institute, Buffalo, NY.

Dysregulation of the cell cycle is a hallmark of many cancers including ovarian cancer which affects more than 21,000 women in the US annually and is a leading cause of gynecologic cancer mortality worldwide. We identified 39 genes involved in cell cycle regulation that are expressed in ovarian tissue and examined 288 common SNPs in a two-stage study. Caucasian cases ascertained at the Mayo Clinic in Rochester, Minnesota (n=385) or a 48-county region in North Carolina (Duke University, n=444) and ovarian cancer-free Caucasian controls (n=941) were genotyped using an Illumina GoldenGate assay. We found that variants in eight genes (ABL1, CDKN1A, CCND3, E2F2, CDK2, E2F2, CDC2 and CDK7) were associated with risk of epithelial ovarian cancer at $p<0.05$ under at least one genetic model. Seven of these SNPs were then assessed in four additional case-control studies from the US, UK and Denmark, with 1689 cases and 3398 controls in a combined replication set. The association between risk of ovarian cancer and ABL1 rs2855192 found in the original case-control set (OR homozygous minor allele vs. homozygous major 2.81, 95% CI 1.29-6.09, p=0.01) was observed in the other American study population (Stanford University) (OR 3.73, 95% CI 1.30-10.7, p=0.02) and remained suggestive in the combined analysis (OR 1.59, 95% CI 1.08-2.34, p=0.02). This appears to be a recessive effect. No other results remained suggestive in the replication populations. As ABL1 has been implicated in multiple processes including cell division, cell adhesion and cellular stress response, these results suggest that characterization of the role of genetic variation in this gene in other ovarian cancer study populations is warranted.

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### 1120/W/Poster Board #778

**European ancestry is positively associated with breast cancer risk both in US and Mexican Latinas.** *L. Fejerman[1], I. Romieu[2], E.M. John[3], E. Lazcano-Ponce[2], S. Huntsman[1], K. Beckman[8], E. Perez-Stable[1,4,5,6], E. Gonzalez Burchard[1,4,9,10], E. Ziv[1,4,6,7], G. Torres-Mejia[2].* 1) Dept Medicine, Univ California, San Francisco, San Francisco, CA; 2) Instituto Nacional de Salud Publica, Cuernavaca, Morelos, Mexico; 3) Northern California Cancer Center, Fremont, CA; 4) Institute for Human Genetics, Univ California, San Francisco, San Francisco, CA; 5) Medical Effectiveness Research Center for Diverse Populations, Univ California, San Francisco, San Francisco, CA; 6) Helen Diller Family Comprehensive Cancer Center, Univ California, San Francisco, CA; 7) Dept Epidemiology and Biostatistics, Univ California, San Francisco, San Francisco, CA; 8) Dept Developmental Biology, Univ Minnesota, Minneapolis, MN; 9) Lung Biology Center, Univ California, San Francisco, San Francisco, CA; 10) Dept Biopharmaceutical Sciences, Univ California, San Francisco, San Francisco, CA.

Women who self-identify as Latinas or Hispanic in the San Francisco Bay Area have a breast cancer incidence that is 35% lower than that for women who self-identify as Caucasians. A large proportion of the difference in incidence disappears in second and third generation immigrants, suggesting a major environmental effect on risk. The degree to which genetic factors play a role is unknown. Latinas are an admixed group with genetic ancestry from Europeans, Indigenous Americans and African populations. We have previously described a significant association between genetic ancestry and risk of breast cancer in a sample of US Latinas from the San Francisco Bay Area who were of Mexican and Central American descent. Higher European ancestry was associated with increased breast cancer risk. Herein, we analyzed a sample of 1,590 Mexican women to determine if our prior results in US Latinas were consistent with those of women from Mexico. We used genetic markers to estimate the ancestry of Mexican women with and without breast cancer and assessed the association between genetic ancestry and breast cancer risk, adjusting for reproductive and other risk factors. We typed a set of 106 ancestry informative markers (AIMs) in 564 Mexican women with breast cancer and 1,026 healthy controls. We estimated genetic ancestry using a maximum likelihood method. Odds ratios (OR) and 95% confidence intervals (CI) for ancestry, modeled as a categorical variable, were estimated using logistic regression and adjusted for known risk factors. Higher European ancestry was associated with increased breast cancer risk in this new and independent sample of Latina women from Mexico. Compared to women with 0-25% European ancestry, the risk was non-significantly elevated among women with 25-50% European ancestry (OR:1.13; 95% CI: 0.84-1.53), and significantly increased for women with 50-75% European ancestry (OR: 1.59; 95% CI: 1.07-2.36), and for women with 75-100% European ancestry (OR: 3.36; 95% CI: 1.10-10.27). The consistent association with genetic ancestry in the US Latinas and Mexican women suggests a potential genetic effect.

### 1121/W/Poster Board #779

**Genetic variation in genes interacting with BRCA1/2 and breast cancer risk in the Cypriot population.** *A. Hadjisavvas[1], M. Loizidou[1], T. Michael[1], E. Bashiardes[2], M. Cariolou[2], K. Kyriacou[1].* 1) Department of Electron Microscopy/Molecular Pathology, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus; 2) Department of Cardiovascular Genetics and Laboratory of Forensic Genetics, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus.

Inability to correctly repair DNA damage is known to play a role in the development of breast cancer. Single nucleotide polymorphisms (SNPs) of DNA repair genes have been identified which modify the DNA repair capacity, which in turn may affect the risk of developing breast cancer. To assess whether alterations in DNA repair genes contribute to breast cancer, we genotyped 62 SNPs in 29 genes in 1,109 Cypriot women with breast cancer and 1,177 age-matched healthy controls. Five SNPs were associated with breast cancer. SNPs rs13312840 and rs769416 in the NBS1 gene were associated with a decrease in breast cancer risk (OR TT v TC/CC = 0.58; 95% CI, 0.37 to 0.92; p = 0.019 and OR GG vs GT/TT = 0.23, 95% CI 0.06-0.85, p = 0.017 respectively). The variant allele of MRE11A rs556477 was also associated with a reduced risk of developing the disease (OR AA vs AG/GG = 0.76; 95% CI, 0.64 to 0.91; p = 0.0022). MUS81 rs545500 and PBOV1 rs6927706 SNPs were associated with an increased risk of developing breast cancer (OR GG vs GC/CC = 1.21, 95% CI, 1.02 to 1.45; p = 0.031; OR AA vs AG/GG = 1.53, 95% CI, 1.07 to 2.18; p = 0.019, respectively). Finally, haplotype-based tests identified significant associations between specific haplotypes in MRE11A and NBS1 genes and breast cancer risk. Further large-scale studies are needed to confirm these results.

### 1122/W/Poster Board #780

**Polymorphisms in genes involved in the progesterone metabolism modify postmenopausal breast cancer risk associated with menopausal hormone therapy.** *R. Hein[1], S. Abbas[1], D. Flesch-Janys[2], J. Chang-Claude[1].* 1) Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Baden-Würtemberg, Germany; 2) Department of Medical Biometrics and Epidemiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Menopausal hormone therapy (HT) is associated with an increased breast cancer risk among postmenopausal women, with estrogen-progestagen combination therapy showing a higher risk increase than estrogen monotherapy. However, few studies focused on potential effect modification of HT associated breast cancer risk by genetic polymorphisms in the progesterone pathway. We assessed effect modification of HT use by five functional single nucleotide polymorphisms (SNP) in the progesterone metabolizing enzymes *AKR1C3* (rs7741), *AKR1C4* (rs3829125, rs17134592) and *SRD5A1* (rs248793, rs3736316). We used a two-centre population-based case-control study from Germany with 2,502 postmenopausal breast cancer patients and 4,833 matched controls. Multivariate conditional logistic regression stratified by centre and year of birth was used to calculate main and interaction effects of HT, i.e. estrogen-progestagen combination therapy and estrogen monotherapy, and SNPs with regard to postmenopausal breast cancer risk. No genetic main effects were observed. Breast cancer risk associated with duration of combination therapy was significantly modified by *SRD5A1_* rs3736316, showing a decreased risk in carriers of the minor allele (pinteraction=0.012). Odds ratios (OR) and 95% confidence intervals (CI) per 5-year-increment of combined estrogen-progestagen use were 1.19 (1.11-1.28), 1.12 (1.05-1.20) and 1.03 (0.89-1.20) in homozygous carriers of the major allele, heterozygous carriers and homozygous carriers of the minor allele, respectively. The risk associated with duration of use of estrogen monotherapy was significantly modified by *AKR1C3_*rs7741 (pinteraction=0.029) and two SNPs in *AKR1C4* (rs3829125: pinteraction=0.015, rs17134592: pinteraction=0.029). The ORs were significantly increased only in homozygous carriers of the *AKR1C3_*rs7741 minor allele (OR=1.29 (95% CI:1.09-1.53)) as well as in homozygous major allele carriers of the two SNPs in *AKR1C4* (rs3829125: OR=1.10 (95% CI:1.02-1.17); rs17134592: OR=1.09 (95% CI:1.02-1.17)). Postmenopausal breast cancer risk associated with combined estrogen-progestagen therapy is modified by SNPs in *SRD5A1* and with estrogen monotherapy by SNPs in the *AKR1C* genes. .As the *AKR1C* enzymes are not only involved in progesterone but also estrogen metabolism by converting estrone to estradiol a potential effect modification associated with estrogen monotherapy is feasible.

### 1123/W/Poster Board #781

**Genetic polymorphisms in MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-14, RECK, TIMP-1, TIMP-3, and TIMP-4 and prostate cancer susceptibility and aggressiveness among African American men.** *W. Hernandez[1], S. Hooker[2], R. Kittles[2].* 1) Cancer Biology Program, Division of Biological Sciences, The University of Chicago, Chicago, IL; 2) Department of Medicine, Section of Genetic Medicine, The University of Chicago, Chicago, IL.

One-third of cancers among men each year will be prostate cancer. The risk and mortality of prostate cancer varies significantly across ethnic groups, in the United States; African American men have the highest rates. These population differences suggest genetics may play an important role. Matrix metalloproteinases (MMPs) modulate cell proliferation, apoptosis, and host immune surveillance. In addition, MMPs have the capacity to promote cell migration and tumor invasion by degradation of the extracellular matrix (ECM). Tissue inhibitors of metalloproteinases (TIMPs) and the reversion-inducing cysteine-rich protein with Kazal motifs (RECK) regulate MMP activity and have been shown to play important roles in several types of cancer initiation and aggressiveness. Here we examined the association between polymorphisms within several MMPs, TIMPs, and RECK and prostate cancer susceptibility and aggressiveness. A total of 88 SNPs were genotyped in 575 men, self-described as African American (controls=318, cases=257), using MALDI-TOF Mass spectrometry analysis (Sequenom Inc., San Diego, CA). We found several SNPs that were independently associated (although nominally) with Pca risk and aggressive disease in African Americans. However, epistasis analysis revealed the strongest associations to Pca risk and aggressiveness. The most dramatic decrease (50-fold, P=0.007) was observed among individuals possessing both minor alleles for rs12365082 and rs2285052 within MMP-8 and MMP-2 respectively. We found statistical evidence of gene-gene interactions likely due to the function of MMPs and their inhibitors, TIMPs and RECK, exhibiting epistasis as a mechanism of regulation and may help explain the inconsistency found in previous studies. Our findings demonstrate that, among African American men, polymorphisms in MMPs, TIMPs, and RECK can significantly influence the risk for prostate cancer and aggressive disease. In addition, our study suggests that an epistatic model should be used to study the effect of SNPs within these genes on prostate cancer risk.

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## 1124/W/Poster Board #782

**DNA REPAIR GENE POLYMORPHISMS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA.** *E. Leal Ugarte[1], JP. Meza Espinoza[1], V. Peralta Leal[1], M. Reyes Segura[1], JG. Perales Hernández[1], HG. Torres Benavides[1], M. Gutiérrez Angulo[2], N. Macías Gómez[3], P. Barros Núñez[4], J. Durán González[5].* 1) Universidad Autónoma de Tamaulipas, Matamoros, Tamaulipas, Mexico; 2) Centro Universitario de los Altos, U de G, Tepatitlán, Jalisco; 3) Centro Universitario del Sur, U de G, Cd. Guzmán, Jalisco; 4) Centro de Investigación Biomédica de Occidente-IMSS,; 5) University of Texas at Brownsville, EE. UU.

Background. The acute lymphoblastic leukemia (ALL) is the most common cancer found among children, accounting for approximately 25-30% of all pediatric tumors. The etiology of the leukemia is not known; nevertheless, the genetic constitution, the damage to the DNA and the incorporation of viral genes are important agents in the origin of the disease. Polymorphisms of DNA repair genes can alter protein structure and may impair DNA repair capacity. Defects in repairing damaged DNA lead to genetic instability and carcinogenesis. This study was performed to evaluate the effect of two polymorphisms of XRCC1 gene on risk of childhood ALL. Materials and Methods. We genotyped polymorphisms of X-ray repair cross-complimenting group 1 (XRCC1) codon 194 (Arg to Trp) and 280 (Arg to His) in 120 children with ALL and 120 healthy controls in Mexican population using PCR-RFLP method. The allele and genotype frequencies of these polymorphisms were compared between cases and controls using Chi-square or Fisher's exact test. RESULTS. The allele frequencies of polymorphism Arg194Trp were Arg-0.86%, Trp 0.14% and Arg-0.81%, Trp 0.19%, for the polymorphism Arg280His were Arg-0.87%, His 0.13% and Arg-0.86%, His-0.14% in controls and patients respectively, the genotype frequencies of polymorphism Arg194Trp were Arg/Arg-0.67%, Arg/Trp-0.28%, Trp/Trp-0.05% and Arg/Arg-0.72% Arg/Trp-0.25%, Trp/Trp-0.03%, for the polymorphism Arg280His were Arg/Arg-0.72%, Arg/His-0.26%, His/His-0.02% and Arg/Arg-0.74%, Arg/His-0.25%, His/His-0.01% in patients and controls respectively. The allele frequencies were consistent with Hardy-Weinberg equilibrium Discussion. Our study shows that Arg194Trp polymorphism has not association with the development of ALL. Although the allelic frequency of this polymorphism was higher in patients than in controls, no statistically significant difference was found. Even several studies have found that the presence of the polymorphism 194Trp has a protective effect against ALL and other cancers; however, in our study we did not find such effect. The genotypic and allelic frequencies of Arg280His polymorphism were similar in controls and in patients. Our findings are consistent with similar studies reported in other populations. In conclusion our study showed no association of polymorphisms 194Trp and 280His in the development of childhood ALL in the Mexican population.

## 1125/W/Poster Board #783

**Does high calcium intake cause prostate cancer? Advances using a Mendelian randomization approach.** *S.J. Lewis[1], L. Chen[1], L. Zuccolo[1], J.A. Lane[1], J. Donovan[1], A. Cox[2], F. Hamdy[3], D. Neal[4], G. Davey Smith[1, 5].* 1) Department of Social Medicine, University of Bristol, Bristol, United Kingdom; 2) Institute for Cancer Studies, University of Sheffield, Sheffield, UK; 3) Nuffield Department of Surgery, University of Oxford, UK; 4) Department of Oncology, University of Cambridge, Cambridge, UK; 5) MRC Centre for Causal Analyses in Translational Epidemiology, University of Bristol, Bristol, UK.

The recent World Cancer Research Fund/ American Institute for Cancer Research review concluded that diets high in calcium probably increased the risk of prostate cancer, but there was no convincing evidence for any specific food items or nutrients investigated. This uncertainty surrounding calcium and prostate cancer probably stems from measurement error and confounding, which are inherent in all observational studies of diet. Mendelian randomization provides an alternative method, which avoids confounding by using genetic variants as proxies for environmental and lifestyle exposures. Genetic variants exist in the calcium sensing receptor (CASR), which are associated with circulating serum ionized calcium concentration; similarly, TRPV5 is a key protein that regulates $Ca2+$ reabsorption, and variants in this gene have been shown to be associated with calcium influx. We investigated whether genetic variants associated with calcium levels were also risk factors for screen-detected prostate cancer by comparing genotype frequencies of 1600 prostate cancer cases to those of over 3000 controls. We found that the CASR rs1801725 GT and TT genotypes, which have been are associated with a small increase in serum ionized calcium concentration relative to the GG genotype were associated with an increased cancer risk OR =1.17 (95% CI = 1.01-1.35). Similarly a variant (rs2110922) in the SCL8A1 gene, which is involved in sodium/calcium exchange, was associated with prostate cancer risk in this study, OR for TG versus GG = 1.12(95% CI= 0.98-1.27), OR for TT versus GG = 1.20 (95%CI=0.99-1.45), p-value for trend =0.04. No strong evidence was found for associations between variants in TRPV5 and prostate cancer risk, however these variants were rare in our study and consequently we had low power to detect associations. In conclusion, we found some evidence that a variant in the CASR gene, which is associated with greater circulating calcium levels, was associated with an increase in prostate cancer risk, and that a variant in a sodium/calcium exchange gene, of unknown function, was associated with prostate cancer risk. Our findings add support to the hypothesis that calcium is implicated in prostate cancer pathogenesis but they require replication in other studies to confirm that they are not false positives. However, as our results are unlikely to have arisen due to confounding, they are an advance on the observational data that have been published to date.

## 1126/W/Poster Board #784

**Matrix metallopreoteinase-13 gene polymorphism in colorectal cancer.** *J.M. Moreno-Ortiz[1,5], M. Gutierrez-Angulo[1,2], M. Partida-Perez[1,5], J. Peregrina-Sandoval[1,3,5], A.S. Suarez-Villanueva[1], M.A. Cardenas-Meza[4], M. Centeno-Flores[4], V.M. Maciel-Gutierrez[4], M.L. Ayala-Madrigal[1].* 1) Instituto de Genetica Humana, Biologia Molecular y Genomica, CUCS, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico; 2) CUALTOS, Universidad de Guadalajara, Tepatitlan, Jalisco, Mexico; 3) Laboratorio de Inmunobiologia, CUCBA, Universidad de Guadalajara, Zapopan, Jalisco, Mexico; 4) Hospital Civil de Guadalajara Juan I. Menchaca, Guadalajara Jalisco, Mexico; 5) Doctorado en Genetica Humana, CUCS, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico.

Colorectal cancer (CRC) is characterized by enhanced expression and activity of several metalloproteinases (MMPs) including MMP13 (collagenase-3). This metalloproteinase has central roles in modulating extracellular matrix degradation through its direct matrix degrading capability as well as having a key involvement in the activation of other MMPs: MMP2, -3 and -9. MMP13 also has an important role in tumor invasion and metastasis. We tested the correlation of a functional MMP13 promoter polymorphism, -A77G, with suceptibility to CRC in a population of West Mexico. MMP13-A77G was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis in 47 CRC patients and 125 healthy controls. The genotype and allelotype distributions of MMP13-A77G in CRC patients were not significantly different from those in healthy controls (P>0.05), and showed the A/A, A/G, G/G genotype frequencies: 61.7%, 34%, 4.3% and 49.6%, 37.6%, 12.8% respectively. Odds ratios and P values using logistic regression were calculated to test for association between genotype, allelotype and disease status. The analysis showed no association, even when stratified for gender, however, the A/A genotype frequency in CRC patients tended to be higher than in the controls. These results seem to exclude an important role of the MMP13-A77G in Mexican CRC patients susceptibility, but further studies on a larger sample are warranted to confirm these findings.

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## 1127/W/Poster Board #785

**Interactions between cigarette smoking and selected candidate gene polymorphisms in risk for colorectal cancer: a case-only analysis.** *M. Pande[1], CI. Amos[2], DR. Osterwisch[1], ML. Frazier[2].* 1) Epidemiology, The University of Texas M. D. Anderson Cancer Center, Houston, TX; 2) Epidemiology, The University of Texas M. D. Anderson Cancer Center, Houston, TX and Program in Human and Molecular Genetics, The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX.

**Objective**: The metabolism of xenobiotics is complex and involves multiple steps and multiple enzymes. Genetic variation in the genes encoding the enzymes as well as the level of exposure to the substrates of these enzymes can alter metabolism and clearance of potential carcinogens and thus may modify cancer susceptibility. This study examined the interaction between cigarette smoking and two non-synonymous metabolic gene single nucleotide polymorphisms (SNPs) *CYP1A1* p.Ile462Val (rs1048943) and *EPHX1* p.Tyr113His (rs1051740) and explored gene-gene interaction between the two SNPs in a series of colorectal cancer cases. The SNPs were selected based on functional significance and suggestion of gene-gene interaction in a prior study. **Methods**: Unconditional logistic regression was used to examine the association between smoking and each of the SNPs and interaction between the two SNPs, in a series of 786 cases of colorectal cancer, using a case only design. **Results**: In the case only analysis, there was significant multiplicative interaction for colorectal cancer risk between smoking and *EPHX1* Y113H (Case only odds ratio [COR] = 1.37, 95% confidence interval [CI] = 1.03 - 1.81, P = 0.03), particularly among heavy smokers with a history of >20 pack years of smoking (COR = 1.52, 95% CI = 1.07 - 2.16, P = 0.02). In addition, there was gene-gene interaction between *EPHX1* Y113H and *CYP1A1* I462V (COR = 1.61, 95% CI = 1.02 - 2.55, P = 0.04). **Conclusion**: Smokers with any variant allele of *EPHX1* were at increased risk for colorectal cancer. Similarly, presence of any variant allele of *CYP1A1* and *EPHX1* together increased susceptibility to colorectal cancer. Genetic and environmental factors may interact to increase colorectal cancer risk; thus the study of gene-environment and gene-gene interactions can help to identify high-risk subgroups.

## 1128/W/Poster Board #786

**Association of OAS1 variants and prostate cancer.** *S.J. Plummer[1], I. Cheng[2], J.S. Witte[3], G. Casey[1].* 1) Dept of Preventive Medicine, USC, Los Angeles, CA; 2) Epidemiology Program, Cancer Research Center of Hawai'i, Honolulu, Hawai'i; 3) Depts of Epidemiology & Biostatistics, and Urology, Institute for Human Genetics, UCSF, San Francisco, CA.

**Background:** The interferon induced antiviral 2-5A pathway has been implicated in the pathogenesis of prostate cancer. Linkage, association, and functional studies implicate *RNASEL*, encoding the terminal enzyme of the 2-5A pathway, as a strong candidate for the Hereditary Prostate Cancer 1 (*HPC1*) gene. We previously reported an association between the *RNASEL* R462Q variant and prostate cancer in a family-based study. The *RNASEL* R462Q variant gene also showed reduced enzymatic activity and deficiency in apoptotic response. However, published results from others regarding the role of *RNASEL* R462Q in prostate cancer risk have been equivocal. One possible explanation for these results is that variants in other genes of the 2-5A pathway might impact the risk of disease. Dimerization and activation of RNase L occurs following binding of 2-5A oligomers produced by the OAS genes in response to viral dsRNA. **Objective:** To further define the role of the 2-5A pathway in prostate cancer, we evaluated the relationship between prostate cancer and genetic variants in *OAS1*--alone and in combination with the *RNASEL* R462Q variant. **Methods:** We studied 1012 men (506 cases of advanced prostate cancer and 506 age, ethnicity, institution-matched controls) that were recruited from medical centers in Cleveland, Ohio between 2001 and 2004. Advanced disease was defined as Gleason ≥ 7, tumor stage ≥ T2c, or PSA ≥10 ng/mL at diagnosis. Five single-nucleotide polymorphisms (SNPs) in *OAS1* and the *RNASEL* R462Q variant were genotyped by the TAQMAN assay and evaluated for their relationship with more advanced prostate cancer. **Results:** Four of the five *OAS1* variants were statistically significantly associated with prostate cancer risk in this population. The strongest association was found with a missense variant, G162S (rs3741981) which significantly reduced the risk of prostate cancer (Odds Ratio CT vs TT=0.67; 95% CI: 0.49-0.90; p=0.009). There was no association with the *RNASEL* R462Q variant in this population and no significant interactions were observed between any of the *OAS1* variants and *RNASEL* R462Q. **Conclusions:** These results indicate that multiple defects in the 2-5A pathway may be involved in the risk of advanced prostate cancer, although further work is needed to determine the relationship between variants in the different genes in this pathway.

## 1129/W/Poster Board #787

**Linking of Utah genealogies to outpatient colonoscopy records defines familial aggregation of colorectal polyps and cancer.**. *T.M. Tuohy[1], R. Pimentel[1], T. Belnap[4], K. Rowe[4], R.W. Burt[1,2], G. Mineau[1,3].* 1) High Risk Cancers Clinic, Huntsman Cancer Institute; 2) Departments of Medicine and; 3) Oncological Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; 4) Department of Oncology, Intermountain Healthcare, Salt Lake City, UT, USA.

**Background:**The Utah Population Database (UPDB) is a shared research resource of the University of Utah. It combines Utah genealogies dating back to the 1700s with data from statewide resources, including a cancer registry, hospital claims, as well as birth and death certificates. A project completed in 2008 linked the UPDB to patient demographic records from Intermountain Healthcare which is a non-profit medical system that serves Utah and Southeast Idaho, providing care to approximately 60% of the resident population. The availability of large numbers of linked, electronically searchable medical records offers unique opportunities for improved clinical and outcomes research. We address the question of the extent to which colonic adenomas and hyperplastic polyps co-segregate with colon cancer risk in non-syndromic families. **Methods:** A pilot project was initiated to use this new linked research infrastructure by ascertaining colorectal polyps from Intermountain clinical data. Deidentified medical information was merged with UPDB family structure and statewide cancer data. These combined data sets provided family structure along with cancer histories to investigate familial aggregation. Cox Regression Analysis was used to assess the relative risk of (a) polyp development and (b) colon cancer for first-, second- and third-degree relatives, by polyp type. Custom kinship analysis tools allowed determination of the excess polyp and cancer risk observed in the kindreds of each case, and differentiated high-risk from population-risk families. **Results:** The queries captured data from over 70,000 positive outpatient colonoscopy procedure and pathology reports from over 58,000 deidentified individuals examined between 1995 and 2009. The queries collected data on age, gender, polyp type, size, pathology and were verified by manual review of a randomly selected sample of 200 cases. We identified high-risk kindreds dating to founders born in the late 1700s, with high relative risks for polyp/cancer. Analysis of discrepancies between excess polyp and excess cancer risks within families allowed us to address the possibility that some cancers may arise in the absence of significant excess polyp risk. **Conclusion:** The combination of a large, electronically searchable medical database (and associated tissue specimens) with a linked genealogical resource offers a powerful platform for the development of hypothesis-driven research.

## 1130/W/Poster Board #788

**Family History-Based Risk of BRCA Mutations in the California Population.** *N. Whitehead[1], Y. Li[1], L. Squires[2].* 1) Statistics & Epidemiology, RTI International, Atlanta, GA; 2) Health, Social and Economics Research, RTI International, Rockville, MD.

In 2005, the US Preventive Services Task Force recommended that women at a high risk of deleterious BRCA mutations, based on family history, be referred by primary care providers for genetic counseling and evaluation for testing. It is estimated that 2% of U.S. women have a family history necessitating referral for genetic counseling. The estimated combined frequency of BRCA mutations in the U.S. is approximately 0.25%. We assessed the proportion of women in California at high risk of carrying a BRCA mutation based on family history. We analyzed data from the 2005 California Health Interview Survey CHIS), a population-based telephone survey that collects state and local data on a range of health topics. The 2005 survey included a detailed cancer family history module. We estimated the probability the respondent carried a BRCA mutation using BRCAPRO. We imputed the current age of family members and their age at cancer diagnosis. We estimated the current age as a function of the respondent's age. We estimated the age of cancer diagnosis of family members who had cancer before age 50 years as a random variable from a uniform distribution with a lower limit of the estimated current age and an upper limit of 50 years. The minimum ages for breast cancer and ovarian cancer onset were set at 25 and 35 years, respectively. The minimum estimated probability was 0.0000014% and the maximum was 94%. The 98.5 percentile for the population was 0.63%. Therefore, <1.5% of the population had an estimated probability of BRCA mutations > 1.0%; < 0.5% had a an estimated probability of BRCA mutations > 10.0%. The CHIS data had some other limitations. The respondent's history of ovarian cancer was not available. No information was available on BRCA mutation testing, so the predictive value of the risk estimate could not be determined. CHIS asks respondents about breast cancer twice -during the regular questionnaire and in the family history module, but only asks for age of diagnosis in the family history module. Therefore, we had to estimate the age at diagnosis for women who reported breast cancer only in the regular questionnaire. The methods we used to estimate the age of cancer diagnosis are biased towards underestimating the age of cancer diagnosis, since cancer incidence increases with age. Since younger age at diagnosis is associated with an increased risk of having a BRCA mutation, we may have overestimated the probability of carrying a BRCA mutation.

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## 1131/W/Poster Board #789

**Joint Effects of Germ-Line p53 Mutation, MDM2 SNP309, and Gender on Cancer Risk in Family Studies of Li-Fraumeni Syndrome.** *C.C. Wu[1], R. Krahe[2], G. Lozano[2], B. Zhang[2], C.D. Wilson[2], E.J. Jo[1], S. Shete[1], C.I. Amos[1], L.C. Strong[2].* 1) Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX; 2) Dept Genetics, Univ Texas MD Anderson CA Ctr, Houston, TX.

Li-Fraumeni syndrome (LFS) is a rare familial cancer syndrome characterized by a high frequency of early-onset and diverse tumor types and an increased frequency of multiple primary tumors. Germ-line p53 mutations have been identified in most LFS families. A high-frequent genetic variant of single-nucleotide polymorphism (SNP) 309 in the MDM2 gene, a direct negative regulator of p53, was recently shown to be a modifier of cancer risk in p53 mutation carriers. Several independent reports in case-control studies showed a significantly earlier age of cancer onset for the SNP309 G-allele carriers than homozygous T-allele carriers by 7-15 years on average. However, mathematical models accounting for p53 mutation, SNP309, and gender have not been explored in family studies. In this study, we used an extension of the usual joint segregation and linkage analysis models for censored age-of-onset traits, which are semi-parametric survival analysis methods based on Cox proportional hazards models. The methods were designed to evaluate the effects of these factors on cancer risk jointly and allow testing of significance for interactions between them. We analyzed the cancer incidence in 19 extended pedigrees with multiple germ-line p53 mutations ascertained through clinical LFS phenotype and included all invasive cancers as a single combined phenotype. The final dataset consisted of 463 individuals and included 127 p53 mutation carriers. The analyses showed that G-allele carriers had an overall 1.6-fold higher relative risk (RR) of developing cancer than non-carriers. Men with p53 mutations had a 18.5-fold higher RR of developing cancer than non-carriers; women with p53 mutations had a 43.8-fold higher RR than non-carriers. Men with both p53 mutations and G-alleles had a 29.7-fold higher RR of developing cancer than those with neither, and women with p53 mutations and G-alleles had a 70.1-fold higher RR than those with neither and a 2.4-fold higher RR than men with p53 mutations and G-alleles. Our investigation revealed that p53 mutation, SNP309 G-allele, and the interaction between p53 mutations and sex had significant effects on cancer risk. In contrast with the outcomes in case-control studies that SNP309 G-allele interacts with p53 mutations, our family study showed that SNP309 was significant only when both p53 and sex were incorporated to the model. This is further evidenced by the correlation (=0.20) between SNP309 and p53*sex in our most parsimonious model.

## 1132/W/Poster Board #790

**Relationship between germ-line copy number variations and sun exposure/host factors in melanoma-prone families without known mutations.** *R. Yang, M.A. Tucker, A.M. Goldstein.* Dept DCEG, NCI, Bethesda, MD.

Genomic copy number variations (CNVs) have recently been recognized as significant sources of genetic variation that may contribute to disease susceptibility. We previously observed that number of copy number gains and number of genes located within CNVs were significantly higher among cutaneous malignant melanoma (CMM) cases compared to unaffected individuals in melanoma-prone families without known mutations. The goal of this study was to examine the association between germline CNVs and major risk factors for melanoma including sun exposure, pigmentation, nevi, and MC1R variables in these melanoma-prone families. We conducted a genome-wide search for CNVs using the Nimblegen 385K whole-genome array-CGH. We analyzed blood-derived genomic DNA from 78 individuals (61 CMM cases and 17 spouses) selected from 30 melanoma-prone families without known mutations. We used the Nexus Copy Number built-in Rank Segmentation algorithm to identify significant CNVs (significance threshold= 0.000001; minimal number of probes per segment=5; log2 ratio=0.2 for low gain, 0.5 for high gain, -0.3 for loss, -0.7 for homozygous loss). We used a T-test and linear regression to test the associations between CNV variables and melanoma risk factors (dysplastic nevi, number of nevi, hair color, eye color, skin color, freckles, solar injury, response to sun exposure, and MC1R variants). We found that, compared to individuals with no or few freckles (mean number of copy number gains/individual, 3.2±2.9), individuals with freckles tended to have more copy number gains (6.2±3.9, P=0.07, moderate freckles; 8.1±5.7, P=0.002, many freckles). Individuals with solar injury also had increased number of copy number gains (4.3±3.7, absent/mild; 6.4±5.2, P=0.11, moderate; 7.8±5.2, P=0.03, severe). We did not find significant associations between any CNVs and the other risk factors evaluated. These results suggest a dose-dependent relationship between freckles and solar injury and germline copy number gains implying that freckles and solar injury may increase melanoma risk through altered genomic rearrangement. We will further identify the unique CNVs that are associated with these exposures and search for candidate genes and pathways that are influenced by these CNVs.

## 1133/W/Poster Board #791

**Building a biorepository for health disparities research using bioinformatics tools.** *G.M. Dunston[1], L. Ricks-Santi[2,3], T. Mason[2], M. Abbas[2], F. Kan[4], M. Faruque[1].* 1) Department of Microbiology Howard University, Washington, DC; 2) National Human Genome Center at Howard University, Washington DC; 3) Howard University Cancer Center, Washington DC; 4) Data Unlimited International, Inc., Rockville, MD.

Background: Biorepositories with linkage to clinical and epidemiological data are valuable resources for facilitating biomedical research. The use of an interactive computer-based laboratory information management system (LIMS) that tracks all aspects of biospecimen collection, processing, and distribution is essential to the development of a biorepository or biobank. For this project, the goals of the National Human Genome Center (NHGC) at Howard University (HU) were: 1) to implement biospecimen collection protocols and long-term storage solutions, and 2) establish a biorepository consistent with the National Cancer Institute (NCI) Best Practices for Biospecimen Resources, and in compliance with the Health Insurance Portability and Accountability Act (HIPAA), Health and Human Services (HHS), and Food and Drug Administration (FDA) regulations. Here we present the experience and approach of the NHGC in cataloguing archival biospecimens and establishing a biorepository for biomedical research that will interface with a high security, web-based study management system for clinical translational research. Methods: First, existing archived biospecimens and paper data were assessed and documented. Archival materials, such as DNA and tissue samples, were inventoried, catalogued, and recorded in a database. Then, a storage organizational strategy was developed and established using a computerized inventory system that tracks the specific position and location of all stored biospecimens. The precise organization of biospecimens encompassed the storage definitions of divisions and sub-divisions, such as location, room, equipment, shelf, rack, box, tube, column, row, and position. Results: Bioinformatics tools and technologies were used to build a biorepository populated largely with specimens, such as DNA and tissue, from African Americans enrolled in cancer (i.e., prostate, breast, and colon), diabetes, asthma, and other health disparity studies at Howard University. Conclusions: The NHGC quality-controlled biorepository at HU is an invaluable resource for molecular epidemiology and clinical translational research focusing on populations of African Descent. Well-documented, secure biospecimens are the direct link of high quality molecular data to biomedical informatics resources. The NHGC biorepository is aimed at expanding and facilitating collaborative research with the global scientific community in achieving personalized medicine.

## 1134/W/Poster Board #792

**Variations in Helicobacter pylori Cytotoxin-Associated Genes are possibly associated with neoplastic progression in gastric cancer.** *C. Rizzato[1], L.J. van Doorne[2], M. Plummer[3], N. Muñoz[3], F. Canzian[1], I. Kato[4].* 1) German Cancer Research Center (DKFZ), Heidelberg, Germany; 2) DDL Diagnostic Laboratory, Fonteijnenburghlaan, Voorburg, The Netherlands; 3) International Agency for Research on Cancer (IARC), Lyon, France; 4) Wayne State University, Karmanos Cancer Institute, Detroit, MI, USA.

Helicobacter pylori (Hp) infection is associated with the development of gastric cancer. Hp is a bacterium that colonizes the human stomach and can establish a long-term infection of the gastric mucosa. Hp infection affects over 50% of the world's population, with a prevalence ranging from 20% in developed countries to over 90% in developing countries. Persistent Hp infection often induces gastritis and is associated with the development of peptic ulcer disease, atrophic gastritis, and gastric adenocarcinoma. Virulent Hp isolates harbor the cag (cytotoxin-associated genes) pathogenicity island (PAI), a 40 kb stretch of DNA that encodes components of a type IV secretion system (T4SS). This T4SS forms a pilus for the injection of virulence factors into host target cells, such as the CagA oncoprotein. In a previous study a very strong association between current infection with cagA-positive Hp strains and the severity of gastric precancerous lesions has been showed. We analyzed the genetic variability in CagA and other selected genes of the Hp PAI, using DNA extracted from frozen gastric biopsies of the participants in a chemoprevention trial in Tachira State, Venezuela, an area with high prevalence of Hp infection and gastric cancer. Because of the high genetic variability of the Hp genome, this requires a thorough optimization of the experimental conditions. The first analysis confirms the presence of high variability in the Hp PAI genes, which warrants further investigations for the risk of neoplastic progression within CagA positive patients.

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## 1135/W/Poster Board #793

**Lifetime environmental exposure to tobacco smoke, genetic susceptibility, and risk of lung cancer in non-smoking Taiwanese.** *C. Chang[1], C. Hsiung[1], G. Chang[2], Y. Tsai[3], Y. Chen[4], M. Huang[5], W. Su[6], W. Hsieh[1], P. Yang[7], C. Chen[8,9], C. Hsiao[1].* 1) Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan; 2) Division of Chest Medicine, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan; 3) Department of Pulmonary and Critical Care, Chang Gung Memorial Hospital, Taipei, Taiwan; 4) Chest Department, Taipei Veterans General Hospital, Taipei, Taiwan; 5) Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 6) Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan; 7) Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; 8) Genomics Research Center, Academia Sinica, Taipei, Taiwan; 9) Graduate Institute of Epidemiology, National Taiwan University, Taipei, Taiwan.

Background: Environmental tobacco smoke (ETS) has been suggested as a risk factor of lung cancer in nonsmokers. The ETS exposure mainly comes from three sources: childhood and adulthood household passive smoking, and adult work-place passive smoking. Although ETS and genetic factors may play important roles on lung cancer risk, the interactive effect between ETS and genetic factors on lung cancer risk needs to be explored. Objects: A large-scale case-control study was conducted to investigate lung cancer risk in relation to childhood and adulthood ETS exposures in Taiwanese nonsmokers. The interactive effect between genetic factors and ETS on lung cancer risk is also explored. Methods: A total of 1,785 non-smoking lung cancer cases and 1,942 non-smoking healthy controls were enrolled from six medical centers in Taiwan between 2002 and 2009. Genetic polymorphisms of hOGG1 Ser326Cys were determined by the MassARRAY system. ETS history was obtained through personal interview based on a structured questionnaire. Multivariate logistic regression analyses were performed to estimate odds ratios (OR) and 95% confidence intervals (CI) for the risk of lung cancer in exposed vs. non-exposed ETS groups. Results: Childhood ETS had non-significant effects on the risk of lung cancer (OR= 1.2, 95% CI= 0.7-2.0) after controlling for adulthood ETS exposure (including adulthood household and work-place). Adulthood household and work-place had independent risk effects on lung cancer (adulthood household: OR= 1.2, 95% CI= 0.9-1.5; work-place: OR=2.8, 95% CI= 2.0-3.8). On the other hand, considering the interaction between hOGG1 gene and ETS for the risk of developing lung cancer, comparing with the Ser/Ser genotype, the Cys/Cys genotype had higher risk in ETS exposure group (OR=2.0, 95% CI= 1.1-3.7), but not in the non-ETS group (OR=1.3, 95% CI= 0.6-2.8). Conclusions: It was concluded that the adulthood ETS has prominent effects on the risk of lung cancer, especially work-place passive smoking. In addition, hOGG1 Cys/Cys genotype has modifying effect on lung cancer risk by ETS exposure status among non-smokers.

## 1136/W/Poster Board #794

**Evaluation of 9p24 risk locus and colorectal adenoma and cancer: a pooled analysis of four studies.** *J.D. Kocarnik[1], C.M. Hutter[1], M.L. Slattery[2], S.I. Berndt[3], L. Hsu[1], D. Duggan[4], J. Muehling[4], B.J. Caan[5], S.A.A. Beresford[1], A. Rajkovic[6], G.E. Sarto[7], J.R. Marshall[8], N. Hammad[9], R. Wallace[10], K. Makar[1], R. Prentice[1], J. Potter[1], R.B. Hayes[11], U. Peters[1].* 1) Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Department of Internal Medicine, University of Utah Health Sciences Center, Salt Lake City, UT; 3) Division of Cancer Epidemiology and Genetics, NCI, Bethesda, MD; 4) Translational Genomics Research Institute, Phoenix, AZ; 5) Division of Research, Kaiser Permanente Medical Care Program, Oakland, CA; 6) Department of Obstetrics & Gynecology, Baylor College of Medicine, Houston, TX; 7) Department of Obstetrics & Gynecology, University of Wisconsin, Madison, WI; 8) Roswell Park Cancer Institute, Buffalo, NY; 9) Barbara Ann Karmanos Cancer Institute, Wayne State University, Detroit, MI; 10) Departments of Epidemiology and Internal Medicine, University of Iowa College of Public Health, Iowa City, IA; 11) New York University School of Medicine, New York City, NY.

An initial genome-wide association study identified a novel susceptibility locus for colorectal cancer on chromosome 9p24 (SNP rs719725). However, replication in different study populations led to inconclusive findings. To further evaluate this relationship, we utilized existing data from four study populations that also had detailed data on environmental factors, to explore gene-environment interactions as potential sources of heterogeneity that could explain the inconsistent results. METHODS: To investigate the full spectrum of colorectal cancer we included both colorectal adenoma and colorectal cancer from four studies (total 3891 cases, 4490 matched controls): the Women's Health Initiative (WHI; 656 colorectal cancer cases, 664 matched controls); the Diet, Activity and Lifestyle Study (DALS; 1461 colon cancer cases, 1813 controls); the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO, 1257 adenoma cases, 1385 matched controls); and a Minnesota population-based case-control study (MinnCCS; 517 adenoma cases, 628 controls). Data were restricted to Caucasians only. We used logistic regression to evaluate the association, with minor allele C as the reference group as reported previously in the literature. In all four studies, the MAF was ~37% and controls were in HWE ($p > 0.26$). RESULTS: In the WHI data rs719725 was statistically significantly associated with risk of colorectal cancer, with OR 1.19 (95% CI 1.01-1.40; p-trend 0.04) per A allele. Similarly, the PLCO data provided a marginally significant finding in the same direction for adenoma risk: OR 1.11 (95% CI 0.99-1.25; p-trend 0.07) per A allele. The other two studies provided OR estimates at or near 1.00. We observed no interactions between rs719725 and several key risk factors for colorectal cancer (age, sex, family history, smoking, NSAIDs, alcohol, BMI, physical activity, folate, calcium, red meat, or HRT use). A pooled analysis of our and previous studies (n = 16) gave an OR of 1.07 (95% CI 1.03-1.11) per A allele. CONCLUSIONS: This study can neither prove nor rule out the existence of a risk locus on chromosome 9p24 that is tagged by SNP rs719725. Along with previous publications, these results suggest that the major allele A may be positively associated with colorectal tumors in some populations. Further replication in larger studies seems to be warranted, perhaps followed by studies to identify the disease-related polymorphism being tagged and its possible function.

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## 1137/W/Poster Board #795

**JUVENILE POLYPOSIS SYNDROME, HISTOPATOLOGICAL DIAGNOSIS AND CLINICAL FEATURES. A CASE REPORT AT THE HOSPITAL PARA EL NINO POBLANO. MEXICO.** *R. Vargas-Gonzalez[1], J.M. Aparicio-Rodriguez[2,5], L. de la Torre-Mondragon[3], G. Victoria-Morales[3], B.A. Llamas-Guillen[4]*. 1) Pathology; 2) Medical Genetics; 3) Surgery; 4) Pediatrics, Hospital para el Nino Poblano, Puebla, Puebla, Mexico; 5) Estomatology, Benemerita Universidad Autonoma de Puebla, México.

Key words: BMPR1A, EGN, SMAD4, genetics, Juvenile Polyposis Syndrome. ABSTRACT. Juvenile Polyposis Syndrome(JPS) is an uncommon hamartomatous disorder with significant gastrointestinal malignant interaction. Mutations in EGN, SMAD4 and BMPR1A genes, implicated in the Transforming Growth Factor pathway. This study confirms the genetics and clinicopathological features of the JPS. INTRODUCTION. JPS is a clinically and genetically heterogeneous condition, a hamartomatous disorder. It affects approximately one in 150 000 new born children. Both sporadic and familial cases with autosomal dominant inheritance are found. To date, there are no clinical,pathological, immunohistochemical or molecular markers that distinguish sporadic from the syndrome associated Juvenile Polyposis. The JPS is regarded as distinct from solitary juvenile polyps which develop less than 5% of children and adolescents, with no malignant potential. HISTOPATHOLOGY. The Patient in this study was diagnosed as JPS with more than 100 polyps distributed throughout the colon, stomach and small intestine. The polyps range from a few millimeters to a few centimeters in size. Macroscopically, a juvenile polyp has a smooth, spherical red head on a narrow stalk. Microscopic changes are mucin-filled cystic dilatation of the epithelial tubules embedded in abundant lamina propria. The tubules are lined by normal columnar epithelium. The cellular infiltrate of the lamina propria includes myofibroblasts, fibroblasts, and macrophages. Muscularis mucosa is not included within the stroma. GENETICS. JPS is an autosomal dominant condition as mentioned before with incomplete penetrance. Research in JPS has identified three specific gene changes causing disruption of the transforming growth factor (TGF b) signal transduction pathway: EGN, SMAD4 and BMPR1A. CONCLUSION. JPS is characterized by anaemia, rectal bleeding, and particularly in the pediatric population disastrous complications of bowel infarction due to intussusception, as the patient in this study. It also has significant gastrointestinal malignancy potential. The new genetic knowledge regarding EGN, SMAD4 and BMPR1A genes allows for genotypic diagnosis in about 50% of patients with JPS. An early diagnosis is important for an early multidisciplinary medical and surgical treatment to offer the patient a better quality of life.

## 1138/W/Poster Board #796

**N-myc downstream regulated gene 1 (NDRG1) is fused to ERG in prostate cancer.** *M.A. Rubin[1], D.S. Rickman[1], A. Sboner[4], P. Perner[1], C.J. LaFargue[1], Y. Pan[1], M.A. Svensson[1], B.J. Moss[1], N. Kitabayashi[1], A. de la Taille8[6,7], R. Kuefer[5], A.K. Tewari[2], F. Demichelis[1,3], M.S. Chee[8], M.B. Gerstein[4,9,10], D. Pflueger[1,5]*. 1) Departments of Pathology & Laboratory Medicine, Weill Cornell Medical College, New York, NY; 2) Department of Urology Weill Cornell Medical College, New York, NY; 3) Institute for Computational Biomedicine, Weill Cornell Medical College, New York, NY; 4) Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT; 5) Department of Urology, University Hospital Ulm, Ulm, Germany; 6) INSERM, Unité 955, Créteil, France; 7) Department of Urology, CHU Mondor, Créteil, France; 8) Prognosys Biosciences Inc., La Jolla, CA; 9) Program in Computational Biology and Bioinformatics, Yale University, New Haven, CT; 10) Department of Computer Science, Yale University, New Haven, CT.

A step towards the molecular classification of prostate cancer was the discovery of recurrent ETS rearrangements, most commonly fusing the androgen-regulated TMPRSS2 promoter to ERG. The TMPRSS2-ERG fusion is observed in around 90% of tumors that over-express the oncogene ERG. The goal of the current study was to complete the characterization of these ERG over-expressing prostate cancers. Using fluorescent in-situ hybridization (FISH) and RT-PCR assays, we screened 101 prostate cancers, identifying 34 (34%) cases with the TMPRSS2-ERG fusion. Seven cases demonstrated ERG rearrangement by FISH without the presence of TMPRSS2-ERG fusion mRNA transcripts. Screening for known 5' partners, we determined that 3 cases harbored the SLC45A3-ERG fusion. To discover novel 5' partners in these ERG over-expressing and ERG rearranged cases, we used paired-end RNA-sequencing (RNA-seq). We first confirmed the utility of this approach by identifying the TMPRSS2-ERG fusion in a known positive prostate cancer case and then discovered a novel fusion involving the androgen-inducible tumor suppressor, NDRG1 (N-myc downstream regulated gene 1) and ERG in two cases. Unlike TMPRSS2-ERG and SCL45A3-ERG fusions, the NDRG1-ERG fusion is predicted to encode a chimeric protein. Like TMPRSS2, SCL45A3 and NDRG1 are inducible by androgen but also by estrogen. This study demonstrates that the vast majority of ERG over-expressing prostate cancers harbor hormonally regulated TMPRSS2-ERG, SLC45A3-ERG, or NDRG1-ERG fusions. Broader implications of this study support the use of RNA-seq to discover novel cancer translocations.

## 1139/W/Poster Board #797

**Clinical Utility in Monitoring Cell Lineage Chimerism after Allogeneic Hematopoietic Stem Cell Transplantation.** *R. Saad*. Pathology, Baylor University Medical Center, Dallas, TX.

Monitoring of the chimeric status of hematopoietic stem cell transplant (HSCT) recipients is of great clinical significance when comparing different conditioning therapies. Short tandem repeats (STR) analysis is the most common method for chimerism monitoring. Engraftment analysis is usually performed on DNA extracted from unfractionated blood or bone marrow samples. Analysis of lineage-specific leukocytes provides useful information in predicting the clinical outcome and plays a critical role in the management of HSCT patient. We have used the GenomeLab Human STR Primer set to assess the dynamics of chimerism within the individual leukocyte subsets in 77 hematopeiotic cell transplant patients after myeloablative and nonmyeloablative regimens. Magnetic anti-CD3, anti-CD15 and anti-CD19 beads were used to capture T cells, myeloid cells and B cells, respectively, from peripheral blood and bone marrow samples prior to isolation of DNA. DNA was extracted on the Beckman Coulter SPRI-TE nucleic acid extractor using the genomic DNA extraction kit. Chimerism analysis performed on specific cellular lineages was most useful in early detection of relapse than unfractionated whole blood analysis due the increased sensitivity in the enriched cell subpopulations. Determination of the level of donor T cells appears to be critical for successful engraftment and could predict graft versus host disease.

## 1140/W/Poster Board #798

**Shortened Telomere Length Correlates with Promoter Methylation Status of p16INK4a/Rb and p53/p21cip1 pathways in Breast Cancer.** *Z. Barekati, R. Radpour, R. Asadollahi, C. Kohler, XY. Zhong.* Biomedicine, University of Basel, Basel, Basel, Switzerland.

Continuous cell growth is a major advanced malignancy hallmark which is correlated with shortened telomere length. Measurement of telomere length is a useful biomarker to elucidate cell replicative history and proliferation in cancerous tissues. The telomere length and promoter methylation status of the p16ink4a/Rb or p53/p21cip1 pathways were examined in different grades of breast cancer. Telomere length was measured in a cohort of 104 samples including 52 cancerous and paired adjacent normal breast tissues by quantitative PCR. Using a novel high-throughput mass spectrometry on MALDI-TOF silico-chips, we determined quantitative methylation changes of TP53, P21 and P16 genes in all studied samples. A two-way hierarchical cluster analysis was used to classify methylation profiles. The result showed significantly shortened telomere length in the cancerous tissues especially in grades two and three of breast cancer ($p<0.001$). Results of the study showed correlation between the ratio of telomere length in the cancerous tissues and the methylation status of analyzed genes. Significant hypermethylation of P16 and P21 promoters in the patients with shortened telomere length was detected ($p<0.001$). No significant correlation has been found between TP53 promoter methylation status and telomere shortening. Inactivation of the p16INK4a/Rb or p53/p21cip1 pathways regarding to the promoter hypermethylation may allow continuous cell division and critical telomere shortening, which induce genome instability, finally leading to malignant transformation. Our results suggest that telomere shortening and inactivation of p16INK4a/Rb or p53/p21cip1 pathways may serve as a potential biomarker for the disease.

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### 1141/W/Poster Board #799

**Evaluation of PTEN and Mcl-1 Expressions in NSCLC Expressing Wild Type or Mutated EGFR.** *S. Berker-Karauzum[1], Z. Cetin[1], G. Ozbilim[2], A. Erdogan[3], G. Luleci[1].* 1) Department of Medical Biology , Akdeniz University Faculty of Medicine, Antalya, Turkey; 2) Department of Pathology, Faculty of Medicine, Akdeniz University, Antalya, Turkey; 3) Department of Thoracic Surgery, Faculty of Medicine, Akdeniz University, Antalya, Turkey.

Signaling pathways activated by Epidermal Growth Factor Receptors are important in lung carcinogenesis. New treatment strategies with Epidermal Growth Factor Receptor targeting drugs provided improvements in management of lung cancer. However molecular mechanisms underlying resistance to these drugs needs to be evaluated. Surgically resected samples were obtained from fifty patients with non-small cell lung cancer. PTEN, Mcl-1 and EGFR protein expression levels were evaluated by Western-blot. Direct sequencing were performed to investigate EGFR tyrosine kinase domain mutations. We detected c.2235-2249 (pGlu746-Ala750del) mutation in exon 19 in two patients with adenocarcinoma histology. Elevated expression levels of both Mcl-1 isoforms (Mcl-1S and Mcl-1XL) and EGFR proteins were found in 15 (30%) and 23 (46%) of the cases, respectively. Reduced PTEN protein expression levels were observed in 17 (34%) of the cases. PTEN expression level was reduced in 26% of cases which showed increased EGFR expression. Also, increased expression of Mcl-1 protein was observed in 26% of cases with EGFR overexpression. One of the cases harbouring pGlu746-Ala750del mutation had increased levels of Mcl-1 and decreased PTEN expression levels. Our results indicate that, in addition to lack of PTEN expression, elevated levels of the Mcl-1 protein might be one of the important intrinsic mechanisms protecting non-small cell lung cancer cells from apoptosis induced by several compounds. Therefore, EGFR mutations in conjuction with evaluation of Mcl-1 and PTEN expression levels in large cohorts might provide important clues for improvements of new treatment strategies in non-small cell lung cancer management.

### 1142/W/Poster Board #800

**Is Human Papillomavirus Viral Load in Cervical Intraepitelial Neoplasia a prognostic factor of disease?** *E. Borrayo Carbajal[1], A.E. Suárez Rincón[2], A.E. Bencomo Álvarez[1], H. Montoya Fuentes[1].* 1) División de Medicina Molecular, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México; 2) Hospital General de Zona No 45, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México.

Human Papillomavirus (HPV) has been proposed as the most important risk factor for the development of cervical cancer and other tumors. Several factors have been related to the development and progression of cervical disease, from those, the viral type is considered the most important and has already been extensively described. Nevertheless, recent publications has proposed viral load (VL) as an effective diagnostic and prognostic marker for cervical neoplasia. In the present work we report a preliminary comparison of the HPV VL of patients with diagnosis of grade 2 Cervical Intraepithelial Neoplasia (CIN2) versus grade 3 Cervical Intraepithelial Neoplasia (CIN3) and Normal Cytology (NC). In this study we used Cervical samples from Mexican patients diagnosed either as CIN2, CIN3 or NC. DNA extraction and PCR amplification, using consensus primers for HPV E1 ORF as previously reported, were performed to determine the positivity to these viruses in each sample. Hybrid Capture II System (HCIIS, DIGENE, Gaithersburg, USA) was used to estimate VL total number. A QPCR melting curve of the GAPDH gene was performed to obtain a number of cells per sample and the data obtained were normalized using the following formula: VL= Antilog [log(RLUx5000)-log(Total No. of Cells)]. The mean and standard deviation of the number of viral copies per cell shows no significant differences in the distributions between CIN2 vs CIN3, NC vs CIN2 and NC vs CIN3 ($p>0.05$). This differs from the role of VL as a prognostic tool previously proposed. It has been suggested that VL plays an important role in the development of cancer. Nonetheless, according to our preliminary data, VL remains constant along the pathological process, as previously described. The debatable association between VL and the risk of developing Cervical Cancer may be due to different standardization and normalization of the absolute number of viral copies in one sample, as well as to different grouping criteria (i.e. CIN1 vs CIN2 or CIN1 vs CIN 2&3, etc.). These phenomena might explain the inconsistent results among different reports. The need for homologated methods in the normalization of data is evident.

### 1143/W/Poster Board #801

**Interleukin-18 gene polymorphisms and the risk of cervical squamous cell carcinoma.** *T.Y. Chang[1], Y.C. Yang[1,2,4], Y.J. Lee[1,3,5], T.C. Chen[2], S.C. Chang[1], H.W. Chan[1].* 1) Medical Research Department, Mackay Memorial Hospital, Taipei, Taiwan; 2) Department of Gynecology and Obstetrics, Mackay Memorial Hospital, Taipei, Taiwan; 3) Department of Pediatrics, Mackay Memorial Hospital, Taipei, Taiwan; 4) Mackay Medicine, Nursing, and Management College, Taipei, Taiwan; 5) College of Medicine, Taipei Medical University, Taipei, Taiwan.

Cervical cancer is strongly associated with infection by oncogenic forms of human papillomavirus (HPV). However, HPV infection alone is not sufficient for progression to cervical cancer. It is now recognized that host immunogenetic background play an important role in the control of HPV infection and the development of cervical cancer. Interleukin-18 (IL-18) is a multifunctional cytokine that induces interferon-gamma secretion and plays an important role in antitumor immunity. The aim of this study is to investigate the association between *IL-18* gene polymorphisms and the risk of cervical cancer in the Taiwanese population. The *-607 C/A* and *-137 G/C* polymorphisms in the promoter region and *+105 A/C* in the exon 5 were genotyped in 127 cervical squamous cell carcinoma (CSCC) patients and 269 age/sex matched healthy controls by using the Pre-Developed TaqMan Allelic Discrimination Assay. The presence and genotypes of HPV in CSCC patients were determined by PCR. We found no significant association between any polymorphisms or haplotypes examined and overall CSCC risk. In addition, no significant association was observed between HPV-16 positive CSCC patients and controls. Our results suggest that specific variations in the *IL-18* gene did not confer susceptibility to CSCC in the Taiwanese population.

### 1144/W/Poster Board #802

**Genetic polymorphisms in xenobiotic metabolizing enzymes and environmental risk factors in colorectal cancer among Filipinos.** *E. Cutiongco[1], C. Padilla[1], C. Ngelangel[2], V. Bañez[3], M. Roxas[4], C. Silao[1], R. Cortez[1], F. Rocamora[1] for The Philippine Cancer Genetics Study Group.* 1) Institute of Human Genetics, National Institutes Health, University of the Philippines, Manila, Philippines; 2) Institute of Clinical Epidemiology, University of the Philippines, Manila, Philippines; 3) Department of Medicine, Philippine General Hospital, Manila, Philippines; 4) Department of Surgery, Philippine General Hospital, Manila, Philippines.

Colorectal cancer is one of the leading cancers of the world. Based on incidence, it ranks fourth in men and third in women, with over 1 million new cases worldwide (World Cancer Report 2008). In the Philippines, cancers of the colon and rectum ranked 3rd overall, 3rd among males and 4th among females. In 2005, there were 8,585 new cases and 5,558 related deaths (Laudico et al 2005). A number of environmental factors have been reported to increase colorectal cancer risk but genetics has also been identified to play a role in cancer susceptibility. Genetic polymorphisms account for variations in genes coding for enzymes that are responsible for the detoxification of various endogenous and exogenous substrates into harmless compounds, or their metabolic activation into toxic and carcinogenic products. Many of these genes are functionally polymorphic and have different allelic variants which result in variations in susceptibility to cancer among individuals. This is a molecular epidemiological study that looks at the putative association of colorectal cancer susceptibility with environmental factors and genetic polymorphisms in GSTM1, GSTT1, GSTP1, NAT1 and NAT2 genes. A total of 500 Filipino patients, 224 with colorectal cancer and 276 cancer-free were included in the study. Corresponding demographics, medical histories, social histories, diet histories, and occupational exposure data were collected for all subjects. Molecular genotyping was done through PCR-RFLP of target DNA regions. Univariate logistic regression analysis of environmental variables revealed wood dust occupational exposure (OR 2.66; 95% CI 1.21-5.83), UV sunlight exposure (>7/month) (OR 1.99; 95% CI 1.16-3.39) and moldy food exposure (>1/ month) (OR 1.61; 95% CI 1.11-2.35) to be associated with colorectal cancer risk. For the genetic polymorphism studies in the genes involved in the xenobiotic metabolism pathway, univariate analysis showed the NAT2*6B allele (dominant model: OR 1.51, 95% CI 1.06-2.16; recessive model OR 1.87, 95% CI 1.05-3.33) to be independently associated with colorectal cancer risk. After multivariate logistic regression of both genetic and environmental factors initially shown to be associated with colorectal cancer, only UV exposure (OR 2.08, 95% CI 1.21-3.58) was shown to be statistically significant.

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### 1145/W/Poster Board #803

**PCA3, the highly specific prostate cancer biomarker, recently evolved within a second gene, BMCC1, which is also upregulated in prostate cancer.** *Z.M. Fang[1], R.A. Clarke[1,2], Z. Zhao[3], A.Y Guo[3], K. Roper[4], L. Teng[2], H. Samaratunga[5], M.F. Lavin[2], R.A. Gardiner[2].* 1) RADIATION ONCOLOGY, St. George Hospital, Kogarah, NSW, Australia; 2) Queensland Institute of Medical Research, Radiation Biology and Oncology, Brisbane, QLD 4029, Australia; 3) Department of Psychiatry and Center for the Study of Biological Complexity, Virginia Commonwealth University, Richmond, VA 23298, USA; 4) Hopkins Marine Station, Stanford University, Stanford, CA 93950, USA; 5) Sullivan & Nicolaides Pathology, Brisbane, Australia University of Queensland Centre for Clinical Research, Brisbane, Australia.

The prostate cancer antigen 3 (PCA3) gene is a highly specific biomarker upregulated in prostate cancer (PCa). We employed cDNA synthesis, RT-PCR and DNA sequencing to identify 4 new transcription start sites, 4 new polyadenylation sites and 2 new differentially spliced exons in an extended form of PCA3. Primers designed from these novel PCA3 exons greatly improve RT-PCR based discrimination between PCa, PCa metastases and BPH specimens. Comparative genomic analyses further demonstrated that PCA3 has only recently evolved in an anti-sense orientation within a second gene, BMCC1 / PRUNE2. BMCC1 has been shown previously to interact with RhoA and RhoC, determinants of cellular transformation and metastasis, respectively. Using RT-PCR we demonstrate that the longer BMCC1-1 isoform - like PCA3 - is upregulated in PCa tissues and metastases and in PCa cell lines. PCA3 and BMCC1-1 levels are also responsive to dihydrotestosterone. Enrichment of two new PCA3 isoforms in PCa tissues improves discrimination between PCa and BPH. The functional relevance of this specificity is now of particular interest given PCA3's overlapping association with the BMCC1 regulator of Rho signalling. Combination testing including PCA3, BMCC1, TMPRSS2-ERG fusions and other PCa biomarkers has real potential for improved diagnosis.

### 1146/W/Poster Board #804

**Association of insertion/deletion polymorphism (Del1518) of MDM2 in Mexican lung cancer patients.** *M. Gallegos[1], L.E. Figuera[2], G. Morgan[3], A.M. Puebla[4].* 1) Dept Med Molec, Guadalajara, CIBO, IMSS, Jalisco, Mexico; 2) Dept. Genetica, CIBO, IMSS; 3) Unidad de radio diagnostico, HE, CMNO, IMSS; 4) Lab. Inmunifarmacologia, CIBO, IMSS.

The MDM2 gene encodes to protein that negatively regulates p53 expression, participate in multiples functions of cell-cycle and modulation of DNA repair. The insertion/deletion polymorphism (Del1518) in the promoter of MDM2 gene has been shown to be associated with lung cancer. In the present study we describe to frequency of Del1518 polymorphism of MDM2 gene in lung cancer patients and health controls from Mexico. In a case-control study of 280 lung cancer patients and 174 controls we observed a frequency of 57% (146/280) and 56% (98/174) of insertion genotype; 38% (107/280) and 34% (59/174) of genotype insertion/deletion and 10% (27/280) and (17/174) of genotype deletion cases and controls respectively, without to be statistically different ($p>0.05$). These results suggest that the Del1518 polymorphism on the MDM2 promoter is not associated with Mexican lung cancer patients.

### 1147/W/Poster Board #805

**Expressions of Peroxiredoxin isoforms during the progression of Endometrial cancer.** *S. Han, I. Oh, M. Jung, W. Choe.* Department of Biochemistry and Molecular Biology, Medical Science and Engineering Research Center for Bioreaction to Reactive Oxygen Species, Biomedical Science Institute, School of Medicine, Kyunghee University, Seoul 130-701, Korea.

Endometrial carcinoma is a common malignant tumor of the human female genital tract, typically affecting women after menopause. Asian nations such as Korea, Japan and China have an incidence that is 4-5 times lower than in western industrialized nations. however, it has been increasing markedly in recent years. One of the family proteins with an antioxidantive function, peroxiredoxin (Prx), so far has revealed six members in mammals. These enzymes share a common reactive Cys residue in the N-terminal region, and are capable of breaking down H2O2 as a peroxidase and involve thioredoxin and/or glutathione as the electron donor. We evaluated the expression of Prx isoforms in normal tissue, endometrial hyperplasia and endometrial cancer. In the present study, We investigated the expression of Prx isoforms in normal endometrium, endometrial hyperplasia and endometrial cancer. We detected that expression of Prx isoforms differs in the normal sample groups : Prx I, II, and III was negative. Prx IV and V was weekly expressed. Prx VI were strongly expressed. Interestingly, endometrial cancer had the strongest expression of Prx I, II, III, IV, and V compared with normal endometrium and endometrial hyperplasia. No difference in staining with intensity by grade of lesion was observed for Prx VI. Therefore, we conclude that Prx I, II, III, IV, and V are upregulated in response to the development of endometrial cancer.

### 1148/W/Poster Board #806

**MicroRNA Profiles in Exosomes Derived from Prostate Adenocarcinomas.** *M. Howell, J. Kimbrough, T. Deng, A. Conrad, P. Ellis, P. Kennedy, T. Tinder, P. Esmay, T. Maney, C. Kuslich.* Caris/MPI, Phoenix, AZ.

MicroRNAs (miRs) are small, non-coding RNAs with critical roles in transcriptional regulation. miRs are present in virtually all nucleated cells, and physiologic expression profiles of miRs, like those of coding RNAs, reflect developmental lineages and differentiation states of specific cells and tissues. Disregulation of miR expression has been correlated with malignant transformation in a variety of cancer types, including prostate cancer (PCa). miR expression profiles in multiple PCa isolates have been shown to differ from those of corresponding non-transformed tissue, suggesting considerable promise for miR signatures as diagnostic biomarkers of PCa. Development of minimally-invasive alternatives to tumor biopsy will further enhance the utility of this diagnostic strategy. One such alternative is to determine miR signatures of tumor-derived exosomes. Exosomes are microvesicles of endocytic origin which, in the plasma of healthy individuals, derive primarily from hematopoietic cells. In cancer patients, tumor-derived exosomes also are present and, since the protein content of exosomes largely reflects that of the parental cell, lineage- and/or tumor-specific antibodies may be used to isolate tumor exosomes for miR analysis. Toward this end, we have begun to implement strategies for immunoselection of PCa-derived exosomes and for the characterization of miR profiles contained therein. Exosomes present in culture medium conditioned by PCa and non-malignant control cell lines have been purified using density gradient centrifugation. The resulting particles were confirmed by EM to possess sizes and morphologies characteristic of exosomes, and immunoblot analysis revealed the presence of multiple spannins known to be enriched in exosomal membranes. Using ELISA and MIA, ten antibodies that bind exosomes purified from several PCa cell lines were identified, and several of these antibodies were used to selectively enrich the respective populations. Total RNA has been isolated from density gradient-purified and immunoselected exosomes, and miRs present in these preparations have been analyzed using: 1) qRT-PCR with selected miR primer and probe combinations; microfluidic TaqMan low-density miR arrays (TLDA); and/or, 3) miR microarrays. Initial analyses reveal significant complexity in these exosomal miR repertoires, and they suggest the potential utility of exosomal miR evaluation in PCa diagnostics.

### 1149/W/Poster Board #807

**The Benefit of Sequencing Analysis to Identify Important KRAS Activating Mutations in Colorectal Cancer.** *U. Jariwala, L. Dejeza-Jamanila, K. Arnold, M. McGinniss, C. Kuslich.* Molecular Diagnostics, Caris Dx, Phoenix, AZ.

KRAS, the human homolog of the Kirsten rat sarcoma-2 virus oncogene, encodes a small GTPase protein, which mediates downstream signaling in the EGFR signaling pathway. Most common activating point mutations occur in codons 12 and 13 and are found in ~40% of colorectal cancers. These mutations render KRAS constitutively active and independent of upstream EGFR activation and are predictive of the failure of EGFR targeted therapies such as cetuximab and panitumumab in patients with metastatic colorectal cancer. The majority of PCR-based mutation analysis methods focus only on mutations contained in codons 12 and 13. Other activating mutations in KRAS have also been identified in codon 61 and these are relevant for predicting response to anti-EGFR therapies. Tumor samples from 394 unselected colorectal cancer patients were analyzed for activating KRAS mutations in exons 2 and 3 by Sanger sequencing. We discovered 58.4% of these tumors were wild type for KRAS while 41.6% of harbored KRAS mutations, a similar overall mutation frequency to what has been previously reported. In addition to finding the 7 mutations most frequently screen for (G12D-14.7%, G12V-8.6%, G12C-4.6%, G12A-2.3%, G12S-1.8%, G12R-0.8%, and G13D-6.3%), we also identified a number of KRAS activating mutations (G12dup, G13R, Q61H, Q61L and Q61K), with a combined frequency of 6.1%, that would not have otherwise been detected by mutation specific PCR-based testing. As these mutations have also been shown to activate KRAS, they too predict failure of anti-EGFR therapies and represent an underserved number of patients that would benefit from therapeutic screening. These data suggest that comprehensive analysis of KRAS mutational status, including additional mutations in codon 13 and codon 61, is warranted for optimal patient selection for cetuximab and panitumumab therapies.

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## 1150/W/Poster Board #808

**_ST14_ variants and low matriptase expression predict poor breast cancer survival.** *J.M. Kauppinen[1,2,3], A. Mannermaa[1,2,3], R. Sironen[1,2,3], Y. Soini[1,2,3], T. Nykopp[1], M. Eskelinen[4], V. Kataja[5,6], V.-M. Kosma[1,2,3].* 1) Institute of Clinical Medicine, Pathology and Forensic Medicine, University of Kuopio, Kuopio, Finland; 2) Department of Pathology, Kuopio University Hospital, Kuopio, Finland; 3) Biocenter Kuopio, Kuopio, Finland; 4) Department of Surgery, Kuopio University Hospital, Kuopio, Finland; 5) Department of Oncology, Kuopio University Hospital, Kuopio, Finland; 6) Department of Oncology, Vaasa Central Hospital, Vaasa, Finland.

The aim of this study was to examine the role of *ST14* (matriptase) genetic variation and expression in breast cancer survival and to assess their clinicopathological correlations. 455 invasive breast cancer cases and 446 healthy controls from Eastern Finnish origin in the Kuopio Breast Cancer Project (KBCP) were genotyped for five SNPs in *ST14* gene. Immunohistochemical analyses were performed in tissue microarrays (TMA) consisting of 377 invasive breast cancers from the KBCP cohort. Three variants in *ST14* gene were found to be prognostic factors independently predicting breast cancer survival, worse outcome associating with the rare homozygous genotype ($P$ = 0.001 in Cox multivariate survival analysis). Statistically significant association of the genotypes with typical prognostic clinicopathological parameters (nodal status, tumour grade and clinical stage, $P$ values 0.036, 0.046 and 0.044, respectively) supports the connection with survival. Also *ST14* mRNA levels were lower among the rare homozygous genotypes compared to the common homozygotes ($P$ = 0.009 in independent samples t-test). Negative/low matriptase expression (IHC) associated with poorer survival ($P$ = 0.046 in Cox multivariate survival analysis). This was also supported by association of matriptase expression with several clinicopathological parameters. We conclude that matriptase genetic variants do have prognostic significance in breast cancer and thus influence progression of the disease.

## 1151/W/Poster Board #809

**The Frequency and Distribution of KRAS Mutations in Various Tumor Types.** *C. Kuslich, U. Jariwala, L. Dejeza-Jamanila, M. McGinniss, K. Arnold.* Molecular Diagnostics, Caris Dx, Phoenix, AZ.

Activating mutations in KRAS, the human homolog of the Kirsten rat sarcoma-2 virus oncogene, mediate constitutively activated downstream signaling in the EGFR signaling pathway. Recent evidence from numerous randomized studies has established that colorectal tumors harboring an activating mutation in the KRAS gene do not derive benefit from epidermal growth factor receptor-directed monoclonal antibodies (e.g. cetuximab or panitumumab). However, a comprehensive analysis has yet been undertaken on activating KRAS mutations in tumors from other tissue types where epidermal growth factor receptor-directed therapy may also be used. In this study, a total of 82 tumors from pancreas (n= 13), liver (n= 23), lung (n=32), stomach (n=6) and breast (n= 8) were screened for KRAS mutations in codons 12, 13, and 61 by direct sequencing. The results of this mutational analysis revealed that 69.2% of pancreatic tumors, 39.1% of liver tumors, 18.7% of lung tumors and 16.7% of stomach tumors had activating mutations in KRAS. The breast tumors analyzed were wild type at codons 12, 13, and 61. The distribution of mutations among the codons of the KRAS gene was different by tumor type. Liver tumors had mutations that were identified in codons 12, 13, and 61 at a similar frequency as evident in colon cancer with codon 61 being mutated in 4.3% of tumors analyzed. The lung tumors analyzed had 15.7% of their mutations in codon 12 and only 3% in codon 13 while stomach tumors only had the G12A mutation. Pancreatic tumors had mutations identified in codon 12, with a wider distribution of point mutations within that codon compared to stomach and lung tumors. While larger selected studies are warranted to investigate these findings in greater numbers in the context of each individual disease and subtype, these results add to the considerations involved in the use of resistance and predictive biomarkers.

## 1152/W/Poster Board #810

**Do MGMT polymorphisms are involved in colon polyps formation?** *K.M. Lamperska[1], A. Przybyla[2], W. Kycler[3], A. Mackiewicz[1,2].* 1) Department of Cancer Diagnostics and Immunology, Wielkopolskie Centrum Onkologii, PoznaÅ", Poland; 2) Department of Cancer Immunology, Medical University PoznaÅ", Poland; 3) Department of oncological surgery, Wielkopolskie Centrum Onkologii.

MGMT plays crucial role in defense again toxic effect of alkylation damages in DNA. It is known more than five polymorphisms of MGMT gene,these SNPs are associated with different types of cancer, increase or decrease its risk. These genetic alternations together with environmental factors may modulate MGMT activity. We investigated polymorphisms in MGMT in blood and colon polyps' tissue and the main aim of our study was to check possible association identified genetic changes with colon cancer risk. The blood samples and colon polyp's tissue were obtained from 254 individuals. All patients filled questionnaire including questions about style of life,dietary and cancer diseases. All recognized polymorphisms were compared with control group consisted of 330 healthy individuals. DNA was isolated from blood sample and polyps tissue using standard method. Polymorphisms in MGMT gene were investigated by PCR-SSCP method. The Pearson's chi-square and Fisher's exact test were used to test the differences in genotype and allele distribution. Logistic regression was employed to calculate odds ratio (OR) and 95% confidence intervals (95%CI) and for calculating interactions. Linkage disequilibrium analysis of identified polymorphisms was performed using free online software Haploview version 4.0. All p values was two-sided. We found two polymorphic variants: L84F and K178R. We found significant differences in L84L, L84F and F84F alleles distribution between cases and control group (p=0, 0001).Higher distribution of 84F allele was observed in smoking patients (p=0, 0405) and older (up 60th) patients (p= 0, 0000). We did not find correlation between this polymorphic variant and gender. The frequency of 178R allele did not show statistical differences.Next polyps were divided according degree of dysplasia and size.We did not observed correlation between 84F and high risk polyps.The study group included 23 colon cancer patients and 27 reported colon cancer in family history but we did not finde correlation between these cases and frequency of 84F variant. Finally we conclude that high frequency of 84F allele in group of patients with colon's polyps may suggest the role of the MGMT variant in colon polyp's aetiology.In the other hand we did not find arguments for connection of variant 84F alone with colon cancer development.

## 1153/W/Poster Board #811

**Ultrahigh sensitive, early detection of relapse in non-small cell lung cancer patients using blood specimen.** *Q. Liu[1], N. Hsu[2], X. Liu[1], Y. Wang[1], L. Chen[1], C. Chen[3], Y. Yen[1].* 1) Department of Clinical and Molecular Pharmacology and Translational Research Lab, City of Hope National Medical Center, Duarte, CA; 2) Department of Surgery, Buddhist Taichung Tzu-Chi Hospital, Taiwan; 3) Cancer Center, China Medical University Hospital, TaiChung, Taiwan.

Non-small-cell lung cancer, accounting for about 85% of all lung cancers, is the leading cause of cancer deaths in the United States and worldwide. Despite advances in standardized treatment, non-small-cell lung cancer often has a poor prognosis due to relapse. Our goal is to identify such relapse at a very early stage through ultra-high sensitive detection of cancer-specific somatic mutations in plasma DNA. Specifically, we will use Pyrophosphorolysis Activated Polymerization (PAP), an ultra-high sensitive nucleic acid detection technology, to detect as few as one copy of cancer-specific mutations in 5 mL of plasma. This assay will be over 1000-folds more sensitive than PCR-based methods. In order to target appropriate mutations, we first applied PCR and Sanger sequencing to search for cancer-specific somatic mutations in the EGFR, KRAS, and P53 genes in frozen cancer tissues but not in their paired normal tissues. We identified 22 such somatic mutations in 28 early stage NSLC patients from Taiwan. Second, we developed highly-sensitive PAP assays to target the identified mutations. Using these PAP assays, we already confirmed their presence in the tumor tissues at high frequencies. Furthermore, we also found their presence at very low frequencies in many of the paired normal tissues. Finally, we are applying these assays to detect as few as one copy of the cancer-specific mutations in 5 mL of plasma drawn at multiple time points. Our ultimate goal is to detect the relapse ≥6 months earlier than clinical features. The success of this study will open new opportunities for early detection of relapse in other cancers with great advantages: Ability to detect a single copy of cancer-specific mutations in a large quantity of plasma, Over 1000-folds more sensitive than any current PCR-based methods, Not affected by metastatic status because DNA mutations are intrinsic signatures of cancer cells, Non-invasive testing.

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## 1154/W/Poster Board #812

**Profiling critical genetic mutations in clinical tumor samples.** *L. MacConaill[1,2], C. Campbell[1,2], S. Kehoe[1,2], C. Hatton[1,2], L. Niu[1,2], M. Davis[1,2], K. Yao[1,2], M. Hanna[1,2], C. Mondal[1,2], L. Luongo[1,2], A. Bass[2], J. Chan[5], M. Fiorentino[4], K. Polyak[1], M. Kieran[3], K. Ligon[4], C. Stiles[3], W. Hahn[1,2], M. Meyerson[1,2], L. Garraway[1,2].* 1) Center for Cancer Genome Discovery, Dana Farber Cancer Inst, Boston, MA; 2) Department of Medical Oncology, Dana Farber Cancer Inst, Boston, MA; 3) Department of Pediatric Oncology, Dana Farber Cancer Inst, Boston, MA; 4) Center for Molecular and Oncologic Pathology, Dana Farber Cancer Inst, Boston, MA; 5) Departments of Pathology and Laboratory Medicine, Clinical Neurosciences, and Oncology, University of Calgary, Alberta, Canada.

Systematic genetic characterization of clinical tumor specimens is necessary to predict patient outcomes and inform targeted treatment options; however, dependable methods for high-throughput mutation profiling in the clinical arena remain nascent and unproven. We report the implementation of novel mutation detection methodologies to allow accurate, robust and reproducible mass-spectrometric genotyping of a substantial set of known cancer somatic mutations. This platform (the “OncoMap”) interrogates over 500 mutations in 34 known cancer genes- oncogenes and tumor suppressors- many of which are known to predict response or resistance to targeted therapies. We previously reported proof-of-principle using a mass-spectrometric genotyping approach (Thomas et al., 2007)- we now demonstrate the utility of OncoMap in DNA derived from clinical frozen and archival (formalin-fixed, paraffin-embedded; FFPE) material, including patient specimens of gastric, esophageal, endometrial, thyroid, breast, lung and colorectal cancer. We assessed the sensitivity and specificity of our assay panel by comparing KRAS mutant samples (identified using Oncomap) to a “ground-truth” set of Illumina sequencing data on barcoded samples. To highlight the utility of OncoMap on clinical specimens, we also performed mutation profiling on 155 FFPE pediatric low-grade gliomas. We detected many mutations at expected frequencies in common cancers. Importantly, we discovered several rare events in genes not previously described in a cancer type, but for which targeted agents are available or in clinical development. We also demonstrate the utility of OncoMap in identifying common oncogene events in a rare subtype of pediatric cancers- these mutations have immediate clinical impact. Our results demonstrate the clinical feasibility of high-throughput, sensitive and cost-effective cancer mutation profiling that queries a large panel of “actionable” somatic events. OncoMap can be used to identify rare mutations in common cancer types, as well as profiling rare cancers for oncogenic and potentially actionable events.

## 1155/W/Poster Board #813

**Frequency of the fifteen most common SNPs of the RB1 gene in Mexican pediatric patients affected with Retinoblastoma tumor.** *M. Macias-Vega[1], J. Juárez[2], J. Ordaz[2], M. Chávez[3], N. Carranza[4], C. Leal[5], P. Gariglio[6], R. Ocadiz[6], O. Pérez[1].* 1) Lab. Oncología Experimental, Instituto Nacional de Pediatría, Mexico City, Mexico City, Mexico; 2) Departamento de Oftalmología, Instituto Nacional de Pediatría.; 3) Laboratorio de Histomorfología, Instituto Nacional de Pediatría; 4) Laboratorio Clínico, Instituto Nacional de Pediatría; 5) Departamento de Oncología, Instituto Nacional de Pediatría; 6) Departamento de Patología molecular, CINVESTAV.

The single nucleotide polymorphisms (SNPs) of the RB-1 gene have been classified into two groups, low frequency (£ 5%); and high frequency (³ 5%). The combination of 15 of them results in the formation of many different haplotypes, some of them have been considered like risk factors for the hereditary tumor, others like prognostic factor and others associated with the variability to the response to treatment. The aim of this study was to screen the frequency of the 15 most common alleles of the RB1 gene in a case-control study including 73 pediatric patients affected with bilateral and unilateral retinoblastoma and were compared with a group of control cases. All samples were genotyped for the 15 SNPs using the detection system Taqman with forward and reverse primers and FAM and VIC labelled probes. Results show that the common allele is homozygote for the 15 markers into the patients group and only in 12 of control cases, although this difference is not high, is important to note that in five markers (C-779216, C-3042283, C-779170, C-33789354, C-31604223), the common allele has a frequency of 100% and in the control cases it happened only in one marker (C-33789354) while only in two of them the frequency was nearly to 100% (C-779170 85%, C-31604223 95%). Hence exist the possibility that those markers could be non polymorphics. On the other hand we found that in patients group 14 markers showed heterozygosity for common and rare alleles unlike control cases where only 7 markers showed heterozygocity for both. With respect to the rare allele in the patients group we founded allelic homozygocity in 11 markers and in the control cases founded homozygocity only in 6 markers. Is clear that the rare allele occurs with most high frequency in the patients group in heterozygocity and homozygocity conditions so the extensive analysis of the correspondent haplotypes formed by those markers is needed to support the recent statements that the presence of them could modified the development of the retinoblastoma in the non hereditary form, the response to treatment and in the clinical prognostic of the disease . CONACYT-71020, INP-07/040.

## 1156/W/Poster Board #814

**Polymorphisms in RAS in Mexican women with benign breast disease and breast cancer.** *A.P. Mendizabal[1], S.A. Gutierrez[2], X.H. Castro[2,3], L.L. Valdez[5], J.A. Morales[1], J.G. Vazquez[4], J. Sanchez[3], M.C. Moran[3].* 1) Centro Universitario de Ciencias Exactas e Ingenierias, Universidad de Guadalajara, México; 2) Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, México; 3) Centro de Investigacion Biomedica de Occidente, Instituto Mexicano del Seguro Social, México; 4) Centro Medico Nacional de Occidente. Instituto Mexicano del Seguro Social, México; 5) Facultad de Ciencias Quimicas, Universidad de Colima, México.

Recent information has revealed new roles in the angiogenic processes linked to the rennin-angiotensin system (RAS) pathways. To date few studies have been done on the association between RAS genes and cancer and the majority focus mainly on angiotensin I-converting enzyme (ACE). For breast cancer there are three reports that include the angiotensin II receptor, subtype 1 (AGTR1), only one for angiotensinogen (AGT) and none for renin gene (REN). In the present study we investigate whether polymorphisms on RAS genes besides ACE are associated with breast cancer. For this purpose we compared three groups: breast cancer, general population and a benign breast disease group (BBD). The polymorphisms studied by RFLP-PCR were: REN (BglI), AGT (M235T), ACE (A245T), and AGTR1 (A1166C). Statistical data concerning REN polymorphism, shows that Breast cancer group had different allelic and genotypic distributions from BBD (p=0.036 and p=0.048 respectively) but similar to general population. With a high frequency of individuals carrying A allele BBD group was also different from general population (genotypic and allelic p=0.001). Analysis from a recessive model shows that homozygous for A allele have an increased risk for BBD (OR 4.14). M235T (AGT) and A1166C (AGTR1) were statistically different only in comparisons between general population and breast cancer groups. Differences in M235T genotype frequencies were significant (p=0.048) with less heterozygous in breast cancer group. Relevant allelic A1166C differences were observed where breast cancer group showed an increase of allele A (p=0.036). C allele carriers present a reduced risk of breast cancer (OR 0.53, p=0.0356). A240T polymorphism (ACE) did not show any statistical difference. AGT and AGRT1 polymorphism distributions between breast cancer and BBD groups were similar, and when compared each of both groups with the general population, different associations where shown. This results could be explained if we consider that the benign diseased breast, although not malignant it has developed different morphology from normal tissue and therefore, genotypic differences should be expected. Results of this work agree with previous observations on the possible involvement of this system in breast cancer but it also suggest its participation in other stages and pathologies like benign disease.

## 1157/W/Poster Board #815

**Proteomics discovery of salivary biomarkers for lung adenocarcinoma.** *G.E. Mercado[1], L. Castellanos[1], K.G. Calderon[1], D. Saavedra[2], G. Ponciano[3], O.G. Arrieta[2], J.P. Reyes[1], J. Gallegos[1], G. Jimenez-Sanchez[1].* 1) National Institute of Genomic Medicine (INMEGEN), Mexico City, Mexico; 2) National Cancer Institute (INCan), Mexico City, Mexico; 3) Smoking Cessation Clinic, School of Medicine, UNAM, Mexico City, Mexico.

Lung cancer is the major cause of cancer-related deaths and has a poor prognosis with a 5-yr overall survival of <10%, mostly due to lack of early stage diagnosis. Cigarette smoking is estimated to be responsible for >80% of the cases. Previous studies have demonstrated that former smokers have a higher risk of lung cancer compared to nonsmokers. Early detection of lung cancer would improve the overall survival of this disease. However, there is no validated screening test for lung cancer. To develop an early detection test, we selected saliva as the biofluid of choice due to its accessibility and protein content that provides diagnostic information on a variety of diseases, including cancer. To identify differentially expressed proteins in saliva from individuals with lung adenocarcinoma, whole saliva samples were collected from 6 patients with lung adenocarcinoma and 6 matched healthy controls (male individuals, >40 years, smokers >10 pack year). Proteins and peptides from whole saliva samples were analyzed by two-dimensional gel electrophoresis identifying 20 differentially expressed protein spots. Mass spectrometry analysis identified 63 peptides and revealed fourteen non-redundant proteins in saliva obtained from lung adenocarcinoma patients. Four of these proteins have been previously reported as serum biomarkers of cancer, and two of them were associated with non-small cell lung cancer. These potential biomarkers are being tested in an independent and larger cohort for validation. Patient-based saliva proteomics is a promising approach to searching for cancer biomarkers. Further characterization of these markers may provide the basis for new, noninvasive tests for screening, detection, and monitoring of high-risk individuals.

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## 1158/W/Poster Board #816

**ARG194TRP AND ARG399GLN POLYMORPHISMS OF XRCC1 GENE IN MEXICAN PATIENTS WITH COLORECTAL CANCER.** *R. Muñiz[1], J. Peregrina-Sandoval[2,3], M. Partida-Pérez[1], M.L. Ayala-Madrigal[1], N.M. Macías-Gómez[4], E. Leal-Ugarte[5], V. Peralta-Leal[5], J.P. Meza-Espinoza[5], S. Cervantes-Ortiz[6], M. Centeno-Flores[6], V. Maciel-Gutiérrez[6], M. Gutiérrez-Angulo[1,7].* 1) Instituto de Genética Humana , Universidad de Guadalajara, Guadalajara, Jalisco, Mexico; 2) CUCBA, UdeG; 3) Hospital Civil Fray Antonio Alcalde; 4) CUSUR, UdeG; 5) Universidad Autónoma de Tamaulipas; 6) Hospital Civil Juan I. Menchaca; 7) CUALTOS, UdeG.

The XRCC1 gene is mapped in 19q13.2-13.3 chromosome and this encoded a 70 KDa protein. This protein is involved in the repair of the single strand breaking which are originated by ionizing radiation or oxygen reactive species and alkylating agents. The polymorphism rs1799782 is localized in exon 6, replace al aminoacid Arg for Trp in position 194 and its effect on the protein is unknown. The polymorphism rs25487 localized in exon 10, it change the aminoacid Arg for Gln in the position 399 of the protein. The functional analysis of this polymorphism has demonstrated that influences the DNA repair. In this study we analyzed the polymorphisms Arg194Trp and Arg399Gln in patients with Colorectal Cancer (CRC). The polymorphisms were detected by PCR-RFLP's assay in 39 DNA samples from patients with CRC and 120 samples of healthy individuals. The genotype frequencies for patients for Arg194Trp were 82% Arg/Arg, 18% Arg/Trp and 0% Trp/Trp and the frequencies for Arg399Gln were 54% Arg/Arg, 36% Arg/Gln and 10% Gln/Gln. The comparative analysis of the allele and genotype frequencies between patients with CRC and healthy group did not show significant differences ($p>0.05$). However, the comparison among genotype, gender and CRC showed significant differences ($p<0.02$). The Arg194Trp and Arg399Gln polymorphisms was not associated with CRC in Mexican patients.

## 1159/W/Poster Board #817

**NBS1 gene mutations as a cancer risk factor.** *J. Nowak[1], M. Mosor[1], I. Ziolkowska[1], D. Januszkiewicz[1,2].* 1) Inst Human Gen, Polish Academy Sci, Poznan, Poland; 2) University of Medical Sciences, Poznan, Poland.

MRE11, RAD50 and NBS1 (MRN) complex is involved in DNA repair and cell cycle checking signaling. NBS1 being a part of MRN complex plays an important role in genome stabilization. Molecular variants of NBS1 gene may therefore constitue a cancer risk factor. Homozygous mutation 657del5 of the NBS1 gene is responsible for the majority of Nijmegen breakage syndrome. Several studies have focused on searching for an association between NBS1 gene mutations and cancer incidence. Heterozygous carriers of the NBS1 657del5 mutation have been shown to have an increased risk for breast cancer, melanoma, colon and rectum cancer. Other studies have found no association between NBS1 gene mutations Hodgkins's or non-Hodgkin's lymphomas. The aim of the study was to analyze the frequency of a panel mutations of NBS1 gene by screening all 16 exons of this gene along with polymorphisms examination. DNA was isolated from peripheral blood of 135 children with acute lymphoblastic leukemia, 270 women with breast cancer, 176 patients with larynx cancer, 93 with second primary tumors of head and neck, 131 with colorectal carcinoma and 1274 healthy individuals. I171V mutation of NBS1 gene was the most frequent and has been found in 23 patients compared to only 8 in healthy individuals. Other mutations of the NBS1 gene have been observed in lower frequencies.Genotyping data from the six polymorphic loci in NBS1 gene, were used to impute haplotypes. Two of the evaluated haplotypes were associated with significantly increased leukaemia risk ($P=0.0038$ and $P<0.0001$). Our results suggest that some specific haplotypes of the NBS1 gene may be associated with malignancies. Since DNA was a isolated from non-malignant cells, all mutations found in cancer patients appeared to be of germinal origin. It can be concluded that I171V mutation of NBS1 gene is associated with predisposition to malignances and NBS1 allele I171V may be a general cancer susceptibility gene of low or middle risk.

## 1160/W/Poster Board #818

**MicroRNA expression and its usefulness in diagnosis, classification and prediction of patient outcome in colorectal cancer.** *C. Nyiraneza[1], C. Sempoux[2], A. Kartheuser[3], R. Detry[3], K. Dahan[1].* 1) Human Gen, Cliniques Univ St Luc/UCL, Brussels, Belgium; 2) Department of Pathology, Cliniques Univ St Luc/UCL, Brussels, Belgium; 3) Department of Colorectal Surgery, Cliniques Univ St Luc/UCL, Brussels, Belgium.

MicroRNAs (miRNAs) are small non-coding RNAs of 19-24 in length that have been shown to regulate gene expression during crucial cell processes such as apoptosis, differentiation and development. Findings over the past five years have shown a significant number of differentially expressed miRNAs in normal and tumor tissues from cancer patients. The aim of this project is to evaluate the relevance of microRNA profiling in diagnosis, classification and prediction of patient outcome in colorectal cancer (CRC). In our preliminary investigations we examined the expression profile of oncogenic miRNAs such as miR21, miR106a, miR106b, and the tumor suppressor miRNAs miR34 in tumor specimens and matched colon mucosa from CRC patients with complete data on DNA mismatch repair system (MMR) deficiency or sufficiency. We correlated these data with clinicopathological features, and with the immunohistochemical expression profiles of p53 protein and its effectors/ or partners such as MDM2, p21/WAF1 and LATS2 protein. Our findings support a causal role for altered miRNAs expression in colorectal carcinogenesis. Focus on the relevance of miRNA expression profiling in diagnosis, classification and prediction of outcome of colorectal cancer patients warrants further investigations.

## 1161/W/Poster Board #819

**Role of metabolic enzyme gene variants and non-genetic factors in breast cancer risk among Filipinos.** *C. Padilla[1], E. Cutiongco[1], C. Ngelangel[2], C. Silao[1], R. Cortez[1], F. Rocamora[1], O. Bisquera[3], E. Montana[4], J. Asprer[4], R. Joson[5], C. Cajucom[6] for The Philippine Cancer Genetics Study Group.* 1) Institute of Human Genetics, National Institutes of Health, University of the Philippines, Manila, Philippines; 2) Institute of Clinical Epidemiology, University of the Philippines, Manila, Philippines; 3) Department of Surgery, Philippine General Hospital, Manila, Philippines; 4) Department of Surgery, Jose Reyes Memorial Medical Center, Manila, Philippines; 5) Department of Surgery, Ospital ng Maynila, Manila, Philippines; 6) Department of Surgery, Medical Center Manila, Philippines.

Breast cancer is the most common cancer in women worldwide. In the Philippines, it ranks as the 2nd most common cancer among both sexes, and first among women, with incidence rates the highest recorded in any Asian population. Like many other cancers, breast cancer has been associated with both genetic and non-genetic factors. Genetic factors found to be associated with breast cancer risk include polymorphisms the in metabolic genes encoding glutathione-S-tranferases and N-acetyltransferases; while a number of reproductive and environmental variables such as contraceptive use, onset of menarche and menopause have also been shown to be related to the disease. To explore the molecular epidemiology of breast cancer among Filipinos, a total of 668 Filipino female patients, 297 with cancer and 371 cancer-free were studied. Corresponding demographics, medical histories, reproductive histories, social histories, diet histories, and occupational exposure data were collected for all subjects. Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) were performed to genotype for polymorphisms in GSTM1 (deletion), GSTT1 (deletion), GSTP1 (c.313A>G) and NAT2 (g.481C>T, g.590G>A, g.803A>G, g.857G>A). After age- and sex- matched multivariate analysis for epidemiological factors alone, the following were found to be associated with risk: increasing age at 1st pregnancy, family history of breast cancer, and intake of scalding hot food. Non-genetic factors found to be protective against breast cancer included patis (fermented fish sauce) consumption and inverted smoking. The only genetic polymorphism shown by univariate logistic regression to be independently associated for breast cancer risk was the GSTT1 null genotype for breast cancer. After multivariate analysis for both environmental and genetic variables, the GSTT1 null genotype was not found to be significantly associated with breast cancer and only family history of breast cancer in a 1st degree relative significantly increased risk.

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## 1162/W/Poster Board #820

**ADIPOQ 45T/G polymorphism in Mexican patientes with Colorectal Cancer.** *M. Partida[1], M.L. Ayala-Madrigal[1], J. Peregrina-Sandoval[2,5], N.M. Macías-Gómez[3], J.M. Moreno-Ortiz[1], A.S. Suárez[1], M.A. Cárdenas[4], E. Cabrales[5], F. Cerda[5], M. Gutiérrez-Angulo[1,6].* 1) Instituto de Genética Humana, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico; 2) Laboratorio de Inmunobiología, CUCBA, UdeG; 3) CUSUR, UdeG; 4) Hospital Civil Juan I. Menchaca; 5) Hospital Civil Fray Antonio Alcalde; 6) CUALTOS, UdeG.

Epidemiology studies showed an association between obesity and colorectal cancer and they have been suggested that hormones derived from adipose tissue can significantly influence on the growth and proliferation of tumors, in fact, some researchers reported an association between the levels of adiponectin and the increase in the risk of colorectal cancer. The polymorphism +45T/G is localized in exon 2, is a synonymous mutation and it has been suggested that is disequilibrium linkage with another polymorphism. This variant has been associated with breast, endometrium, stomach, lung and colorectal cancer (CRC). In this study we analyzed the polymorphism +45T/G in patients with CRC and healthy individuals. We genotyped 45 patients with CRC and 111 healthy individuals. The analysis of the polymorphism were done by PCR-RFLP's assay. The genotype frequencies for patients were 76% TT, 24% TG and 0% GG, and the frequencies for healthy individuals were 67% TT, 30% TG and 3% GG. The allele and genotype frequencies were in Hardy-Weinberg equilibrium. The association analysis did not show any significant differences, however, the allele G was less frequent in patients than healthy individuals (18% and 12%, respectively). The polymorphism +45T/C was not associated with CRC risk in Mexican patients.

## 1163/W/Poster Board #821

**TGFBR1 haplotypes and risk of non-small cell lung cancer.** *B. Pasche[1], Z. Lei[2], R. Liu[2], J. Zhao[2,3], Z. Liu[2], X. Jiang[2], W. You[4], X. Chen[2,5], X. Liu[2], K. Zhang[6], H. Zhang[2].* 1) Div Hematology/Oncol, Univ Alabama, UAB Comprehensive Cancer Center, Birmingham, AL; 2) Laboratory of Medical Genetics, School of Basic Medicine & Biological Sciences, Medical College of Soochow University, Suzhou, P. R. China; 3) Department of Surgery, The First Affiliated Hospital, Medical College of Soochow University, Suzhou, P. R. China; 4) Division of Clinical Medicine, Wuxi Third People's Hospital, Wuxi, P. R. China; 5) Department of Surgery, Shanghai Hospital for Pulmonary Diseases, Shanghai, P. R. China; 6) Department of Biostatistics, School of Public Health, The University of Alabama at Birmingham, Birmingham, AL.

Transforming growth factor beta (TGF-β) receptors are centrally involved in TGF-β-mediated cell growth and differentiation and are frequently inactivated in non-small cell lung cancer (NSCLC). Constitutively decreased type I TGF-β receptor (TGFBR1) expression is emerging as a novel tumor-predisposing phenotype. The association of TGFBR1 haplotypes with risk for NSCLC has not yet been studied. We tested the hypothesis that single nucleotide polymorphisms (SNPs) and/or TGFBR1 haplotypes are associated with risk of NSCLC. We genotyped six TGFBR1 haplotype tagging SNPs (htSNPs) by PCR-restriction fragment length polymorphism (PCR-RFLP) assays and one htSNP by PCR-single strand conformation polymorphism (PCR-SSCP) assay in two case-control studies. Case-control study 1 included 102 NSCLC patients and 104 healthy controls from Suzhou. Case-control study 2 included 131 patients with NSCLC and 133 healthy controls from Wuxi. Individuals included in both case-control studies were Han Chinese. Haplotypes were reconstructed according to the genotyping data and linkage disequilibrium (LD) status of these seven htSNPs. None of the htSNP was associated with NSCLC risk in either study. However, a four-marker haplotype CTGC was significantly more common among controls than among cases in both studies (P=0.014 and P=0.010, respectively) indicating that this haplotype is associated with decreased NSCLC risk (adjusted OR, 0.09; 95% CI, 0.01-0.61 and adjusted OR, 0.11; 95% CI, 0.02-0.59, respectively). Combined analysis of both studies shows a strong association of this four-marker haplotype with decreased NSCLC risk (adjusted OR, 0.11; 95% CI, 0.03-0.39). This is the first evidence of an association between a TGFBR1 haplotype and risk for NSCLC.

## 1164/W/Poster Board #822

**Prognostic Importance of Single Nucleotide Polymorphisms in IL-6, IL-10, IFN-γ, TGF-β1 and TNF-α Genes in Chronic Myeloid Leukemia (Ph+).** *M. Pehlivan[1], S. Pehlivan[2], K. Ozdilli[3], F.O. Savran[4], L. Kaynar[5], T. Sever[2], M. Yilmaz[1], B. Eser[5], Y. Duvarci-Ogret[4], C. Kis[1], V. Okan[1], M. Cetin[5], M. Carin[4].* 1) Gaziantep University, Medical Faculty, Hematology, Gaziantep, Turkey, MD; 2) Gaziantep University, Medical Faculty, Medical Biology and Genetics, Gaziantep, Turkey, PhD; 3) Halic University, Institute of Health Sciences, Istanbul, Turkey, MD; 4) Istanbul University, Medical Faculty, Medical Biology and Genetics, Istanbul, Turkey, PhD; 5) Erciyes University, Medical Faculty, Hematology, Kayseri, Turkey, MD.

The aim of this study was to explore the association between polymorphisms of five cytokines and clinical parameters in patients with Philadelphia positive (Ph+)-chronic myeloid leukemia (CML). We analyzed five cytokines ( IL-6, IL-10, IFN-γ, TGF-β1, TNFα ) in 60 cases with CML (Ph+) and 74 healthy controls. Cytokine genotyping was performed by the polymerase chain reaction sequence-specific primer method. Univariate and multivariate analyses were performed to test for correlations with clinical outcomes. All data were analyzed using SPSS version 14.0 for Windows. The median follow up time was 49.3 months (range 6.1-168.4) and the median duration of imatinib was 39.5 months (range 5.2-103.4) for these patients. No significant differences were detected between CML group and healthy controls in the distributions and numbers of genotypes and alleles in TNF-α, TGF-β1, IL-10 and IFN-γ genotypes whereas GG allele in IL-6 was found to be significantly high in CML but GC allele was found to be low (p=0.010, 0.002, respectively). The frequency of a high IL-6 (-174) producing GG genotype was significantly more common in the patients compared to the controls (p=0.010). Analyzing associations with event-free survival time, we found the GG genotypes of IL6-174 to be related to a lower EFS [AA-AG versus GG Cox proportional hazard ratio 0.140 (p=0.019, [95% CI: 0.027-0.726]). While the polymorphism in IL6 gene was observed to diverge from the Hardy-Weinberg Equilibrium (HWE), it was observed that the polymorphisms of other cytokine genes did not diverge from HWE. Conclusions: The relationship between cytokine genotyping and clinical parameters in CML (Ph+) was investigated for the first time in this study. Our results suggest that IL6 and IL-10 may be useful markers for CML prognosis. These results need to be replicated in larger series and examined in CML.

## 1165/W/Poster Board #823

**Analysis of the Molecular Factors Controlling Human Osteosarcoma Response to Chemotherapy.** *K. Sol-Church, D.L. Stabley, S.M. McCahan, D. Kamara, A. Kolb.* Nemours Biomedical Research, Alfred I duPont Hospital for Children, Wilmington, DE.

Osteosarcoma (OS) is the most common primary malignant bone tumor in children and young adults. Despite the progress made in adjuvant chemotherapy, the overall survival rate remains around 60 percent. New promising therapeutics such as R1507, a fully humanized monoclonal anti-IGF1R antibody available in clinical trials, is effective in inhibiting the growth of a subset of human osteosarcoma tumors engrafted into mice. However, while some of these xenograft tumors respond to therapy, others show little to no growth inhibition. We hypothesized that changes observed in the gene expression and microRNA complement (miRNAome) of these tumors may help elucidate the mechanism of resistance or salvage in OS and identify biomarkers of response that will provide new avenues for the development of prognostic and therapeutic tools. During the present study, we have characterized at the molecular level a panel of six osteosarcoma xenograft tumors that respond differently to R1507 therapy. Our preliminary data suggests that inhibition of IGF signaling induces expression of EGFR, which may maintains tumor growth. Using expression analysis tools, we have identified additional changes in R1507-treated vs. untreated tumors that have potential as prognostic markers of response. In addition, using the hypothesis-free strategy afforded by the SOLiD System Next Generation Sequencing, we have determined the tumors' miRNAome and identified changes induced by R1507 treatment. The combined results from this study will reveal important aspects of tumor biology and identify molecular targets that may be exploited for the development of new therapeutics for OS.

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## 1166/W/Poster Board #824

**Human papillomavirus DNA in plasma of patients with HPV16 DNA-positive uterine cervical cancer.** *S. Takako[1], Y. Naohiro[2], Y. Kentaro[1], M. Kiyonori[1], M. Hideaki[1].* 1) Obstetrics and Gynecology, Nagasaki University Graduate School , Nagasaki, Japan; 2) Molecular Microbiology and Immunology, Nagasaki University Graduate School, Nagasaki, Japan.

Introduction : The squamous cell carcinoma antigen (SCCA) is considered the most accurate serologic tumor marker for uterine cervical carcinoma. However, serum SCCA levels were found to correlate significantly with clinical severity of atopic dermatitis and chronic renal failure. The present study was conducted in patients with human papillomavirus (HPV)16 DNA-positive uterine cervical cancer to determine the plasma level of HPV16 DNA and the diagnostic values of plasma HPV DNA in these patients. Materials and methods : Thirty-six HPV16-positive patients with cervical intraepithelial neoplasia (CIN) or uterine cervical SCC were recruited in this study. The diagnosis was cervical cancer in 11 patients, high-grade squamous intraepithelial lesions (HSIL) in 21, low-grade squamous intraepithelial lesions (LSIL) in 1 and negative for intraepithelial lesion or malignancy (NILM) in 3 patients. Before any treatment, blood samples were collected from all patients. For analysis of HPV DNA in plasma of patients with cervical cancer, qPCR fluorescent assay for HPV16 was performed using HPV16 primers and SYBR Green dye using the LightCycler 480 SW1.5 apparatus. Results : Plasma HPV16 DNA was detected in only 27.3% of patients with HPV16-positive cervical cancer and in none of normal controls. The copy number of plasma HPV16 DNA was significantly higher in patients with invasive cancer than in those with cervical intraepithelial neoplasia (CIN3), microinvasive cancer and in normal individuals. Summary : These results indicated that plasma HPV DNA level could be potentially used as a marker of low-invasive cervical cancer tumors in patients with normal SCCA levels before treatment.

## 1167/W/Poster Board #825

**de novo methylation in DLBCL frequently occurs proximal to genes that are poorly expressed in normal progenitor cells.** *X. Wang[1], T. Greiner[2], B.L. Pike[1], D.J. Weisenberger[3], K.D. Siegmund[4], U. Sinha[5], M. Muschen[6], P.W. Laird[3], J.B. Fan[7], J.G. Hacia[1].* 1) Department of Biochemistry and Molecular Biology, University of Southern California Los Angeles, CA, USA; 2) Departments of Pathology and Microbiology University of Nebraska Medical Center Omaha, NE, USA; 3) Department of Surgery University of Southern California Los Angeles, CA, USA; 4) Department of Preventive Medicine University of Southern California Los Angeles, CA, USA; 5) Department of Otolaryngology, Head and Neck Surgery University of Southern California Los Angeles, CA, USA; 6) Leukemia Research Program Children's Hospital Los Angeles Los Angeles, CA, USA; 7) Illumina, Inc. San Diego, CA, USA.

Epigenetic profiling studies have identified methylated loci in diffuse large B-cell lymphoma (DLBCL). However, it is unclear what proportion represents de novo methylation acquired during tumorigenesis in contrast to methylation present in normal precursor B-cells. We used BeadArray assays to identify methylated loci in activated B-cell like (ABC-DLBCL), germinal center B-cell-like (GCB-DLBCL), and normal precursor B-cells. We identified 81 candidate loci predicted to show de novo methylation in >20% of GCB-DLBCL cases. All candidate loci were proximal to genes that are poorly expressed or silent in normal germinal center B-cells. Our results are consistent with the hypothesis that DNA methylation is more frequently involved in the maintenance rather than the initiation of gene silencing in cancer. We propose that similar to de novo genetic changes found in tumors, the majority of de novo methylation events we observed are neutral and not causally involved in tumoriogenesis. However, this would not affect their potential value as clinical biomarkers. A major challenge remains to identify the subset of de novo epigenetic changes that are functionally significant in DLBCL.

## 1168/W/Poster Board #826

**Digital Counting of Gene Copy Number in Clinical Settings.** *S. Ishikawa[1,2], T. Sakatani[2], M. Fukayama[2], H. Aburatani[1].* 1) Genome Science Division, Univ. of Tokyo, Tokyo, Japan; 2) Department of Pathology, Graduate School of Medicine, Univ of Tokyo, Tokyo, Japan.

Detection gene copy number is important in several clinical settings. Technology for sensitive and robust detection of copy number aberration is especially indispensable in the situation where the abnormal fraction is lower with more normal component. High-density oligonucleotide microarray and qPCR may be routinely used for detection of copy number aberration. However, these techniques are not reliable enough to detect copy number change with high normal background. To address these issues, we introduced two methods for "digital" copy number count, (1) Illumina Genome Analyzer and (2) Fluidigm Nanofluidic Chip for genome-wide and targeted approach. (1) To assess copy number by counting frequency of short read within defined window, we developed algorithm to modify raw data using genome-wide hit number of these short reads and other experimental conditions. Digital count of short read generated by Illumina Genome Analyzer successfully got HCT116 cell copy number comparable with but more reliable than high density oligonucleotide microarray. (2) We did serial dilutions of genome sample mixed with TaqMan® reagents and finely partitioned each dilution within 48 separate, on-chip 770 grids of reaction chambers. As signal is produced only in chambers containing copies of the target sequence, the positives in each chamber grid are digital-counted and evaluated for statistical significance. The presentation will includes the detection of CNV and cancer gene amplification and its comparative experiments using high density oligonucleotide microarray. Nanofluidic digital array technology can reliably quantitate an absolute copy number with high normal background.

## 1169/W/Poster Board #827

**Genetic variants in the candidate genes of the apoptosis pathway and susceptibility to chronic myeloid leukemia.** *D. Kim[1, 2], W. Xu[3], C. Ma[3], X. Liu[4], K. Siminovitch[4], H.A. Messner[2], J.H. Lipton[2].* 1) Hematology/Oncology, Samsung Medical Center, Seoul, Korea; 2) Chronic Myelogenous Leukemia Group, Department of Hematology/Medical Oncology, Princess Margaret Hospital, University Health Network, University of Toronto,Toronto, Ontario, Canada; 3) Department of Biostatistics, Princess Margaret Hospital, University Health Network, University of Toronto,Toronto, Ontario, Canada; 4) Analytical Genetics Technology Centre, University Health Network, Toronto, ON, Canada.

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder, characterized by the presence of BCR/ABL fusion gene. It is unclear which cellular events drive BCR/ABL gene translocation or initiate leukemogenesis in CML. Previous observations showed that intriguing detection of BCR/ABL fusion gene at a very low level in the blood of normal healthy individuals, suggesting that only a minor fraction of spontaneous Ph translocations progress to CML, or that BCR/ABL fusion gene is essential but not sufficient to lead to the leukemogenesis without additional second cellular or molecular events. Also, Bcl-2 promotes survival of hematopoietic stem cells and more frequent expression of bcl-2 was known to be associated with progression of CML from chronic phase to advanced stage. Accordingly, apoptosis-related pathway may involve in the leukemogenesis of CML. In the present study, we evaluated 80 SNP markers involved in the 6 major candidate pathways that have been proposed to provoke leukemogenesis in CML such as pathways of apoptosis (n=30), angiogenesis (n=7), myeloid cell growth (n=14), xenobiotic metabolism (n=13), WT1 signaling (n=7), interferon signaling (n=4) and others (n=5) in 170 CML patients and 182 healthy controls. Sequenom MassARRAY platform was used for genotyping and Haploview version 3.32 and WHAP version 2.09 were used for statistical analysis. In a single marker analysis, following SNPs were identified to be associated with increasing risk of CML including *VEGFA*, *BCL2*, *CASP7*, *JAK3*, *CSF3* and *HOCT1*. In the multivariate logistic model with these SNPs and covariates, only *BCL2* (rs1801018) was significantly associated with the susceptibility to CML (p=0.05; adjusted-OR 2.16, [1.01-4.68]). In haplotype analyses, haplotype block of *BCL2* consistently showed significant association with increasing susceptibility to CML. Risk allele analysis showed that a greater number of risk alleles from *BCL2* SNP correlated to increasing risk of CML (overall p=0.1, OR 1.84, [1.06-3.22] for 3-4 risk alleles vs. 0-1 risk alleles). In summary, the current study indicated that BCL2 SNP seemed to be associated with increasing susceptibility to CML.

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## 1170/W/Poster Board #828

**Cumulative Genetic Risk Predicts Platinum/Taxane-Induced Neurotoxicity.** *S. McWhinney-Glass[1, 5], S.J. Winham[2,5], J.Y. Revollo[1,5], J. Paul[3], R. Brown[4], A. Motsinger-Reif[2,5], H.L. McLeod[1,5].* 1) Schools of Pharmacy and Medicine, University of North Carolina, Chapel Hill, NC; 2) Bioinformatics Research Center and Department of Statistics, North Carolina State University, Raleigh, NC; 3) Cancer Research UK Clinical Trials Unit, University of Glasgow, Glasgow; 4) Department of Oncology, Imperial College London, London; 5) Institute for Pharmacogenomics and Individualized Therapy, University of North Carolina, Chapel Hill, NC.

Platinum/taxane-based chemotherapy is the standard of care for many cancers, including lung and ovarian. However, the utility of these chemotherapeutic drugs is often limited due to severe neurotoxicity. Previous studies have identified potential clinically-relevant genetic factors associated with chemotherapy-induced neurotoxicity, but the value of these variants is limited by a lack of replication. Additionally, interventions such as the administration of neuroprotectant agents to minimize or circumvent the neurotoxic effects require further studies to determine if there is a significant reduction of symptoms. For these reasons, we sought to identify genetic risk factors for chemotherapy-induced neurotoxicity. We conducted a candidate-gene association study to evaluate 1569 SNPs in 58 candidate genes in 914 ovarian cancer patients from the SCOTROC1 ovarian cancer Phase III trial treated with platinum-based chemotherapy. For analysis, the dataset was randomly split into test/validation sets, each set composed of 50% of the controls (grades 0/1 neurotoxicity) and 50% of the cases (grades 2/3/4 neurotoxicity). Significant associations with four SNPs in *SOX10, BCL2, OPRM1*, and *TRPV1* that confer a significant risk for chemotherapy-induced neurotoxicity were identified and replicated in the validation sample. Permutation testing was used to correct for multiple testing and variable selection concerns. Individuals with 0 risk genotypes have a reduction in incidence of neurotoxicity of 84.9% compared to individuals with all 4 risk genotypes. According to our multiplicative model, the odds of developing neurotoxicity increase by a factor of 2.34 for every increase of risk genotypes possessed (corrected p-value=0.007). The strength of this model indicates the strength of an additive trend in risk genotypes. None of the 4 significant SNPs were associated with progression-free survival or overall survival. This decouples the theoretical link between neurotoxicity and tumor control. Indeed, this data indicates that intervention or prevention strategies for platinum/taxane neurotoxicity based on these four genetic variants are not likely to adversely influence the efficacy of the chemotherapeutic agent. A genetic test for the risk genotypes in *SOX10, BCL2, OPRM1,* and *TRPV1* may serve as a valuable prediction tool for clinicians to approximate the level of risk for neurotoxicity for each individual based on the number of risk genotypes that they harbor.

## 1171/W/Poster Board #829

**Glutathione S-transferase P1 genetic polymorphism: A potential risk factor for the development of childhood ALL in Poland.** *J.J. Pietrzyk, A. Madetko-Talowska, M. Bik-Multanowski.* Chair of Pediatrics, Jagiellonian University, Krakow, Poland.

The presence of some metabolically important genetic polymorphisms may contribute to the risk of acute lymphoblastic leukemia (ALL), which is the most common pediatric malignancy. Glutathione S-transferases are enzymes involved in detoxication of xenobiotics, including environmental carcinogens. The aim of this study was to evaluate the role of GSTP1*B (c.313a>g) polymorphism of glutathione S-transferase P1 gene which results in reduced activity of glutathione S-transferase P1, as a risk factor for ALL in Polish population. Methods The study population consisted of 1000 healthy newborns representative for Polish population. DNA samples were isolated from dried blood spots (remainder parts of newborn screening cards). Genotyping for the detection of the GSTP1*B polymorphism was performed using PCR-RFLP method. Population allelic frequency of the polymorphism was compared with the one observed in a group of 400 children with ALL (data published in Pediatr Blood Cancer 2009;3:364-368). Fisher's exact test and odds ratio with 95% confidence interval were used for data analysis. Results We found that GSTP1*B (313G) allele was significantly more frequent among the individuals with ALL in comparison to the general population (OR:1,54; 95% CI 1,30-1,83; p-value: 0,0001). Details on genotyping are shown in the table.

| | Genotypes/Cases: c.313aa/c.313ag/ c.313g | Allelic frequency (c.313g) |
|---|---|---|
| ALL | 140 / 219 / 41 | 0.37 |
| Controls | 510 / 417 / 73 | 0.28 |

Our results suggest that GSTP1*B genetic variant is associated with increased risk of ALL. Further studies are needed to confirm the above findings in other populations. Study was sponsored by government research grants NN407232934 and PBZ-KBN-090/P05/06/2003.

## 1172/W/Poster Board #830

**Direct detection of rare circulating tumor cells in blood by competitive allele-specific TaqMan-based PCR (castPCR).** *C. Chen, R. Tan, J. Chan.* Genomic Assays R & D, Life Technologies, Foster City, CA.

Circulating tumor cells in blood from metastatic breast and lung cancer patients have been reported as a surrogate marker for tumor response and shorter survival. Detection of these rare tumor cells in blood is the key to early cancer diagnosis. Here we report a new competitive allele-specific TaqMan-based PCR (castPCR) method for rare mutation detection. castPCR combines allele-specific TaqMan qPCR with allele-specific MGB blockers to suppress amplification of the wild type allele, resulting in better specificity. We have successfully designed and validated castPCR assays for 48 cancer-related SNPs of Ras, EGFR, Kit, pTEN, and p53. Results demonstrate that castPCR not only maintains the wide dynamic range, high sensitivity, and reproducibility but also improve the specificity of allelic TaqMan assays for up to 1 in 1,000,000. Results of detecting circulating tumor cells in cancer patient samples will be presented.

## 1173/W/Poster Board #831

**Yields of viral and circulating nucleic acids using the new large volume QIAamp Circulating Nucleic Acid Kit.** *M. Horlitz, T. Hartinger, A. Lucas, J. Schaper, M. Sprenger-Haussels.* QIAGEN GmbH, Hilden, Germany.

Introduction: Fragmented DNA and mRNA originating from tumors or infections circulate as cell-free nucleic acids in plasma, serum and other cell-free body fluids. Access to these molecules for analysis could allow for specific detection of certain disease states based on a simple blood sample. In this study the extraction efficiency of a new large volume nucleic acid extraction kit for free-circulating DNA & RNA, miRNA, and nucleic acids from viral particles, was compared to established extraction methods. Materials/ Methods: Endogenous circulating nucleic acids were extracted from pooled plasma using the QIAamp Circulating Nucleic Acid (CNA) Kit (5 ml sample; N=12). DNA yield was quantified by 18S rDNA-specific duplex qPCR (two separate targets, 66 bp and 500 bp long) ; mRNA yields were measured by qPCR specific for GAPDH, c-fos, β-globin. The QIAamp MinElute Virus Kit (1 ml; N=12) served as the reference protocol. For endogenous miRNA extraction from plasma, an optimized protocol was tested (3 ml; N=12). miRNA yields were quantified by QIAGEN miScript (miR16, miR103) and Applied Biosystems TaqMan (miR16, miR30b) assays. HBV, HCV, HIV-1 particles (all WHO standard material) were added to pooled EDTA plasma in a 6-step, 2-fold dilution series (N=16 per concentration). After nucleic acid extraction using the CNA Kit (5 ml sample, 30 μl elution), viral nucleic acids were detected by qPCR to determine hit rates. Results: For both free-circulating DNA and free-circulating mRNAs, the yield was 6-9-fold higher compared to the MinElute Virus protocol for all genetic targets. The optimized miRNA extraction protocol was found to deliver significantly increased miRNA yields ($\Delta$Ct from 2 to 7) compared to the CNA standard protocol for the 4 miRNA assays used. For viral nucleic acids, 95% detection rates calculated by probit regression analysis were: HBV DNA 0.3 IU/ml, HCV RNA 7.2 IU/ml, HIV-1 RNA 14.2 IU/ml. Conclusions: The results demonstrate that the new extraction protocol can serve as a sample preparation solution for processing up to 5 ml plasma, it allows for small elution volumes, and can extract and concentrate (volume ratio sample/eluate of up to 250:1) circulating cell-free DNA and mRNA, circulating miRNA, and viral nucleic acids for applications in biomarker and viral infection research. The applications presented here are for research use only. Not for use in diagnostic procedures.

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### 1174/W/Poster Board #832

**DIFFERENTIAL ASSOCIATION OF CYTOCHROME P450 3A4 GENOTYPES WITH ONSET OF BREAST TUMORS.** *D. McDaniel[1], T. Thurber[2], C. Berry[2], X. Zhou[3], S. Bigler[3], R. Vance[4], W. Barber[5].* 1) Dept Surgery/Neurology; 2) School of Medicine; 3) Pathology; 4) Dept Medicine/Cancer Center; 5) Dept. Surgery, Univ Mississippi Med Ctr, Jackson, MS.

Background: Growth of >50% of breast tumors are hormone-dependent and breast adipose tissue is a local source for both estrogen (E) and E-receptors. Estrogens are produced through the activity of Cytochrome P450 aromatase. Particularly, P450 3A4 is involved in the metabolism of several drugs including Tamoxifen, used in breast cancer therapy. The goal of our study was to validate the role of CYP 3A4 gene polymorphism and the association with tumor stage, grade and hormonal receptor status including estrogen-receptors, progesterone-receptors and human epidermal growth factor (HER-2/neu) in African American and Caucasian patients with breast cancer. Methods: Patients who had undergone biopsy procedures for diagnosis or for partial or radical mastectomy were recruited in this study. Genotypes were detected using SNP analysis. The mRNA transcripts were screened by a RT-PCR. Clinical data including tumor staging, pathology grades and family history were evaluated. Results: The GG alleles were significantly increased in African American patients requiring biopsy as compared with healthy controls (p<0.001). However, there was no significant difference between the genotypes comparing the benign vs. ductal carcinomas in situ (DCIS) or infiltrating ductal carcinomas (IDCA). The African American patients with a pathology diagnosis of fibroadenomas, 50% carried the AA alleles as compared with 23% AG and 28.6% GG alleles. Patients (58%) with stage II tumor diagnosis carried the GG genotype as compared with 20% and 29% AA and AG genotypes respectively. The HER-2/neu receptors were increased in patients with AG or GG. The mRNA expression was reduced in patients with IDCA vs. DCIS or benign tumors (Benign vs. IDCA, p<0.0009; DCIS vs. IDCA, p<0.005). Likewise, it lower in patients with HER-2/neu positive tumors (p<0.0024, OR-2.13). Conclusion: A novel aspect of our report is that the expression levels of CYP 3A4 is associated at least with disease sub-category. The data suggest that testing for the determination of CYP 3A4 alleles in concert with the receptors would increase the power of early diagnosis, and early management of patient care.

### 1175/W/Poster Board #833

**Interaction of CYP1B1 gene variant (N453S) with carcinogen metabolism in cigarette smoke increases lung cancer risk.** *M. Haznadar[1], T. Church[2], M. Geisser[2], K. Anderson[3], N. Caporaso[5], S. Hecht[4], B. Van Ness[1].* 1) GCD, University of Minnesota, Minneapolis, MN; 2) Environmental Health Sciences, University of Minnesota, Minneapolis, MN; 3) Epidemiology, University of Minnesota, Minneapolis, MN; 4) Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN; 5) NCI, Bethesda, MD.

The purpose of this study was to examine single nucleotide polymorphisms (SNPs) in genes important to the metabolism of NNAL and PheT, carcinogenic metabolites from cigarette smoke. In a previous case-control study, a significant correlation was found between the serum levels of total NNAL and a prospective lung cancer risk. Given the association of NNAL levels as a marker of metabolism of smoking carcinogens, and SNPs in cytochrome P450 genes associated with drug and carcinogen metabolism, we examined risk by interaction of SNPs and metabolic biomarker levels.

A case-control study was nested in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. 100 lung cancer cases and 100 controls who smoked at time of enrollment were selected. The baseline serum levels for total NNAL, cotinine, and PheT were analyzed in all subjects. DNA was extracted from banked lymphocytes and SNPs in 3 genes involved in NNAL and PheT metabolism were determined (CYP1B1, EPHX1, GSTP1). Logistic regression was used to determine if there was an interaction between each SNP and the levels of carcinogenic biomarker as a risk for lung cancer.

A SNP in CYP1B1 (N453S) was found to significantly interact with NNAL in increasing the risk for lung cancer. The minor variant doubles the slope of the risk for lung cancer with increasing serum NNAL levels. The main effect (OR=0.133; 95%, CI=0.024,0.737) and interaction effect (OR=1.020; 95%, CI=1.002,1.038) are both statistically significant for SNP N453S. This SNP has been shown to decrease intracellular levels of CYP1B1 protein, presumably by decreasing its stability and half life. Most of the carcinogenic effects of polycyclic aromatic hydrocarbons present in tobacco smoke are mediated by the aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor that regulates tobacco-induced expression of carcinogen metabolic enzymes. While CYP1B1 has not previously been implicated in NNAL metabolism, it does alter induction of CYP1A1 by AhR, and CYP1A1 has been implicated in the activation of NNAL and DNA adduct formation.

A common polymorphism of CYP1B1 may play a role in the risk of lung cancer through interaction with NNAL metabolism. This interaction provides a novel example of pathways influencing gene-environmental exposure as a risk for this disease.

### 1176/W/Poster Board #834

**Generation of Controls for an Allele Specific PCR Assay for Detection of the T315I Mutant Allele in the BCR-ABL1 Kinase Domain Found In Imatinib Resistant Patients with Chronic Myeloid Leukemia.** *D. Payne[1], R. Garcia[1], C. Tirado[1], E. Keohanne[2].* 1) UT Southwestern, Dallas, TX; 2) University of Medicine and Denistry, New Jersey.

Background: Chronic myeloid leukemia (CML) patients resistant to Imatinib (IM) have mutations in the Kinase Domain (KD) of the BCR-ABL1 protein. Thus far, a minimum of 40 different KD mutations have been identified with T315I as the most frequent KD mutation in CML patients. Methods: We generated controls for the wild type (WT315) and T315I mutant alleles in the BCR-ABL1 Kinase Domain present in IM resistant patients. Initially, a BCR-ABL-1 cDNA was generated from a K-562 cell line. To generate the wild type (WT315) and T315I controls, an ASO-PCR assay designed by Willis et al. (2005) was used for detection of both wild type and mutant alleles. The cDNA generated from the K-562 cell line served as template for the forward primer shortabl (F) and reverse primer WT315 (R) to generate the WT315 control. Similarly, the T315I control was generated by using WT315 DNA as template for shortabl F and the reverse mutant primer T315I + 5 R. Results: Using a BCR-ABL1 PCR assay, a 332 base pair (bp) fragment was amplified with the K-562 DNA confirming the presence of the BCR-ABL1 sequence. Subsequent allele specific amplification showed a 160 bp fragment corresponding to the wild type control, WT315 amplified by primers shortabl F and WT315 R using K562 cDNA as the template. Later, a 160 bp band corresponding to the mutant control was amplified by primers shortabl F and T315I + 5 R using WT315 DNA as the template (the single base mismatch between the mutant primer and wild type template was overcome by decreasing annealing temperature to 55°c, decreasing number of PCR cycles to 25, and by adding 5 bases to the reverse primer past the 3' end of the wild type allele). ASO-PCR validation showed: a 160 bp band corresponding to the amplified T315I DNA by the primers shortabl F and T315I R + 5, while there was no amplified band for the T315I DNA by the primers shortabl F and WT315 R. In this study, the ASO-PCR had a detection limit of 1/100. DNA sequencing and detection of the T315I mutation from a patient further validated controls. Conclusion: The ongoing problem with many molecular assays is the relative absence of positive control material. We used several approaches for generating control material all of which failed prior to the final approach. The last approach incorporates an extended primer with the single base pair change followed by 5 bases. This approach is a simple and an inexpensive method for developing control material for ASO PCR assays.

### 1177/W/Poster Board #835

**Identification of regulatory genetic variation that affects drug response in childhood acute lymphoblastic leukemia (ALL).** *A. Kiialainen[1], J. Nordlund[1], L. Milani[1], A. Lundmark[1], U. Liljedahl[1], G. Lönnerholm[2], A-C. Syvänen[1].* 1) Department of Medical Sciences, Uppsala University, Uppsala, Sweden; 2) For the Nordic Society of Pediatric Hematology and Oncology (NOPHO), and Department of Children's and Women's Health, Uppsala University, Uppsala, Sweden.

Acute lymphoblastic leukemia (ALL) is the most common form of childhood cancer. Although treatments for ALL currently exist, some patients do not benefit from them, while others suffer from unnecessary side effects. In order to gain insight into regulatory genetic variation that affects drug response in ALL, we have recently determined the allele-specific expression (ASE) levels of over 8000 genes in RNA-samples from 197 ALL patients whose in vitro drug responses against ten anti-cancer drugs are known (Milani et al., Genome Research 2009). By genotyping SNPs in coding regions of genes in DNA and RNA (cDNA) and comparing the allelic ratios in RNA with those in genomic DNA from heterozygous individuals, we have detected ASE with high confidence in 400 genes. Guided by the allele-specific differences in gene expression, we have chosen a subset of 56 genes displaying ASE for re-sequencing. We are sequencing the whole genes, including exons, introns, and some of the 5' and 3' non-coding sequence. We are using Nimblegen sequence capture arrays for template preparation of DNA samples from ALL patients and the Genome Analyzer (Solexa/Illumina) for sequencing the genes. We align the sequences and perform SNP calling with the MAQ software (Heng Li, Sanger Institute). In the 3 Mb target region that we are sequencing, we find up to 4000 SNPs per sample. After sequencing, we plan to genotype the regulatory variants discovered by sequencing in a larger set of patient samples. We will correlate the genotypes with the allele-specific expression levels as well as with the drug response patterns of the patient cells.

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## 1178/W/Poster Board #836

**Sensitive detection of KRAS mutations using mutant-enriched PCR and reverse-hybridization teststrips.** *G. Kriegshaeuser[1], B. Holzer[2], B. Rauscher[1], E. Schuster[2], F. Kury[1], R. Zeillinger[2], C. Oberkanins[1].* 1) ViennaLab Diagnostics, Vienna, Austria; 2) Molecular Oncology Group, Department of Obstetrics and Gynaecology, Medical University of Vienna, Austria.

The KRAS gene encodes the human cellular homolog of a transforming gene isolated from the Kirsten rat sarcoma virus. Like the other members of the ras gene family, the KRAS protein is a 21 kD GTPase and an early player in many signal transduction pathways. While wildtype KRAS plays a vital role in normal cellular signaling processes, mutated forms of the gene are potent oncogenes that are found in many human cancers. The most critical region of the KRAS gene for oncogenic activation are mutations in codons 12 and 13, causing the protein to accumulate in the active GTP-bound state. KRAS mutations were shown to be also predictive for the response to cancer therapy with certain anti-EGFR monoclonal antibodies (panitumumab, cetuximab) and tyrosine kinase inhibitors (erlotinib, gefitinib). We have developed a novel reverse-hybridization assay (StripAssay) targeting 10 frequent mutations in codon 12 and 13 of the KRAS gene. The test is based on PCR in the presence of a suppressor for wild-type KRAS amplification (mutant-enriched PCR), followed by hybridization of PCR products to teststrips presenting a parallel array of allele-specific oligonucleotide probes. The hybridization and detection steps can be carried out fully automated using available instrumentation. The performance of the StripAssay was evaluated on DNA obtained from cultured cell lines, from formalin-fixed paraffin-embedded (FFPE) tissue and from stool. Using serial dilutions of DNA from various KRAS-mutated tumor cell lines into normal DNA, each of the 10 mutations was shown to be detectable at levels as low as 1%. DNA samples containing various proportions of mutated KRAS were analyzed by the StripAssay in direct comparison to real-time PCR, dideoxy sequencing and pyrosequencing. While all methods correctly identified samples containing 25% mutated DNA, dideoxy sequencing and pyrosequencing failed to detect levels of 12.5% or lower. Both the StripAssay, as well as real-time PCR, unambiguously identified 10%, 5% and 1% of KRAS-mutated DNA in the presence of excess wild-type DNA. The simultaneous detectability of 10 different mutations with excellent sensitivity will make the StripAssay a very useful tool for the assessment of the KRAS mutation status in cancer patients. (oberkanins@viennalab.co.at).

## 1179/W/Poster Board #837

**Association of XPD Polymorphisms with Severe Toxicity in Non-Small Cell Lung Cancer Patients in a Chinese Population.** *W. Wu[1], W. Zhang[2], R. Qiao[2], D. Chen[1], H. Wang[1,3], Y. Wang[1], S. Zhang[1], G. Gao[1], A. Gu[2], J. Shen[2], J. Qian[1], W. Fan[1], L. Jin[1], B. Han[2], D. Lu[1].* 1) State Key Laboratory of Genetic Engineering and Key Laboratory of Contemporary Anthropology, School of Life Sciences, Fudan University, Shanghai, China; 2) Department of Respiratory Disease, Shanghai Chest Hospital, Shanghai Jiaotong University, Shanghai, China; 3) Department of Neurological Surgery, Brain Tumor Research Center, The First Clinical Medical School of Harbin Medical University, Harbin, China.

Purpose: Platinum agents cause DNA cross-linking and adducts. Xeroderma pigmentosum group D (XPD) plays a key role in the nucleotide excision repair pathway of DNA repair. Genetic polymorphisms of XPD may affect the capacity to remove the deleterious DNA lesions in normal tissues and lead to greater treatment-related toxicity. This study aimed to investigate the association of three polymorphisms of XPD at codons 156,312, and 711 with the occurrence of grade 3 or 4 toxicity in advanced non-small cell lung cancer patients. Experimental Design: We used matrix-assisted laser desorption /ionization time-of-flight mass spectrometry to genotype the three polymorphisms in 209 stage III and IV non-small cell lung cancer patients treated with platinum-based chemotherapy. Results: The variant homozygotes of XPD p.Arg156Arg (rs238406) polymorphism were associated with a significantly increased risk of grade 3 or 4 hematologic toxicity [adjusted odds ratios (OR) = 3.24, 95% confidence interval (CI) = 1.35-7.78, P for trend =0.009], and more specifically, severe leukopenia toxicity (P for trend = 0.005). No statistically significant association was found for the three polymorphisms and grade 3 or 4 gastrointestinal toxicity. Consistent with these results of single-locus analysis, both the haplotype and the diplotype analyses revealed a protective effect of the haplotype 'CG' (in the order of p.Arg156Arg- p.Asp312Asn) on the risk of grade 3 or 4 hematologic toxicity. Conclusions: This investigation, for the first time, provides suggestive evidence of an effect of XPD p.Arg156Arg polymorphism on severe toxicity variability among platinum-treated non-small cell lung cancer patients.

## 1180/W/Poster Board #838

**The association of promoter variations in *HMOX1* and *UGT1A1* genes with colorectal cancer.** *A. Jiraskova[1,2], J. Novotny[3], L. Novotny[4], B. Pardini[5], A. Naccarati[5], P. Vodicka[5], H.A. Schwertner[6], J. Hubacek[7], L. Vitek[1,2].* 1) 4th Department of Internal Medicine; 2) Department of Clinical Biochemistry and Laboratory Diagnostics; 3) Department of Oncology; 4) Institute of Hygiene and Epidemiology, and 3rd Department of Internal Medicine, 1st Faculty of Medicine, Charles University in Prague, Czech Republic; 5) Institute of Experimental Medicine, Academy of Sciences of the Czech Republic; 6) Clinical Research, Wilford Hall Medical Center, San Antonio, USA; 7) Institute of Clinical and Experimental Medicine, Prague, Czech Republic.

Background: Enzymes heme oxygenase-1 (HO-1, encoded by *HMOX1*) and bilirubin UDP-glucuronosyltransferase (*UGT1A1*), both involved in the heme catabolic pathway, play an important role in the oxidative stress defence. HO-1 is the rate-limiting enzyme in heme catabolism that converts heme to biliverdin. Two functional polymorphisms, a (GT)n repeat variation and T-413A single nucleotide polymorphism (SNP), in the promoter of *HMOX1* gene have been associated with different clinical phenotypes. *UGT1A1* gene product catalyses glucuronidation of serum bilirubin. (TA)n repeat variation modulates *UGT1A1* transcriptional activity and quantity of the enzyme available to conjugate serum bilirubin, which is known as a potent antioxidant. *UGT1A1* and *HMOX1* promoter variations affecting serum bilirubin levels may be predictive of genetic susceptibility to oxidative damage and cancer. The objective of this study was to determine whether these variations affect development of colorectal cancer (CRC). Methods: The study was performed on 442 CRC patients (182 female) and 493 healthy age- and sex-matched controls (203 female). The (GT)n and (TA)n dinucleotide variations were determined by fragment (size-based) analysis using an automated capillary DNA sequencer (CEQ8000, Genetic Analysis System, Beckman Coulter). The length variations of (GT)n repeats were classified into short S ($n < 27$), medium M ($n = 27\text{-}32$) and long L ($n \geq 33$) subgroups. Results: No significant differences in frequencies of L-allele and L-allele carrier (S/L, M/L, L/L), M-allele and M-allele carrier (M/M, M/L, M/S), and S-allele and S-allele carrier (S/S, S/L, S/M) between control and CRC group were found. Similarly, no significant association between A-413T SNP and development of CRC was observed. However, gender-associated differences of (TA)n polymorphism in *UGT1A1* were observed. Higher frequency of (TA)6/(TA)6 genotype was found in CRC male patients (OR = 1.52; p = 0.019), but not in CRC female patients (OR = 1.08; p = 0.78). Conclusions: Results of our study suggest that (GT)n variations and A-413T SNP in *HMOX1* do not provide protection against CRC. Nevertheless, (TA)6/(TA)6 genotype of *UGT1A1*, associated with lower serum bilirubin levels, may increase a risk for development of CRC in male population.

## 1181/W/Poster Board #839

**The epidermal growth factor receptor (EGFR) and K-ras gene mutation detection in cancer cell lines using a multiplex genomic SNP/Insertion and Deletion(Indel) genotyping system.** *R. Fang, D. Wang, J. Shewale, P. Brzoska, M. Furtado.* Applied Markets, Applied Biosystems, Foster City, CA.

Human cancer cell lines have proven to be excellent models for studying many aspects of cancer development and also provided a powerful system for assessing the sensitivity to anticancer drugs. The genotype information about mutations for genes which are involved in cancer development in these cells will be valuable for cancer research. We have developed a multiplexed SNP and Indel detection assay for 29 and 5 somatic mutations in epidermal growth factor receptor EGFR and K-ras genes, respectively, which are frequently found in human cancers. The genotyping system is based on an initial PCR amplification followed by an oligonucleotide ligation reaction; the alleles were detected by capillary electrophoresis. The system is sensitive, accurate, easy to use and amenable for automation and high throughput processing. DNA was extracted from tumor cell lines using PrepFiler kit and genotyped. DNA preparations from 48 ATCC and 60 NCI tumor cell lines were also genotyped. The EGFR and K-ras mutations were discovered in some of the investigated tumor cell lines with specific pattern. These mutated genotypes were further confirmed by the Sanger sequencing chemistry. The information generated from this study will be definitely useful when these cell lines are used as models for cancer study and cancer drug screening.

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## 1182/W/Poster Board #840

**Pharmacogenomic modeling for predictions of patient outcomes in myeloma.** *B. Van Ness[1], M. Haznadar[1], C. Ramos[1], M. Gosse[1], V. Kumar[2], M. Steinbach[2], B. Durie[3], J. Crowley[4], A. Hoering[4], D. Johnson[5], G. Morgan[5], S. Janz[6].* 1) Dept Genetics, Cell Biol & Dev, Univ Minnesota, Minneapolis, MN; 2) Dept Computer Science and Electrical Engineering; 3) Hematology/Oncology, Cedars-Sinai Cancer Center, Los Angeles, CA; 4) Cancer Research & Biostatistics, Seattle, WA; 5) Heamatology-Oncology, Institute of Cancer Research, London, UK; 6) Dept Pathology, Univ Iowa, Iowa City , IA.

Purpose: While myeloma is still considered a fatal disease, new therapeutic approaches, including combination of drugs that target different intracellular pathways, have shown marked improvements in response and survival. Yet there are wide variations in responses seen among the patient population. One major source is the variation in chromosomal abnormalities and the genes deregulated among the plasma cell (PC) tumors. Another may be in inherited variations (SNPs) that can have a major influence on drug distribution, transport and metabolism. We are taking several approaches to model the impact of both tumor genetic variations and inherited genomic variations on patient outcomes. Methods: Using well established cell lines we are examining variation in cellular response to dexamethasone, melphalan, bortezomib (Velcade), and bcl-inhibitors. Variation in cytotoxicity assays are correlated to common patterns of gene expression or genome wide SNPs to develop models of pathways influencing cellular response. Our laboratory has developed a transgenic mouse model targeting overexpression of c-myc and bcl-xL in late B cells, that results in early expansion of non-malignant PCs, followed by development of clonal plasma cell malignancies in 100% of the mice. This model is being used along with established HAPMAP cell lines to examine response. Results and Conclusions: Cell surface markers, chromosomal abnormalities, and gene expression profiles of the mouse plasma cell tumors demonstrate a very similar profile and heterogeneity to human myelomas. The mouse PC tumors can be adoptively transferred to syngenic recipient mice, and grown in culture. The advantage of this model is that the impact of tumor heterogeneity can be assessed in a common genetic background in the mice with an intact immune system. Disease progression is variable among human and mouse tumors, and our results show association with patterns of gene expression, as well as heterogeneity of response to current drug therapies. Through the Bank On A Cure project of the International Myeloma Foundation, we show that inherited genomic variations in genes associated with drug response also influence therapeutic outcomes, including: survival, disease related complications (extent of bone disease), and adverse events (thrombosis). The presentation will include analytical approaches, and statistical refinements necessary to assess predictive value and integration of the various models.

## 1183/W/Poster Board #841

**Excision repair cross-complementing and Breast Cancer.** *M. Hosseini[1], M. Houshmand[2], A. Ebrahimi[3].* 1) Asst Prof, Dept Science, Islamic Azad Univ, Tehran, Iran; 2) National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran; 3) Special Medical Center, Tehran, Iran.

Excision repair cross-complementing 1, are important in DNA nucleotide excision repair and lie on chromosome 19q13.2-q13.3has been associated with breast cancer risk, but previous studies have been relatively small yet. Excision repair cross-complementing 1 gene is reported to be associated with increased risk of breast cancer. This study evaluates the influence of genetic polymorphism of Excision repair cross-complementing 1 on breast cancer susceptibility. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect the polymorphism, and the genotypes identified were assigned as homozygous wild type (GG), heterozygous variant (GT), and homozygous variant (TT). Probably a statistically significant increased risk in carriers of homozygous T allele was found in woman ($P < 0.001$) in comparison with G . However, no significant association between the genotype and breast cancer risk was observed among women with strong family history yet. We genotyped 560 cases of primary breast cancer and population controls, all age of Iranian women, for the Excision repair cross-complementing1, polymorphism studied. Small overall association was found between ERCC1 and breast cancer risk, for the TT carriers compared to the GG carriers until today.

## 1184/W/Poster Board #842

**Subtype discovery and network analysis in neuroblastoma by gene expression profiling.** *F. Abel, D. Dalevi, R. Jornsten, S. Nilsson.* Mathamatical Statistics, Mathematical Scienses, Gothenburg, Vastra Gotaland, Sweden.

Neuroblastoma (NB), a childhood tumor originating from neural crest cells in the sympathetic nervous system, has a complex biological heterogeneity depending on clinical stage and age at diagnosis. Around 30% of tumors show amplification of the oncogene MYCN, and around 15% show mutations or amplification of the familial predisposition gene Anaplastic Lymphoma Kinase (ALK). To determine whether the previously established types of neuroblastoma tumors could be identified by gene expression profiling, we used oligonucleotide microarrays (HU133A from Affymetrix) to analyze the pattern of genes expressed in tumor tissue from 45 patients originating from Ireland, Belgium and Sweden. Principal Components Analysis (PCA) identified each of the prognostically important neuroblastoma subtypes, including Type 1 constituting of favourable tumors with high NTRK1 expression, Type 2A constituting of unfavourable tumors with high frequency of 11q deletion and elevated ALK expression, and Type 2B constituting of unfavourable tumors with very high expression of MYCN and ALK. In addition, a fourth neuroblastoma subgroup was identified based on its unique expression profile. This fourth group was comprised of high stage tumors with a high degree of patients that were dead-of-disease. Moreover, the fourth subgroup showed low expression of MYCN and ALK, but very high expression of two tyrosine kinases which have shown to play roles as oncogenes in other cancer forms. In order to identify progression-associated genes and to elucidate the underlying cellular networks in the four different subgroups, we calculated the mutual dependence of each gene pair based on the expression profiles from all 45 patients (i.e. mutual information). In conclusion, we intend to develop a single platform of expression profiling to enhance the accurate subgroup and risk classification of neuroblastoma patients. Moreover, we intend to map the disease-causing cellular pathways within each tumor subgroup in order to identify suitable clinical targets.

## 1185/W/Poster Board #843

**Polymorphisms of methylenetetrahydrofolate reductase gene and susceptibility to adult lymphoblastic leukemia in Chinese population.** *L. Bao[1], C. Wu[2], L. Lv[2], H. Fu[2].* 1) Divison Human Gen, Cincinnati Child Hosp Med Ctr, Cincinnati, OH; 2) Fudan University, Shanghai, China.

Methylenetetrahydrofolate reductase (MTHFR) is a major enzyme in the folate metabolism. Two common polymorphic variants on the MTHFR gene, C677T and A1298C, result in reducing MTHFR enzymatic activity and may also influence the susceptibility for developing acute lymphoblastic leukemia (ALL). However, results from previous studies in different populations remain controversial with some showing the genetic variants as risk factors for ALL while others suggesting them as protective variants against ALL. Most reported investigations focused on relationship of the MTHFR variants with development of childhood ALL. Here, we report a case-control study of 127 adult ALL cases in Chinese population to study the correlation between the MTHFR genetic variants and adult ALL susceptibility. Polymerase chain reaction-restriction fragment length analyses show that the frequency of the C677C/1298AC haplotype was lower among ALL patients compared the one in controls, conferring a 2.9-fold decrease in risk of ALL [odds ration (OR), 0.35; confidence interval (CI) 95%, 0.165-0.727]. No significant differences in frequencies of either of the MTHFR genotypes or other haplotypes between ALL and control groups were found. Our findings suggest that it is the MTHRF C677C/A1298C halpotype rather than any of the MTHFR genotypes alone or other haplotypes that may decrease susceptibility for developing ALL in Chinese adults.

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## 1186/W/Poster Board #844

**Glutathione S-transferase P1 variants (SNP at codon 105) in combination with the p53 codon 72 polymorphism and COX-2 variants (G-765C) on the risk of esophageal squamous cell carcinoma: A case-control study in Iranian population.** *F. Biramijamal[1], G. Irvanloo[2].* 1) Medical Genetics, NIGEB, Tehran, Iran; 2) Cancer Institute, Tehran-Iran.

Background: The incidence of esophageal squamous cell carcinoma (ESCC) is high in Iran. Increasing risk of esophageal cancer is depend on geographic differences. The specific genetic factors which are involved in development of this type of cancer is not clear exactly. It is shown that single nucleotide polymorphisms (SNPs) are important to change susceptibility of esophageal cancer. These variations in specific genes including Cyclooxygenase-2 (COX-2), p53 tumor suppressor gene and Glutathione S-transferase P1, affect on risk of ESCC, however, environmental factors must be considered during investigation. The p53 protein is considered a key combination in countering stress messages such as DNA damage and is involved in the etiology of malignant disease. The polymorphism at codon 72 of the p53 gene (CCC, proline/CGC, Arginine) is frequent among different populations and involved in various types of cancers. The Cyclooxygenase-2 (COX2) gene plays an important role in inflammation and carcinogenesis process. It is shown that polymorphism of this gene at promoter (-765 G>C) associated with cancers. In addition, Glutathione S-transferase P1 (GST-P1) is important enzyme in detoxification and polymorphism at codon 105 (Ile>Val) is involved in susceptibility of different types of cancers. In this study, we analyzed the effect of genetic polymorphism of GST-P1 at codon 105 in combination with p53 and COX-2 genes variants on the risk of ESCC in Iran. Methods: In this study, we investigated GST-P1, p53 and COX-2 polymorphisms among ESCC patients compared with healthy controls. PCR-RFLP performed for genotyping (for 199 healthy controls and 60 patients) and confirmed by sequencing. Conclusions: Our finding suggested that Ile allele of GST-P1gene combined with C allele of COX-2 gene, and, Arg allele of p53 gene increased risk of ESCC. Additionally, the results of the study indicated that COX-2 genotype alone may play an important role in developing esophageal tumor among ESCC patients.

## 1187/W/Poster Board #845

**A common SNP associated with non-Hodgkin Lymphoma influences protein binding at the *H2AFX* promoter.** *K.L. Bretherick[1], S. Leach[1], S.B. Montgomery[2], J.P. Banath[3], S.J.M. Jones[2], P.L. Olive[3], A.R. Brooks-Wilson[1].* 1) Genome Sciences Centre, British Columbia Cancer Research Centre, Vancouver, BC, Canada; 2) Wellcome Trust Sanger Institute, Hinxton, UK; 3) Medical Biophysics Department, British Columbia Cancer Research Centre, Vancouver, BC, Canada.

The tendency for non-Hodgkin lymphoma (NHL) tumors to carry chromosomal translocations suggests that this cancer may arise from an attenuation of DNA repair systems that protect against such defects. This is supported by the finding that a common G/A SNP (rs2509049) located upstream of *H2AFX*, which encodes a histone involved in response to DNA double-stranded breaks, is associated with risk for NHL. Bioinformatic analyses predict that the G allele is part of a binding site for the replication-associated E2F-1 transcription factor, whereas the A allele is more consistent with a binding site for CREB1, which is not replication specific. *H2AFX* is expressed from two transcripts, a 0.6 kb transcript with processing signals typical of replication-linked histone genes, and a 1.6 kb poly(A)-tailed transcript that is transcribed independently of replication. We predict that rs2509049 genotype influences expression of *H2AFX*, altering DNA repair capacity and modifying NHL risk. To assess this hypothesis, we examined the impact of rs2509049 A and G alleles on transcription factor binding and gene expression. Gel shift assays with purified nuclear extract from HeLa and BJAB cell lines suggest differential protein binding at the A and G alleles. Specifically, the A allele exhibits low affinity for two protein complexes, one of which also binds to the G allele with higher binding affinity. Relative allelic expression assessed by quantitative sequencing of cDNA from rs2509049 heterozygous cell lines revealed no difference in expression of the two alleles. This analysis, however, did confirm the presence of an intron in the 1.6 kb transcript which, although annotated in the UCSC Genome Browser, has not previously been reported in the literature. Differential protein binding between A and G alleles supports the hypothesis that rs2509049 is a functional SNP. It is possible that the expression analyses are not sensitive enough to detect subtle differences between genotypes, or that this SNP affects another aspect of gene expression such as timing or tissue specificity. Further analysis of functional differences between genotypes may explain the association with NHL risk.

## 1188/W/Poster Board #846

**Rapid and Sensitive detection of K-RAS Mutations using PNA-Mediated Real-Time PCR Clamping.** *M. Cho, J.J. Choi, H. P.* Institute of Bioscience, PANAGENE Inc., Yuseong-gu, Daejeon, Korea.

K-RAS mutations may indicate prognosis and drug response, and many new cancer therapies are being targeted to the K-RAS pathway. Many studies suggest that in lung cancer patients the K-RAS mutation status is strong predictor of resistance to therapy with tyrosine kinase inhibitors. Therefore, it is important that a minority of K-RAS mutations detect fast and accuracy for predict drug response and prognosis. To detect a minority of mutant K-RAS alleles among abundant wild-type alleles, we developed a high sensitivity and simple method for detection of K-RAS point mutations using PNA-mediated real-time PCR clamping. PNA mediated real-time PCR clamping relies on the following two unique properities of PNA probes. 1) PNA-DNA duplexes generally have greater thermal stability than the corresponding DNA-DNA duplex and 2) PNA oligomers are not recognized by DNA polymerases and consequently can serve as sequence selective clamp during PCR amplification. In this paper, one-step PNA-mediated real-time PCR clamping method was optimized for accuracy detection of K-RAS mutations in exon 12 and 13 with high sensitivity. The PNA clamps used in this study was a 21 to 23mer complementary to wild type K-RAS sequence and 5uM concentrations of PNAs. Our PNA-mediated real-time PCR clamping method was simple, robust and sensitive with detection limit approximately 0.1% mutant alleles using 1ng to 50ng of normal DNA as the template. It is particularly useful for tumors containing abundant non-neoplastic cells. We concluded that PNA mediated PCR clamping might be a useful supplement of standard PCR amplification in low concentration mutation alleles. PNA-mediated real-time PCR clamping is a rapid, reliable, and economical method for K-RAS mutation detection in clinical diagnosis.

## 1189/W/Poster Board #847

**Lack of germ-line mutations in SDHD in familial carcinoid tumors.** *L. Deng[1], V. Palka[1], O. Hashmi[1], L. Liang[1], J. Tischfield[1], C. Shao[1], N. Gardner[2].* 1) Dept Genetics, Rutgers Univ, Piscataway, NJ; 2) School of Nursing, Rutgers Univ, Newark, NJ.

Carcinoids are neuroendocrine tumors, mainly located in gastrointestinal and bronchial-pulmonary systems. While carcinoid tumors are relatively rare, with an incidence reported to be 4 per 100,000 in United States and 1.9 / 100,000 worldwide, there is a much higher incidence among the first degree relatives of the carcinoid patients, indicating the involvement of genetic components in its initiation and pathogenesis. Mutations in succinate dehydrogenase complex, subunit D (SDHD), were reported to be associated with paraganglioma, pheochromocytoma and gastrointestinal stromal tumors. To test whether germ-line mutations in SDHD are responsible for familial carcinoid tumors, we collected peripheral blood samples from 15 individuals in eight families with familial carcinoid tumors and performed direct sequencing of SDHD. All exons and exon-intron junctions in SDHD were sequenced. No mutation was detected in any of the subjects analyzed, indicating that SDHD mutation carriers are not common among carcinoid patients, even for familial cases. Sequencing analysis of other candidate genes, such as MEN1, is being conducted.

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## 1190/W/Poster Board #848

**Integrated Network Analysis of Genetic and Epigenetic factors in Glioblastoma.** *H. Dong[1,2], L. Luo[2], H. Siu[1], Y. Xiao[3], S. Hong[1], G. Peng[1], X. Fang[4], R. Chen[5], D. Wheeler[5], L. Jin[1], M. Xiong[2].* 1) State Key Laboratory of Genetic Engineering and MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai, 200433, China; 2) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77030, USA; 3) Department of Computing and Information Technology,Shanghai (International) Database Research Center, Fudan University Shanghai, 200433,China; 4) School of mathematical sciences, Peking University, Beijing, 100871, China; 5) Dept. of Molecular and Human Genetics Baylor College of Medicine, Houston, TX, 77030, U.S.A.

Glioblastoma arises from complex interactions between a variety of genetic, epigenetic alterations and environmental perturbations. To perform integrated genetic and epigenetic analysis in glioblastoma studies, a total of 601 genes were sequenced for detection of somatic mutations in 179 tumor and matched normal tissues pairs; expressions of 12,042 genes were measured in 243 tumor tissue samples and 10 normal tissue samples and 1 cell line; expressions of 470 human miRNA were profiled in 240 tumor tissue sample and 10 normal tissue samples and a total of 2,994 genes were examined for methylation in 239 tumor tissue samples and 1 cell line in TCGA pilot project. We used system biology approach as a general framework to develop several novel statistical methods for joint genetic and epigenetic data analysis. We identified significant association of somatic mutations in five genes TP53, PTEN, EGFR, FKBP9, CHEK2 strongly affected the expression of 147 differentially expressed genes, 68 of which were under-expressed in tumor tissue and 79 were over-expressed in tumor tissue. The mutations in these five genes also significantly affected the expression of 5 human miRNAs: mir-106a, mir-17-5p, mir-20a, mir-504, mir-99b, which in turn target 32 of the 165 genes. We also identified significant association of LOH in seven genes NRAP, MKI67, C10orf54, C9orf66, MYO3A, PRAME, EGFR, which influenced expressions of 201 genes and 25 miRNA, a total of 98 miRNA with p-value<1.06E-4 and 1,221 genes with p-value<4.15E-6 were differentially expressed. We constructed miRNA interaction networks including 333 miRNAs and 563 interactions after a Bonfferroni correction p-values of 8.51E-7 for declaring interaction. We constructed miRNA co-expression networks including 375 miRNAs and 441 edges by partial correlation methods. We also constructed gene co-expression and interaction networks. We used paired expression profiles of miRNAs and mRNAs to identify functional miRNA target relationships and found 1,094 miRNA-target gene pairs discovered by regression and sequence-based prediction algorithm. We analyzed the network properties and test the association of network with cell function and disease phenotype. We finally identified an integrated network that connected significant mutations, CNVs, altered gene expression, miRNA and methylation, which act together to alter regulation and metabolism and finally lead to the emergence of glioblastoma.

## 1191/W/Poster Board #849

**The regulation of epithelial characteristics by miR-200 promotes the development of late stage metastasis.** *D. Dykxhoorn[1,2], Y. Wu[2], H. Xie[3], A. Lal[3], F. Petrocca[2], D. Martinvalet[2], B. Lim[3,4], J. Lieberman[2].* 1) The John T. Macdonald Foundation of Human Genetics and the Department of Microbiology and Immunology, Miami Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL 33136 USA; 2) Immune Disease Institute and Department of Pediatrics, Harvard Medical School, Boston, MA 02115 USA; 3) Stem Cell and Developmental Biology, Genome Institute of Singapore, Singapore; 4) Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115 USA.

The development of metastasis requires that cells from the primary tumor acquire genetic or epigenetic variations that allow them to successfully complete the steps in the metastatic process including intravasation, survival and arrest in the circulatory system, extravasation and the formation of macroscopic secondary tumors. MicroRNAs have been shown to play important roles in many cellular processes and dysregulation of their expression patterns can have pathogenic consequences including promoting tumor and metastatic development. To determine if miRNAs play a role in the latter stages of metastatic development, tumor colonization of distant sites, miRNA expression patterns were analyzed from a series of isogenic mouse breast cancer cell lines with distinct metastatic capabilities. Several members of the miR-200 family were found to be highly expressed only in the cells capable of forming macroscopic metastasis. This was surprising since miRNAs from this family had been previously shown to promote epithelial characteristics through the targeted silencing of the transcriptional repressor Zeb2 and, as a consequence, increased expression of E-cadherin. The transgenic expression of miR-200 in mammary tumor cells that were normally unable to colonize distant sites resulted in decreased Zeb2 and Snail expression, increased E-cadherin expression, and the formation of lung and liver metastasis. These results suggest a role for the miR-200 family in enhancing the epithelial characteristic of cells and promoting the transition from micrometastasis to macroscopic tumors.

## 1192/W/Poster Board #850

**Modulation of Biomarkers of Oxidative Stress by Berry Treatment in High "At-risk Normal" Tissues from Oral Squamous Cell Carcinoma Patients.** *J.M. Ferguson[1,4], T.J. Knobloch[1,2], L.K. Uhrig[1,2], B.C. Casto[1,2,5], B.M. Warner[1,2,4], D.E. Schuller[1,3,5], E. Ozer[1,3,5], A. Agrawal[1,3,5], C.M. Weghorst[1,2,5].* 1) The Ohio State University, Columbus OH; 2) College of Public Health; 3) College of Medicine; 4) College of Dentistry; 5) Comprehensive Cancer Center.

PURPOSE: The oral cavity experiences high levels of exogenous and endogenous oxidative stress. Elevated levels of reactive oxygen species have been associated with oral squamous cell carcinoma (OSCC), and can activate carcinogenic transcription factors and signal transduction pathways. Black raspberries represent a food-based chemopreventive agent rich in polyphenolic compounds, which have been shown to reduce oxidative stress and inflammation. Cell culture and animal studies have shown altered gene expression in tumors following exposure to lyophilized black raspberries (LBR) or their extracts. Consistently, these chemopreventive studies show the ability of LBR to modulate genes prominently associated with oxidative stress. In OSCC cells exposed to LBR extract in culture, we have shown a down-regulation of a series of stress related biomarkers. To transition these *in vitro* findings into an *in vivo* setting, we have used the hamster cheek pouch (HCP) model of OSCC to demonstrate suppression of oral tumor lesion incidence and multiplicity (Anticancer Res 22, 2002) following dietary LBR treatment. The role of these pre-clinical studies is to translate into a human patient-based clinical investigation. Consequently, the aim of our ongoing Phase 1 Clinical Trial is to evaluate the molecular changes in oral cavity tissues following short-term, locoregional, oral exposure to LBR troches.

METHODS: According to protocols approved by the IRB of The Ohio State University, biopsy-confirmed OSCC patients were administered three LBR troches 4x/day (4.3g cumulative dose) between pre-surgical enrollment and their normally scheduled surgery. Biopsies were obtained from tumor and distant high "at-risk normal" tissues at enrollment and during surgical resection, after an exposure range of 6.8-20.8 days. Using a partial interim cohort of patients, a validated Biotrove OpenArray assay was used to assess stress related genes for LBR-dependent expression changes.

RESULTS: Decreased expression of multiple stress related genes (*HMOX1, HSPB1, NFE2L2, and NQO1*) was seen in the high "at-risk normal" tissue samples after LBR treatment, indicating that berry exposure may be reducing the risk of carcinogenesis by eliminating the harmful effects of reactive oxygen species.

## 1193/W/Poster Board #851

**Comparison of gene expression in primary breast tumors from node-negative and node-positive women.** *L.A. Field[1], B. Love[2], B. Deyarmin[1], J.A. Hooke[3], R.E. Ellsworth[4], C.D. Shriver[3].* 1) Clinical Breast Care Project, Windber Research Institute, Windber, PA; 2) Invitrogen, Carlsbad, CA; 3) Clinical Breast Care Project, Walter Reed Army Medical Center, Washington, DC; 4) Clinical Breast Care Project, Henry M. Jackson Foundation for the Advancement of Military Medicine, Windber, PA.

Background: Lymph node status is one of the most useful prognostic indicators in breast cancer and influences the course of treatment that will be used. Nodal status is currently determined using sentinel lymph node biopsy (SLNB), an invasive surgical procedure that can disrupt the lymphatic system and lead to secondary complications such as seroma and neuropathy. In this study, we have compared gene expression profiles from primary breast tumors from node-negative and node-positive women to determine if a molecular signature exists that can distinguish between patients whose breast tumors have metastasized to the lymph nodes and those that have not. Such a discovery could prevent the unnecessary need for SLNB in those women without lymph node metastases and potentially allow for the development of therapeutics to prevent metastasis from the breast to the lymph nodes. Methods: Tumor cells were removed from primary breast tumors from patients with (n=20) and without (n=20) lymph node metastases using laser microdissection and RNA isolated using the RNAqueous Micro kit (Ambion). RNA was amplified in two rounds using the MessageAmp II aRNA Amplification kit (Ambion). Fragmented aRNA was applied to Affymetrix HG U133A 2.0 arrays. A Mann-Whitney U test was used to determine significance. Microarray data were generated in the same manner for a second validation set containing 20 node-positive and 22 node-negative patients. Results: The probe lists for the two sets of microarray data were compared and 22 probes representing 21 genes were found to be common to both lists ($P < 0.05$). Fifteen of these genes had higher expression in primary breast tumors from node-negative patients while 6 genes had higher expression in node-positive patients. Discussion: The gene expression differences identified here may be responsible for determining whether cells from the primary breast tumor will disseminate to the lymph node. Genes with higher expression in node-negative patients function in nucleosome assembly, metabolism, insulin secretion and signaling, transcription, transport, and apoptosis. Genes with higher expression in tumors with lymph node metastases function in regulation of transcription, signal transduction, transport, cell differentiation, and microtubule organization and movement. These genes may represent new molecular targets to which drugs can be developed to prevent metastasis of breast cancer to the lymph node.

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## 1194/W/Poster Board #852

**Gender-dependent differences in UGT2B17 expression in human tissues.** *C. Gallagher, R. Balliet, J. Muscat, P. Lazarus.* Penn State Cancer Institute, Departments of Pharmacology and Public Health Sciences, Penn State College of Medicine, Hershey, PA, USA.

UGT2B17 is a phase II metabolizing enzyme involved in the glucuronidation of androgens as well as exogenous compounds including the tobacco carcinogen NNAL and cancer treatment drugs including suberoylanilide-hydroxamic-acid (SAHA). UGT2B17 is expressed in many tissues including liver, lung, and prostate and is down-regulated by androgens in prostate tissue. Due to the fact that this gene metabolizes and is regulated by androgens, we hypothesized that there may be gender differences in the expression and activity of this gene. We performed reverse-transcription PCR on 77 human liver RNA samples and assayed the expression of UGT2B17 cDNA using real-time PCR. We then assayed microsomes from these same human liver samples (HLMs) for activity against NNAL and SAHA using ultra-performance liquid chromatography (UPLC). We demonstrated that liver specimens from men with at least one intact UGT2B17 allele exhibited an 8-fold higher expression of UGT2B17 mRNA than women (P-value < 0.001). In addition, HLMs from men with at least one intact UGT2B17 allele exhibited a 2-fold higher glucuronidation activity against NNAL (P-value < 0.02) and a 2-fold higher glucuronidation activity against SAHA (P-value = 0.001). When stratifying this data by UGT2B17 deletion genotype, similar patterns were observed for glucuronidation of both substrates; individuals with 2 copies of the UGT2B17 gene exhibited higher glucuronidation activity than individuals who have 1 or 0 copies of the UGT2B17 gene for both men and women. Due to the fact that the deletion polymorphism of UGT2B17 has been shown to be associated with increased lung cancer risk specifically in females, expression of UGT2B17 was also examined in adjacent normal lung tissue from 60 lung cancer patients (35 males and 25 females). In contrast to the results from liver, women have 2-fold higher levels of expression of UGT2B17 then men in lung tissue (P-value = 0.027). These data are consistent with a hormonal regulatory mechanism for UGT2B17 in humans and suggest that glucuronidation activities, at least for UGT2B17, may differ with gender. These results suggest that expression and activity of UGT2B17 differ between men and women, and that the differences vary by tissue type, similar to previous observations in rodents. This data may have implications in gender differences in cancer risk and may also have pharmacological implications in the dose of cancer therapy drugs (such as SAHA) given to men and women.

## 1195/W/Poster Board #853

**Germline epimutations in suspected Lynch syndrome.** *A. Gylling[1], S. Ollila[2], O. Vierimaa[3], H.J. Järvinen[4], J.-P. Mecklin[5], P. Peltomäki[1].* 1) Dept of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Department of Biological and Environmental Sciences, Genetics, University of Helsinki, Helsinki, Finland; 3) Department of Clinical Genetics, Oulu University Hospital, Oulu, Finland; 4) Second Department of Surgery, Helsinki University Hospital, Helsinki, Finland; 5) Department of Surgery, Jyväskylä Central Hospital, Jyväskylä, Finland.

In a majority of families/cases suspected of having hereditary nonpolyposis colorectal cancer/Lynch syndrome, but not fulfilling the Amsterdam criteria, point mutations or large genomic rearrangements in DNA mismatch repair (MMR) genes are not found. The role of germline epimutations was evaluated in 43 index patients who were mutation-negative by genomic sequencing, testing for a prevalent population-specific founder mutation and large genomic rearrangements, and selectively lacked MMR protein expression (MLH1, 31; MSH2, 7; MSH6, 5) in tumor tissue. Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) was used to detect epimutations. Germline epimutations of MLH1, one of which coexisted with a genomic deletion, occurred in 3 patients (3/31, 10%) and were accompanied by monoallelic expression in mRNA. No epimutations were found in MSH2 or MSH6. A previous study (Ligtenberg et al 2009) reported that deletions of the most 3' exon of the EPCAM/TACSTD1 gene can lead to secondary methylation and inactivation of MSH2, which is often mosaic in somatic tissues. In analogy, a similar mechanism might be valid for MLH1 through deletion of the most 3' exon of LRRFIP2. No germline deletions were found in the LRRFIP2 gene among 31 cases with absent MLH1 protein in tumor tissue, including the 3 epimutation cases, or in the TACSTD1 gene in 7 cases lacking MSH2 protein in tumors. In conclusion, germline epimutations explain a definite but relatively small proportion (10%) of Lynch syndrome suspected cases lacking the MLH1 protein in tumor tissue. In contrast to point mutations and large genomic rearrangements, which are associated with strong family histories for Lynch syndrome, epimutations occur in patients with multiple early-onset tumors without any significant family history. The identification of such defects is important for the diagnosis, counseling and management of the patients and their families.

## 1196/W/Poster Board #854

**The Hippo Pathway Effector, Yap, Functions as a Potent Oncogene in Ovarian Cancer.** *C. Hall[1], P. Szafranski[2], M. Zhao[2], R. Wang[2], S. Orsulic[4], S. Goode[1,2,3].* 1) Dept of Molecular & Human Genetics; 2) Dept of Pathology; 3) Dept of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX; 4) Women's Cancer Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA.

The recently discovered Hippo pathway regulates cell polarity and tumorigenesis in Drosophila, yet its relevance to human cancer remains unclear. Human homologs of each member of the pathway from the cell membrane to the nucleus have been identified and Yap, a downstream transcriptional coactivator, has been reported to function as both a tumor suppressor and an oncogene. To determine the role of the Hippo pathway in Human Ovarian Cancer (OvCa) we assessed the level of nuclear Yap (active) and phospho-Yap (inactive) in an OvCa tissue array. We found that patients with either high levels of nuclear Yap or low levels of phospho-Yap have approximately 40% lower 5 year survival (p=.011 and .032). Expression of activated Yap, but not dominant negative or knockdown of Yap, in an immortalized ovarian surface epithelium cell line conferred resistance to apoptosis, faster wound healing, and the ability to grow in soft agar. Together, these data support the role of Yap as an oncogene in OvCa as in Drosophila. In order to determine the mechanism of Yap activation, we tested several hypotheses. First, we found that Yap is phosphorylated in 10/10 cell lines indicating that the upstream Hippo pathway remains functional in OvCa. Using QPCR we did not detect increased Yap in 10 OvCa cell lines and 16 OvCa tumors indicating Yap mRNA is not overexpressed in OvCa. In these same samples we also did not detect Yap mutations indicating Yap is not activated by mutation. Finally, we tested if loss of 14-3-3-sigma, a known inhibitor of Yap, could result in activation of Yap even in the presence of inhibitory signals from the Hippo pathway. 14-3-3-sigma is known to be lost by promoter hypermethylation and this loss correlates with poor patient outcome in OvCa. We find a very strong correlation between the presence/absence 14-3-3-sigma and the cytoplasmic/nuclear localization of Yap in 10 OvCa cell lines. We are currently testing if treatment with azacitidine can restore 14-3-3-sigma expression and proper Yap localization in OvCa cell lines. In support of this hypothesis, Bast et al. recently demonstrated that azacitidine pretreatment of patients with platinum resistant OvCa can reverse platinum resistance and improve overall survival. Together, this data represents the first clinical correlation for the Hippo pathway, the establishment of Yap as potent oncogene in OvCa, and new potential therapeutic avenues for the treatment of ovarian cancer.

## 1197/W/Poster Board #855

**Analysis of the genomic structure of chronic myelogenous leukemia using a paired-end-tag (PET) sequencing approach.** *A.M. Hillmer[1], K.P. Ng[2], A.S.M. Teo[1], Y. Fei[1], W.H. Lee[1], P.N. Ariyaratne[1], A. Shahab[1], V. Cacheux-Rataboul[1], G. Bourque[1], X. Ruan[1], W.K. Sung[1], C. Chuah[2], S.T. Ong[2], Y. Ruan[1].* 1) Genome Inst Singapore, Agency of Science Technology & Research, Singapore, Singapore; 2) Cancer & Stem Cell Biology Program, Duke-NUS Graduate Medical School, Singapore, Singapore.

A balanced, reciprocal translocation involving the long arms of chromosomes 9 and 22 is the major genomic alteration in the etiology of chronic myelogenous leukemia (CML), and gives rise to the Philadelphia chromosome and Bcr-Abl fusion gene. Although targeted therapy against the Bcr-Abl kinase has led to highly effective treatment for patients with chronic phase (early-stage) CML (CP), patients with blast phase (late-stage) CML (BP) inevitably develop drug-resistance (DR), and almost always succumb to their disease. The etiologies of disease progression from CP to BP, and DR are unknown, but it is likely that structural rearrangements in the CML genome contribute to these phenomena. We have developed a paired-end-tag (PET) sequencing strategy to study structural variations in the human genome. In addition to the identification of copy number changes, which can also be discovered by array based technologies, the PET sequencing approach has the capability to identify and characterize copy number neutral structural changes. We have applied this technology to construct comprehensive karyo-genomic maps from the K562 BP cell line, as well as four patients representing individuals with CP and BP CML, and DR. We found the Bcr-Abl fusion gene in all samples, except those from patients with a major molecular response to therapy. These results confirm the capability and validity of the technology to identify structural changes in a genome-wide manner. The data also provide unique insights about CML genomic architecture at different stages of disease, and provide a comprehensive list of structural variations following disease progression. These variations are of potential relevance for an improved understanding of the pathogenesis of CML progression and/or DR.

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## 1198/W/Poster Board #856

**Phosphorylation of PI3K/AKT/mTOR signaling pathway members is heritable and associated with variants on chromosomes 3 and 14.** *J.E. Hutz*[1,2], *H.L. McLeod*[2]. 1) Institute of Pharmacogenomics and Individualized Therapy, University of North Carolina, Chapel Hill, NC; 2) Division of Statistical Genomics, Washington University in St. Louis, St. Louis, MO.

The PI3K/AKT/mTOR signaling pathway is activated in a large percentage of all cancers, and its activation is almost universally associated with poorer clinical outcomes. Many somatic mutations in pathway members have been identified, but little is known about the effects of inherited variation in this signaling pathway. To assess whether pathway activation is a heritable trait, phosphorylated and total levels of three key proteins in the pathway (AKT1, p70S6K, 4E-BP1) were measured by ELISA in 122 related lymphoblastoid cell lines from 14 CEPH families. Interestingly, the phenotypes with the highest heritability were the ratios of phosphorylated to total protein for two of the pathway members: AKT1 ($h^2$=0.18) and p70S6K ($h^2$=0.23). Despite moderate heritabilities and sample size, linkage analysis identified several intriguing peaks. The AKT1 phenotype had a maximum LOD score of 1.77 on chromosome 14, near the *AKT1* gene, as well as a peak of 1.44 on chromosome 3p. The chromosome 3 peak overlapped with the best peak for the p70S6K phenotype (LOD=2.23). CANDID, a candidate gene selection algorithm, identified *miR-203*, *AKT1* and *HSP90AA1* as the top candidates on chromosome 14 and *RAF1*, *VHL*, *KAT2B*, *RAB5A* and *GRM7* as the top candidates on chromosome 3. These cell lines were genotyped for tag SNPs as well as potentially functional SNPs in these genes, and a mixed model was used to test for association. Statistically significant evidence for association was found for SNPs in *HSP90*, *RAF1*, and *KAT2B*. Analysis of these SNPs is underway and will soon produce data for (1) possible functional significance, (2) replication in a separate set of cell lines, and (3) association with cancer-related phenotypes in a clinical sample. This data provides the first evidence that phosphorylation levels of key signaling proteins are influenced by inherited variants. The completion of further analyses of these variants may yield valuable insights into cancer pathogenesis.

## 1199/W/Poster Board #857

**Silencing of TPM3 by RNA interference influences invasive potential of human hepatocellular carcinoma cell lines.** *C. Hye-Sun*[1,2], *X. Hai-Dong*[1,2], *S. Seung-Hun*[1,2], *J. Seung-Hyun*[1,2], *C. Yeun-Jun*[1,2]. 1) Department of Microbiology, The Catholic University of Korea, Seoul, Korea; 2) Integrated Research Center for Genome Polymorphism,The Catholic University of Korea, Seoul , Korea.

**Purpose:** In previous study, we already founded that Tropomyosin 3(TPM3) has the oncogenic potential in Hepatocellular carcinoma (HCC) using array-CGH with 1Mb resolution. To elucidate biological functions and molecular mechanisms of TPM3 in HCC, we performed silencing of TPM3 expression using RNA interference (RNAi) method.

**Experimental design:** TPM3-siRNAs were transfected into the HCC cell lines, HepG2 and SNU-475 overexpressing TPM3. To verify the sequence specificity of siRNA, we prepared negative control groups (siRNAs with no significant homology to any known sequences in human genome) and mock control group (just treated reagent). The cells were analyzed by real-time RT-PCR, western blotting for genes expression such as TPM3. Cell growth capacities were detected by colonyformation and anchorage-independent assay. Cell proliferation activity and Cytotoxicity with anti-cancer drug were measured by WST-1 and MTT assay, respectively. Moreover effects of TPM3 on metastatic potentials were examined using matrigel-coated or uncoated polycarbonate membrane.

**Results:** After the TPM3-siRNA transfection, the expression of the TPM3 mRNA was significantly reduced compared to negative control. Western blotting also revealed an obvious reduction of TPM3 protein level in the TPM3 siRNA group. Down-regulation of TPM3 inhibited HCC cell growth, anchorage-independent growth and increased sensitivity for cancer-drug. Furthermore silencing TPM3 resulted in suppression migration and invasion capacities.

**Conclusions:** On the basis of our in vitro data, we suggested that TPM3 plays an important role in the metastatic process in HCC. RNA interference-mediated depletion of TPM3 may be an attractive strategy for the treatment of malignancy HCC.

## 1200/W/Poster Board #858

**Aberrant expression of *let-7* microRNA leads to overexpression of Dicer in oral squamous cell carcinomas.** *A. Jakymiw*[1], *R. Patel*[1], *N. Deming*[1], *I. Bhattacharyya*[2], *C. Stewart*[2], *P. Shah*[1], *D. Cohen*[2], *E. Chan*[1]. 1) Oral Biology, University of Florida, Gainesville, FL; 2) Oral and Maxillofacial Surgery and Diagnostic Sciences, University of Florida, Gainesville, FL.

Recently, several reports have demonstrated that Dicer, an RNase III endonuclease involved in microRNA (miRNA) maturation, is up-regulated in prostate and precursor lesions of lung adenocarcinomas. Furthermore, Dicer protein levels have been reported to be regulated by *let-7*, a miRNA whose expression level has been reported to be reduced in lung cancer. Objectives: To determine whether Dicer is aberrantly expressed in oral squamous cell carcinomas (OSCCs) due to altered expressions of *let-7*. Methods: Dicer protein and mRNA levels were examined by Western blot analysis and real-time-PCR, respectively, in a panel of head and neck squamous cell carcinoma (HNSCC) cell lines, including OSCC cell lines, and compared to normal primary gingival epithelial cells (pGECs). The *let-7* expression levels were analyzed by real-time-PCR. In addition, Dicer protein levels were examined in paraffin embedded OSCC tissues by indirect immunofluorescence studies. Lastly, an OSCC cell line was transiently transfected with either chemically synthesized *let-7* or small interfering RNA (siRNA) targeting Dicer to evaluate the effect on Dicer levels and cell proliferation. Results: Dicer protein was found to be overexpressed in a panel of HNSCC cell lines compared to pGECs. Moreover, Dicer protein was found to be high in OSCC tissues in comparison to normal gingival epithelial tissues. Interestingly, the levels of Dicer mRNA were not significantly up-regulated in all the HNSCC cell lines suggesting a post-transcriptional form of gene regulation. *Let-7* levels were reduced in HNSCC cell lines in comparison to pGECs and transfection of *let-7* resulted in the downregulation of Dicer expression. Lastly, silencing of Dicer inhibited cell proliferation. Conclusion: Dicer is up-regulated in OSCCs due to the aberrant expression of *let-7* miRNA and appears to be important for cell proliferation.

## 1201/W/Poster Board #859

**Development of stem cell based genetic pre-clinical models of astrocytomas.** *D. Kamnasaran*[1,2]. 1) Department of Pediatrics, Laval University, Quebec, Canada; 2) Pediatrics Research Unit, Laval Hospital Research Centre, Quebec, Canada.

Astrocytomas (gliomas) are the most frequent tumors prevalent in adults, and the second most common type of tumors among children. Unfortunately, despite current therapies the prognosis is still poor. One of the major reasons for such poor prognosis is the current paucity of genetic pre-clinical models that recapitulate the genetic, pathological and treatment responses reminiscent in the human tumors. Current in-vitro models such as glioma stem cells are very problematic as a result of having low clonality and variable therapeutic responses. In an effort to explore alternative robust in-vitro models of astrocytomas, we have developed the first non-neural stem cell based genetic model of astrocytomas from murine embryonic stem cells. Specifically, we observed that the "synthetic astrocytes" derived from murine embryonic stem cells are more representative in the origin of astrocytomas, even at high clonality, compared to mature somatic astrocytes. Furthermore, these "synthetic astrocytes" seem to be very similar to immature or progenitor astrocytes. Similar findings are also observed with "synthetic astrocytes" differentiated from murine neural stem cells. Overall, these "synthetic astrocytes" have an intrinsic property of enhanced proliferation that may potentiate an ease towards transformation. We have noted that the genetic alterations of these synthetic astrocytes with candidate astrocytomagenesis genes can potentiate intracranial astrocytomas in Nod-Mice, unlike mature astrocytes harboring the same cocktail of mutations. In addition, we undertook functional random mutagenesis screens on these "synthetic astrocytes" using gene trapping, and have been able to develop novel genetic pre-clinical models of astrocytomas. This body of work augments the use of stem-cell based pre-clinical models to decipher the genetic and pathological basis of astrocytomagenesis. Moreover, these models can also be utilized for future therapeutic targeting applications.

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## 1202/W/Poster Board #860

**Paclitaxel suppresses the viability of MCF-7 through AMPK-mediated EF1α and FoxO3 regulation.** *J. Kim, J. Lee, J. Jung, S. Lee, S. Park, H. Kim.* Korea University College of Medicine, Seoul, Korea.

Paclitaxel (Taxol), a potent drug of natural origin isolated from the bark of the Pacific yew, is widely used in the treatment of ovarian, lung and breast cancer. Up to now, however, there is little information regarding the anti-cancer mechanism of paclitaxel. In order to understand the molecular mechanisms, we investigated the relationship between Taxol and AMPK, a key energy sensor kinase. In results, paclitaxel suppressed the viability of breast cancer MCF7 cells and increased phosphorylation of AMPK. Taxol also down-regulated EF1αwell known transcription factor. Paclitaxel increased the phosphorylation of FoxO3 (Forkhead box O3), a transcription factor for tumor suppression, and its downstream target p21. Moreover, phosphorylation of FoxO3 was suppressed in the presence of AMPK specific inhibition, Compound C, suggesting the involvement of AMPK in paclitaxel-mediated FoxO3 phosphorylation. Furthermore, knock-down of either EF1α or AMPK make cancer cell more susceptible to paclitaxel-mediated FoxO3 induction and caspase-3 activation. Finally, paclitaxel-induced EF1α suppression was not observed in AMPK knout-down cells. All together, these results suggest that paclitaxel may have anti-tumor effects in breast cancer through activation of the AMPK-EF1α-FoxO3 signal pathway.

## 1203/W/Poster Board #861

**Copy number alterations in Korean stomach cancers.** *Y.I. Kim, W.S. Park, S.W. Nam, S.H. Lee, N.J. Yoo, J.Y. Lee, S.H. Kim, B.H. Lee, Y.S. Choi, S.Y. Kim.* Department of Pathology, College of Medicine, The Catholic University of Korea, Seoul, Korea.

Gastric carcinoma is one of the most frequent malignant tumors in Korea. Development of gastric carcinoma is associated with environmental factors and genetic factors. Traditionally, studies on the development of stomach cancers were focused on genetic or epigenetic alterations of specific genes found in malignant tissues. These studies revealed many tumor-related genes participating in the development of stomach cancers. However, role of other area in human genome is still unknown in terms of stomach cancer development. To delineate genome-wide alterations in stomach cancers, we studied copy number alterations in Korean gastric cancers using microarry-based CGH (aCGH). As a result, we found sixty loci of common copy number gain and twenty loci of common copy number loss. Among the copy number gains, MYC, CCNE1 and ERRB2 genes were included. JAK2, CDKN2A and CDKN2B were found in the loci showing copy number loss. The aCGH findings were correlated with the results of quantitative real-time PCR using genomic DNA extracted from tumor tissues and fluorescent in situ hybridization (FISH) using formalin-fixed paraffin-embedded tumor tissues.

## 1204/W/Poster Board #862

**Microsatellite variability, CGI methylation and differential expression patterns in colon cancer.** *M.J. Kovach, C.B. Wiese.* Biological & Environmental Sci, Univ Tennessee, Chattanooga, TN.

The overall goal of this study is to investigate the hypothesis that the genes in the molecular pathway(s) of cancer progression are subject to transcriptional regulation by microsatellite repeat sequence variability. It is our hypothesis that the accumulation of these variants through defects in mismatch repair or the normal aging process contribute to cancer progression by influencing gene expression and chromatin remodeling as it relates to the positioning of regulatory elements, mRNA stability and methylation status of CpG islands. We propose that microsatellite repeat elements represent a normal, but as yet uncharacterized, mechanism of gene regulation in which polymorphisms in microsatellite repeats function to control the local secondary structure of the genome and mRNA transcripts; structures predicted to be important in the recruitment and binding of transcriptional accessory and processing proteins that influence the rate of transcription and transcript stability. Fourteen colon cancer cell lines were evaluated for Microsatellite Instability (MSI), CGI methylation and gene expression of 6 candidate oncogenes. Replication Error prone (RER+) cell lines showed a higher level of MSI than RER- cell lines. More specific, an increase in mean percent variation was observed in RER+ samples for microsatellites located in the first intron (32%) and middle introns (24%). The higher variability noted for microsatellites in the first intron may reflect important mutations in the pathogenesis of cancer. Microsatellites located within the last intron and 3'UTR had the least tolerance for mutations; sequence conservation suggestive of a region of functional and/or structural importance. There was an inverse relationship between the level of MSI and length of the repeat unit; mononucleotide and dinucleotide repeats showed the greatest variation. Among dinucleotide repeats, a 32% increase in variability was observed for those capable of complimentary base pairing. Methylation studies indicated that CpG islands overlapping promoters were hypomethylated, suggestive of the pattern of active gene expression. In contrast, CpG islands located internally were largely hypermethylated and consistent with the reported global hypermethylation of internal regions of genes. Abnormal methylation, and predicted gene repression of *THBS1* and *STK11*, known tumor suppressor genes involved in angiogenesis inhibition, is consistent with the establishment of cancer and tumor growth.

## 1205/W/Poster Board #863

**Comprehensive regulatory mechanisms of the MSMB gene associated with prostate cancer susceptibility.** *H. Lou[1], H. Li[1], M. Yeager[2,3], S. Anderson[1], M. Dean[1].* 1) Cancer & Inflammation Program, NCI/FCRDC, Frederick, MD; 2) Core Genotyping Facility, Advanced Technology Program, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD; 3) Division of Cancer Epidemiology and Genetics, Center for Cancer Research, NCI, NIH, Department of Health and Human Services (DHHS), Bethesda, Maryland.

The significant single-nucleotide polymorphism (SNP) located at 10q11.2 (rs10993994), 57 bp upstream of the transcription start site of the *MSMB* gene, is the most strongly associated marker with prostate cancer risk identified by genome-wide association studies (GWAS). Recent functional work has shown that the different alleles of the promoter SNP, rs10993994, influence *MSMB* expression. To investigate the regulatory mechanisms of the *MSMB* gene, we determined the methylation status of the *MSMB* gene by using bisulphite sequencing. A higher methylation level in a 273 bp GC rich region 2.8 kb upstream of the ATG of the *MSMB* was observed in prostate cancer DU145 cells which have a low level of *MSMB* expression. In contrast, lower methylation levels were confirmed in cell lines with high *MSMB* mRNA expression. In an additional experiment, we investigated the distal and proximal promoter sequences of the *MSMB* gene to characterize the regulatory elements. The distal elements inhibited promoter activity in prostate cancer cells, but enhanced promoter activity in breast cancer cells. A 320 bp length of the proximal promoter sequence of the *MSMB* gene exhibited highest promoter activity. Together, our mapping study and functional analyses implicate the regulation of expression of *MSMB* as a plausible mechanism accounting for the association identified at this locus. Both genetic and epigenetic mechanisms are involved in the regulation of *MSMB* activity.

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## 1206/W/Poster Board #864

**Genetic Predisposition for unilateral Vestibular Schwannoma.** *N. Lüblinghoff, W. Maier, R. Lazig, R. Birkenhäger.* Otorhinolaryngology H&N Surg, University Medical Center Freiburg, D-79106 Freiburg, Killianstr. 5, Germany.

Background: A vestibular schwannoma (VS) is a benign tumor originating from Schwann cells (SC) of the vestibular nerve and located in the cerebello pontine angle or the inner auditory canal. The tumor results from an overproliferation of SC that wrap around nerve fibres, often causing gradual hearing loss, tinnitus and dizziness. It can also interfere with the facial nerve causing paralysis by compression. Early detection of the tumor is sometimes difficult because the symptoms may be subtle and may not appear in the beginning. There are two types of VS: unilateral and bilateral. The genetic background of this tumor is not completely known. Until now there is only a single gene known for bilateral VS leading to neurofibromatosis type 2 (NF2). This gene is located on Chromosome 22, about 60 % of the patients have mutations in the NF2 gene. The average age of onset is 18 to 24 years. Almost all patients develop bilateral VS by the age of 30. Unilateral VS account for approximately 8 % of all cranial tumors. Symptoms may develop at any age, but usually occur between the age of 30 to 60. The exact cause of unilateral VS is unknown, most occur spontaneously. It is our aim to identify genes and factors that are specifically involved in the development of unilateral VS. Methods: Our study group consists of 79 patients (from 77 independent families) of which 17 have bilateral VS (NF2), 58 a unilateral VS and 2 an initial suspicion of a VS. All 79 patients are clinically clearly defined. Genetically we performed a mutation analysis on the NF2 gene by PCR based individual exon and intron-transitions sequencing of the NF2 gene. A genome-wide haplotype analysis was accomplished on the whole genome and on the tumor tissue by utilizing the Affymetrix GeneChip 500 K SNP mapping array. Results: So far we identified 5 different missense/nonsense and splice mutations in NF2 gene which all lead to a loss of function of the gene product Merlin and the appearance of VS [IVS2+1G>A (2x) ,c.809A>G-(Glu270Gly), c.532C>T(p.Gln178ter), c.1396C>T(p.Gln466ter), IVS15+1G>A]. We further identified several polymorphisms in the NF2 gene that do not seem to cause VS. Conclusions: Until now we do not clearly know whether genomic predispositions or exclusively somatic mutations are responsible for unilateral VS; or if additional genomic alterations e.g. loss of LOH's areas or suppressor genes are responsible for the development of this special kind of VS.

## 1207/W/Poster Board #865

**The role of NER pathway in DNA recovery of head and neck squamous cell carcinomas.** *I. Majsterek[1,2], D. Pytel[2,3], M. Markiewicz[1], T. Sliwinski[1], P. Rusin[1], J. Olszewski[4], A. Morawiec-Sztandera[5], J. Szemraj[6].* 1) Department of Molecular Genetics, University of Lodz, Lodz, Poland; 2) Department of Chronopharmacology, Medical University of Lodz, Lodz, Poland; 3) The Abramson Family Cancer Research Institute, Department of Cancer Biology, University of Pennsylvania, Philadelphia, PA, USA; 4) Department of Otolaryngology and Oncology, Medical University of Lodz, Lodz, Poland; 5) Department of Head and Neck Cancer, Medical University of Lodz, Lodz, Poland; 6) Department of Medical Biochemistry, Medical University of Lodz, Lodz, Poland.

Head and neck squamous cell carcinomas (HNSCC) comprise about 6% of all malignant neoplasms. One of the major risk factor of HNSCC is tobacco smoking. There are approximately 4 thousand chemical compounds identified in tobacco smoke. Many of them like polycyclic aromatic hydrocarbons (PAHs) and aromatic amins (AA) are well known carcinogens which form adducts with DNA. Cells remove those adducts mainly by using nucleotide excision repair (NER). We examined DNA repair efficiency in peripheral blood lymphocytes obtained from HNSCC patients and healthy subjects as well as HTB-43 larynx and CRL-1628 tongue squamous cancer cells. NER activity was assessed in the cell extracts by use of an UV-irradiated plasmid as a substrate. After in vitro repair the incorporation of [gamma 32P]-dAMP during strand resynthesis as equivalent to NER efficiency was quantified by densitometry. Results obtained from these experiments indicate that there is a decrease in the efficacy of NER in HTB-43, CRL-1628 cells and HNSCC lymphocytes compared to healthy controls. In conclusion, we suggest that NER pathway is critical for DNA recovery of HNSCC cells what in turn may be responsible for higher susceptibility to mutagenesis and cancer transformation. This work was supported by grant N301 099 32/3581 from Polish Ministry of Science and Higher Education.

## 1208/W/Poster Board #866

**IL18, LUM, PTGER4 and SPHK1 are transcriptional targets of ETV6, a tumour suppressor implicated in childhood leukemia.** *C. Malouf[1,2], S. Langlois[1], J. Larose[1], D. Sinnett[1,3].* 1) Division of Hematology, Research Center CHU Sainte-Justine, Montreal, Canada, H3T 1C5; 2) Department of Biochemistry, Faculty of Medicine, University of Montreal, Montreal, Canada, H3T 1J4; 3) Deparment of Pediatrics, Faculty of Medicine, University of Montreal, Montreal, Canada, H3T 1C5.

Acute Lymphoblastic Leukemia (ALL) is the most frequent pediatric cancer accounting for ~25% of all cases. The most frequent genetic alteration that has been associated with ALL is the t(12;21) ETV6-AML1 translocation, which results in the formation of the chimeric transcription factor ETV6-AML1. Deletion of the residual allele of ETV6 has been observed in 20-40% of pre-B ALL, which strongly suggests that ETV6 functions as a gene tumour suppressor. ETV6 is a transcriptional repressor that belongs to the ETS family of transcription factors with very few known targets. We previously identified 87 genes that were co-modulated following expression of ETV6. The expression of four of these genes, interleukin-18 (IL18), lumican (LUM), prostaglandine E2 receptor EP4 subtype (PTGER4) and sphingosine kinase 1 (SPHK1) was significantly correlated with that of ETV6. Using gene reporter assays and chromatin immunoprecipitation in Jurkat (leukemic T lymphocyte) and HeLa (cervical) cells, we confirm that IL18, LUM, PTGER4 and SPHK1 are transcriptional targets of ETV6. ETV6 has two functional domains specific to the Ets family, a pointed helix-loop-helix domain mediating protein interactions and oligomerization and an ETS DNA-binding domain. We found that both functional domains are necessary to repress the activity of the IL18, LUM, PTGER4 and SPHK1 promoters. Transcriptional repression of these targets by ETV6 takes place in a small region close to the basal promoter and, unlike other Ets factors, does not require a consensus ETS-binding site (EBS). The validation of four novel transcriptional targets of ETV6 that are frequently up-regulated in childhood ALL will help us to elucidate the molecular functions of ETV6 in physiological and pathophysiological conditions.

## 1209/W/Poster Board #867

**Molecular analysis of endometrial tumorigenesis in Lynch syndrome and sporadic cases.** *T.T. Nieminen[1], A. Gylling[1], W.M. Abdel-Rahman[2], K. Nuorva[3], M. Aarnio[4], L. Renkonen-Sinisalo[5], H.J. Järvinen[5], J-P. Mecklin[4], R. Butzow[6,7], P. Peltomäki[1].* 1) Med Gen, Univ Helsinki, Helsinki, Finland; 2) College of Health Sciences, University of Sharjah, Sharjah, United Arab Emirates; 3) Department of Pathology, Jyväskylä Central Hospital, Jyväskylä, Finland; 4) Department of Surgery, Jyväskylä Central Hospital, Jyväskylä, Finland; 5) Second Department of Surgery, Helsinki University Hospital, Helsinki, Finland; 6) Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Helsinki, Finland; 7) Department of Pathology, Helsinki University Central Hospital, Helsinki, Finland.

PURPOSE. Endometrial carcinoma (EC) is common in the population and the most frequent extracolonic malignancy in hereditary nonpolyposis colorectal carcinoma (HNPCC)/Lynch syndrome. We characterized endometrial hyperplasias, precursor lesions of endometrioid EC, to identify molecular markers of malignant transformation and tumor progression. EXPERIMENTAL DESIGN. Serial specimens of normal endometrium, simple hyperplasia (SH) , complex hyperplasia without atypia (CH), complex hyperplasia with atypia (CAH), and EC obtained during a ten-year surveillance of DNA mismatch repair (MMR) gene mutation carriers (together 71 samples) were analyzed for MMR protein expression, microsatellite instability (MSI), and promoter methylation of tumor suppressor genes. Results were compared to a sporadic reference series of endometrial specimens taken for non-malignant reasons (133 samples). RESULTS. Among MMR gene mutation carriers, decreased MMR protein expression was present in 7% in normal endometrium, 40% in SH, 100% in CH, 92% in CAH, and 100% in EC. MSI frequencies were lower (6%, 17%, 67%, 39%, and 64%, respectively). Among 24 tumor suppressor genes, the number of methylated loci increased from normal endometrium to SH to complex hyperplasia (CH/CAH) in both Lynch syndrome and reference series. The most frequently methylated genes were CDH13, RASSF1A, and GSTP1. In MMR gene mutation carriers, MMR and methylation defects appeared up to 12 years prior to EC. CONCLUSIONS. Increased methylation (and abnormal MMR in Lynch syndrome patients) classifies normal endometrium and SH into one category, and CH, CAH, and EC into another, suggesting that - contrary to a traditional view - CH and CAH are equally important as precursor lesions of EC. Furthermore, in genetically predisposed individuals, molecular changes are detectable in even normal endometrial tissue several years before EC.

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### 1210/W/Poster Board #868

**Understanding genetic selection in the breast cancer genome.** *N.I. Park[1], P.K. Rogan[2,3], J.H.M. Knoll[1,4].* 1) Pathology; 2) Biochemistry; 3) Computer Science, University of Western Ontario; 4) London Health Sciences Centre, London, ON, Canada.

Confronted with frequent chromosome instability and gene mutation, the minimal genome of a tumor cell would have to maintain the ability of self-renewal and differentiation, plasticity, unlimited growth potential, and the ability to form tumors de novo. We are examining selective forces in the breast cancer genome, with respect to specific pathways, to determine if the minimal genome encodes proteins that are consistently targets of therapeutic drugs. Computational analysis of the genomes of 243 previously reported breast tumors (*Gen Res.*2006.16:1465) revealed 2,089 unstable (amplified and/or deleted) and 2,114 stable (or quiescent) genomic regions. Unstable regions contained 1,500 single copy (sc) intervals (>10bp), corresponding to 427 protein coding genes (CCDS database). Stable regions contained 84,843 sc intervals >100bp in length and 9,839 sc intervals >500bp long, corresponding to 2,503 genes. This corresponded to 1,891 (>100bp) and 622 (>500bp) genes in the NCBI Entrez database. 428 genes were identified which were common to both sets of sc interval-related, quiescent regions. This set was characterized according to gene ontology and pathway analysis. GO analysis showed that stable regions were enriched relative to unstable ones for genes encoding nucleosome assembly proteins, interferon receptor binding, intermediate filament components, monooxygenase activity, and neuropeptide signaling (p≤0.01). This gene set is enriched for pathways in which some of the functions are targeted by therapeutics: NK-cell mediated cytotoxicity (Cetuximab), cytokine-cytokine receptor interaction, antigen processing and presentation (Pegasys), toll-like receptor signaling, regulation of autophagy, cell adhesion pathways and Jak-STAT signaling (Avastin). Analyses of gene ontologies and pathways for coding genes in >500bp sc quiescent intervals suggest enrichment of genes encoding oxygen transport, defense response, tubulin binding, molybdopterin cofactor metabolism, and regulation of DNA repair (p≤0.01). *PARP4* was among the genes enriched for DNA repair regulation and DNA metabolism. The GO and pathways for >100bp sc quiescent intervals include several other members of this gene family. PARP-inhibitors appear to be effective for targeting ER/PR/HER2 negative breast tumors. This study suggests that conservation of this minimal gene set may explain the effectiveness of potential chemotherapeutic agents and interactions in breast cancer.

### 1211/W/Poster Board #869

**Copy number variation in low- and high-grade breast tumors.** *H. Patney[1], J. Kane[1], J. Weyandt[1], J. Hooke[2], C. Shriver[2], R. Ellsworth[3].* 1) Clinical Breast Care Project, Windber Research Inst, Windber, PA; 2) Walter Reed Army Medical Center, Washington DC; 3) Henry M. Jackson Foundation for the Advancement of Military Medicine, Windber, PA.

**Objective** Histological grade classifies breast carcinomas into low-, intermediate- and high-grade. Developmental pathways for high-grade disease remain controversial; clinical data have been used to describe both linear models, in which high-grade tumors evolve from low-grade precursors, as well as models that depict low- and high-grade carcinomas as distinct molecular diseases. Genome-wide copy number alterations were examined in low- and high-grade breast tumors to identify genetic changes associated with the etiology of low- and high-grade breast carcinomas. **Methods** Tumors were diagnosed and characterized by a single, dedicated breast pathologist. DNA was isolated after laser microdissection from low- (n= 20) and high-grade (n=57) tumors. Genotype data were generated using Human Mapping 250K Sty arrays (Affymetrix). Copy number alterations and LOH were detected using Genotyping Console 3.0.2 (Affymetrix). **Results** The number of chromosomal arms with alterations was significantly higher (P<0.005) in high- (mean 12 chromosomal arms, range = 0-30 arms) compared to low-grade (average 5 chromosomal arms, range = 1-18 arms). The most frequent alterations in low-grade disease were gains of chromosomes 1q and 16p and loss of 16q in 55%, 40% and 60% of samples, respectively. These alterations were frequently simple and included the entire chromosomal arm. Alterations on chromosomes 2, 3, 4, 9, 10, 14, 16 18, 19 and 21 were rare or undetected. In contrast, patterns of alterations were complex in high-grade tumors, frequently with alternating gains and losses on a single chromosomal arm. The most common alterations in high-grade tumors were gain of chromosome 1q (63%) with gain of 8q, 17q and 20q in >40% of tumors. Chromosome 8p was the most frequently lost arm (49%) with additional loss of chromosomes 5q and 17p in >40% of tumors. Loss of 16q occurred significantly (P<0.005) less frequently in high (25%) compared to low-grade tumors (60%). **Conclusion** Low- and high-grade breast tumors are genetically different. Gain of chromosome 1q was common in both low- and high-grade tumors, and may be an early event in tumorigenesis. Given the significantly lower rate of loss of chromosome 16q in high- compared to low-grade tumors, high-grade disease may not arise from dedifferentiation of low-grade but rather represent a separate disease defined by unique genetic alterations.

### 1212/W/Poster Board #870

**Screening for germline mutations of DNA mismatch repair (MMR) genes in colorectal cancer patients: Implications for a population specific detection strategy for Lynch syndrome.** *M. Ravnik-Glavac[1,2], G. Berginc[2], D. Glava[2].* 1) Institute of Biochemistry, Faculty of Medicine, Vrazov trg 2, University of Ljubljana, Ljubljana, Slovenia; 2) Department of Molecular Genetics, Faculty of Medicine, Vrazov trg 2, University of Ljubljana, Ljubljana, Slovenia.

Colorectal cancer (CRC) is one of the commonest cancers wordwide. The risk factors for CRC are both environmental and genetic. Approximately 75% of CRCs are sporadic; the rest are hereditary or belong to a familial syndromes. Identification of familial forms of CRC and their genes have enabled the development of several models of carcinogenesis. Molecular diagnostics tools are available on a routine basis for the commonest hereditary colon cancer syndromes including Lynch syndrome. Microsatellite instability (MSI) is present in more than 90% of Lynch syndrome cases, and is therefore a feasible marker for the disease. Mutations in MLH1, MSH2, MSH6 and PMS2, which are one of the main causes of deficient mismatch repair and subsequent MSI, have been linked to the disease. In order to establish the role of each of the 4 genes in Slovenian Lynch syndrome patients, we performed MSI analysis on 593 unselected CRC patients and subsequently searched for the presence of point mutations, larger genomic rearrangements and MLH1 promoter hypermethylation in patients with MSI-high tumours. We detected 43 (7.3%) patients with MSI-H tumours, of which 7 patients (1.3%) harboured germline defects: 2 in MLH1, 4 in MSH2, 1 in PMS2 and none in MSH6. Twenty-nine germline sequence variations of unknown significance and 17 deleterious somatic mutations were found. MLH1 promoter methylation was detected in 56% of patients without detected germline defects and in 1 (14%) suspected Lynch syndrome patients. Due to the specific absence of germline defects in MSH6, we adapted the Lynch syndrome detection strategy for the Slovenian population of CRC patients, whereby germline alterations should be first sought in MLH1 and MSH2 followed by a search for larger genomic rearrangements and PMS2 mutations, and when no germline defects are found, mutation analysis of the MSH6 gene should be performed. Our study demonstrates that the incidence of MMR mutations in a population should be known prior to the application of one of several suggested strategies for detection of Lynch syndrome.

### 1213/W/Poster Board #871

**Evaluation of hormone receptor, erbb2 and chromosome 16 status in transition areas from in situ to invasive breast carcinomas.** *R.M. Rodrigues-Peres[1], S.F.M. Derchain[2], J.K. Heinrich[1], K.P. Serra[2], J.H. Yoon[3], G.A. Pinto[1], M. Alvarenga[2], F.A. Soares[4].* 1) CAISM - Women's Hospital - State University of Campinas - UNICAMP, Campinas, SP, Brazil; 2) Department of Obstetrics and Gynecology, Faculty of Medical Sciences, State University of Campinas - UNICAMP, Campinas, SP, Brazil; 3) Faculty of Medical Sciences, State University of Campinas - UNICAMP, Campinas, SP, Brazil; 4) Hospital do Câncer AC Camargo, São Paulo, SP, Brazil.

There is evidence indicating that the transition from ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC) is regulated by a restrict number of genes. It remains unknown whether erbB-2 and hormone receptor status predict the invasive potential of DCIS. By the other hand, as chromosome 16 is one of the most altered chromosomes in breast cancer and frequently presented in an aneuploid status, we investigated whether its ploidy status could be used as a marker of genetic instability in in the specific areas of breast tumors which share both components of DCIS and IDC, concomitantly, indicating a possible transition. 107 cases of breast malignancies harboring contiguous regions of DCIS and IDC were selected. TMA (tissue microarrays) were mounted with separate cores, one from the DCIS and other from the neighboring IDC, from the original paraffin block. ErbB-2 and ER/PR status were assessed using conventional immunohistochemistry techniques and chromosome 16 status was verified through FISH using a centromeric probe. Clinicopathological data from the patients and from the disease were retrieved out of the patients' medical records. Results: Chromosome 16 status did not differ between the areas. Approximately 35% of the invasive areas were erbB-2 2+ or 3+, compared to 47% of their in situ counterparts. The expression of erbB-2 did not differ in the in situ and in the invasive components of the breast tumors (p=0.12). The PR status was not statistically different comparing the in situ and invasive regions of the tumors (p=0.06). By contrast, there was a slight imbalance in ER status (p= 0.04). The intraclass correlation coefficient (ICC) disclosed good agreement in sample-by-sample comparisons of erbB-2 (ICC = 0.61), PR (ICC= 0.61) and ER (ICC=0.70) expression in the in situ and invasive components. Our findings suggest that the expression of erbB-2 and ER/PR does not differ accross the in situ and invasive components of breast malignancies, especially in the transition region from one component to the other. It is expected that the molecular alterations which take place before the morphologic tissue changes persist in the invasive forms of breast tumors. The ocurrance of concomitant epigenetic factors may explain why some in situ tumors develop to an invasive phenotype, while other in situ tumors with similar molecular characteristics do not have the same evolution, supporting the hypothesis for a multifactorial basis for disease progression.

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## 1214/W/Poster Board #872

**Loss of nucleolar tumor suppressor function plays a role in breast cancer initiation.** *S. Rossetti[1], A.T. Hoogeveen[2], J. Esposito[1], N. Sacchi[1].* 1) Cancer Genetics Program, Roswell Park Cancer Inst, Buffalo, NY; 2) Dept. Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands.

Altered nucleolar function and aberrant transcription of ribosomal genes (rDNA) are common in cancer. However, whether increased ribosomal RNA (rRNA) synthesis is an initiating cause rather than a mere consequence of cell transformation is poorly understood. MTG16a (CBFA2T3), a member of the MTG family of transcriptional co-repressors, is a suppressor of breast tumorigenesis with a nuclear/nucleolar localization. We found that knock down of MTG16a in human mammary epithelial cells leads to impaired acinar morphogenesis in vitro, a phenotype indicative of cell transformation. We could specifically trace this phenotype to the loss of MTG16a nucleolar function by expressing a mutant MTG16 protein able to re-localize wild type MTG16a from the nucleolus to the nucleoplasm. We further demonstrated that MTG16a plays a role in rDNA repression by gain-of-function and loss-of-function evidence. We identified two candidate mechanisms of MTG16a-mediated rRNA repression. First, MTG16a can interact both with the rDNA promoter and the major component of the Nucleolar Remodeling Complex (NoRC), which is known to impose repressive chromatin changes at rDNA regulatory regions. Second, we demonstrated that MTG16a effectively counteracts MYC-driven, RNA Polymerase I-mediated, rRNA transcriptional activation in cells over expressing MYC. In conclusion, our data support the conclusion that loss of MTG16a tumor suppressor function in the nucleolus plays a role in breast cancer initiation by inducing increased rRNA synthesis. This work was supported by the Susan Komen Foundation (NS and SR).

## 1215/W/Poster Board #873

**Epigenetic silencing of a tumor suppressor network reveals doubled edged effects of master cell signals.** *N. Sacchi, S. Rossetti.* Cancer Genetics Program, Roswell Park Cancer Institute, Buffalo, NY.

Epigenetic silencing of tumor suppressor genes is common in breast cancer cells. We found that an aberrant signaling of retinoic acid (RA) via the RA receptor alpha (RARA) results in the concerted epigenetic silencing of a tumor suppressor gene network downstream of RARA. This network includes the RA receptor beta 2 (RARB2), which mediates RA growth-inhibitory action, and TGFBR2, the main receptor of transforming growth factor beta (TGFB). Unexpectedly, we observed that both RA and TGF beta signals, which have anticancer effects in normal cells, exacerbate the tumor phenotypic features of cancer cells that underwent loss of RARB2 and TGFBR2 tumor suppressor activities. Apparently, as a consequence of epigenetic silencing of canonical receptors, master signals such as RA and TGFB, exploit alternate targets to promote, rather than inhibit, tumorigenesis. This work was partially supported by the National Cancer Institute grant NCI R01-CA127614-01 (NS).

## 1216/W/Poster Board #874

**Identification of Novel VHL-Regulated Proteins Using Proteomic Profiling in Transgenic *Drosophila*.** *T.N. Seagroves[1,2], S. Doronkin[3], I. Djagaeva[3], L.T. Reiter[4].* 1) Dept. of Pathology, University of Tennessee Health Science Center, Memphis, TN; 2) Center for Cancer Research; 3) Dept. of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN; 4) Dept. of Neurology, University of Tennessee Health Science Center, Memphis, TN.

Von Hippel-Lindau (VHL) syndrome affects ~1/36,000 individuals as a result of mutations in the tumor suppressor gene *VHL*. Type 1 VHL patients develop renal cell carcinoma (RCC) without pheochromocytoma; type 2A or 2B patients develop pheochromocytoma without or with high risk for RCC and pancreatic tumors, respectively. The majority of patients also develop hemangioblastomas in the nervous system. As RCC is highly refractive to current therapies, and up to 80% of sporadic RCCs harbor mutations in *VHL*, understanding how VHL promotes tumorigenesis is critical to develop novel therapeutics. VHL is known to mediate the proteosome-dependent turnover of the Hypoxia-Inducible Factor (HIF) transcription factors, directly contributing to the VHL phenotype. However, there have been limited proteomic screens to identify novel VHL-regulated proteins, and, no profiling has been attempted using a whole model organism without tissue bias. We utilized the GAL4/UAS system to create *Drosophila* (fruit flies) that over-express high levels of VHL or Vhl (the fly ortholog) globally in response to heatshock. We also developed two methods using flies to assay for biological function of the 25 VHL-regulated candidate proteins identified in our screen. First, we analyzed the consequences of hypoxic exposure or genetic manipulation of the HIF/VHL pathways upon the migration of border cells in the *Drosophila* ovary, which is a model of metastasis. In response to 1%$O_2$ or VHL/Vhl over-expression, border cells entered a diverse migratory mode, ranging from blocked border cell migration (BCM) to an unprecedented acceleration. In contrast, loss of function or over-expression of *sima* (the ortholog of HIF-1$\alpha$) modulated BCM in a non-linear, dose-dependent manner. Mosaic clone analysis revealed that HIF transcriptional activity is specifically required in the leading cell of the BC cluster since loss of either *sima* or *tgo* (the HIF-1$\beta$ ortholog) produced BCM phenotypes. Second, we found that modulation of Vhl expression in the peripheral sensory neurons of larvae disrupted dendritic arborization (DA). We are currently assaying how proteins identified in our profiling screen modulate these VHL/Vhl-dependent BCM and DA phenotypes. Functional analysis of candidate proteins in *Drosophila* may ultimately reveal pathways conserved in humans that are amenable to therapeutic intervention.

## 1217/W/Poster Board #875

**P21 (Waf1/Cip1) Gene Polymorphism, Protein Expression of P53, P21 and MDM2 in Esophageal Squamous Cell Carcinoma in Northeastern Iran.** *N. Taghavi[1], F. Biramijamal[1], MR. Abbaszadegan[3], M. Sotoudeh[2], H. Khademi[2], R. Malekzadeh[2].* 1) Dept. of Medical Genetics, National Institute of Genetic Engineering & Biotechnology (NIGEB), Tehran, Iran; 2) Digestive Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran; 3) Division of Human Genetics, Immunology Research Center, Bu-Ali Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran.

Background: The incidence of Esophageal Squamous Cell Carcinoma [ESCC] is very high in northeastern Iran. However; the genetic predisposing factors in this region have not been clearly defined. P21 (waf1/cip1) gene involves in cellular growth arrest, induced by p53 tumor suppressor gene. The p21 polymorphism, in codon 31 (p21 C98A, dbSNP rs1801270) may affect the protein expression and play a role in cancer susceptibility. Methods: The role of p21 polymorphism on the risk of ESCC in relation to P21, P53 and MDM2 protein expression was evaluated in a case-control study in northeastern Iran. The current analyses include 60 ESCC patients (aged 35-83), and 60 age and sex matched controls, enrolled from 2006-2007. Genotyping of p21 polymorphism was determined using PCR-RFLP method and the expression of p21, p53 and MDM2 proteins was analyzed by immunohistochemistry for 38 of the recruited cases and controls. Results: The p21 polymorphism was not associated with ESCC formation ($P>0.05$). However, tobacco smoking may have synergistic interaction with P21 polymorphism in ESCC carcinogenesis. The expression of p21 and p53 proteins was significantly higher in cases ($P=0.031$ for p21 and $P=0.0001$ for p53). P21 genotype did not correlate with p21 or p53 protein expression ($P> 0.05$). In addition, we found a significant difference in cases between smokers and non-smokers in p53 overexpression ($P=0.045$). Conclusion: Our data suggests; the p21 polymorphism at codon 31, is not genetic susceptibility biomarker for ESCC in northeastern Iran. However, its interaction with tobacco smoking, suggests some environmental factors may be involved in association with the p21 polymorphism in ESCC development in northeastern Iran. This polymorphism and p53 overexpression may consequently be a candidate genetic marker for screening ESCC risk in association with exposure to particular environmental carcinogens in this region of Iran.

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## 1218/W/Poster Board #876

**Silencing of tumor suppressor genes *RASSF1A*, *SLIT2* and *P16*, through genomic loss and promoter hypermethylation in hereditary breast carcinomas.** *T. Tapia[1], C. Alvarez[1], E. Castillo[1], A. Corvalan[2], M. Camus[2], M. Alvarez[2], MP. Carvallo[1].* 1) Department of Cell and Molecular Biology, P. Universidad Catolica de Chile; 2) Centro de Cancer, Faculty of Medicine, P. Universidad Catolica de Chile, Santiago, Chile.

Breast cancer is one of the most common cancers in women worldwide and the second cause of death by cancer in Chile. The molecular mechanisms responsible for the progression of breast cancer remain still unknown, although several studies have been performed in order to identify genes participating in this process. It has been well established that tumor suppressor gene silencing is one important event in tumor development, and that it may occur through epigenetic modifications or genomic alterations. We analyzed 50 breast tumor biopsies through array-CGH and found several chromosomal losses involving tumor suppressor genes. Among these *RASSF1A* (chromosome 3p21.3) and *SLIT2* (chromosome 4p15.2), have been reported to be silenced by hypermethylation of its promoters in several tumors including sporadic breast carcinomas. Another gene silenced by this epigenetic alteration is $P16^{INK4A}$, a tumor suppressor gene that actively regulates cell cycle progression. We studied the methylation status of *RASSF1A, SLIT2* and *P16* gene promoters in 47 hereditary breast tumors. These regions were amplified by MS-PCR, from sodium bisulphite modified tumor DNA, by using specific primers for the methylated and not methylated conditions. Our results show that 61.3 % of hereditary tumors present promoter hypermethylation for *RASSF1A* , 86 % for *SLIT2* and a 2.6 % for *P16*. Five tumors showing genomic loss at the *RASSF1A* locus also presented promoter hypermethylation. In the case of *SLIT2* , correlation between genomic deletion and methylation was found in two tumors. These observations suggest inactivation of both alleles by different mechanisms in these two genes. Six tumors showed only the methylated *SLIT2* product, and five tumors showed only the methylated *RASSF1A* product, not presenting genomic losses at these loci. These observations are in agreement with an epigenetic modification of both alleles as a molecular mechanism for gene silencing. The present observations are consistent with the participation of *RASSF1A* and *SLIT2* hereditary breast tumor progression. Financed by Fondecyt 1080595.TTapia is a Conicyt Doctoral fellow.

## 1219/W/Poster Board #877

**Rare, evolutionarily unlikely missense substitutions in ATM confer increased risk of breast cancer.** *S.V. Tavtigian[1], D. Babikyan[1], A. Hartmann[2], S. Healey[3], F. Le Calvez-Kelm[1], F. Lesueur[1], G.B. Byrnes[1], S.C. Chuang[1], M. Hashibe[1], A. Thomas[4], C. Voegele[1], J. Hall[5], S. Sangrajrang[6], J.L. Hopper[7], M.C. Southey[7], I.L. Andrulis[8], E.M. John[9], P.J. Oefner[2], G. Chenevix-Trench[3], Australian Cancer Study, BCFR, kConFab.* 1) Genetic Cancer Susceptibility, Intl Agency Res Ctr, Lyon, France; 2) Institute of Functional Genomics, University of Regensburg, Regensburg, Germany; 3) Queensland Institute of Medical Research, Brisbane, Australia; 4) Department of Biomedical Informatics, University of Utah School of Medicine, Salt Lake City, UT, USA; 5) Institue Curie, Recherche, INSERM U612, Orsay, France; 6) Research Division, National Cancer Institute, Bangkok, Thailand; 7) Centre for MEGA Epidemiology, University of Melbourne, Carlton, Victoria, Australia; 8) Cancer Care Ontario, Fred A. Litwin Center for Cancer Genetics, Samuel Lunenfeld Research, Mount Sinai Hospital, Toronto, ON, Canada; 9) Northern California Cancer Center, Fremont, CA, USA and Department of Health Research and Policy, Stanford University School of Medicine, Stanford, CA, USA.

The susceptibility gene for Ataxia telangiectasia, ATM, is also an intermediate-risk breast cancer susceptibility gene. However, the spectrum and frequency distribution of ATM mutations that confer increased risk of breast cancer have been controversial. To assess the contribution of rare variants in this gene to risk of breast cancer, we pooled data from seven published ATM case-control mutation screening studies including a total of 1544 breast cancer cases and 1224 controls with data from our own mutation screening of an additional 987 breast cancer cases and 1021 controls. Using an in silico missense substitution analysis that provides an ordered ranking of missense substitutions from evolutionarily most likely to least likely, we carried out analyses of protein truncating variants, splice junction variants, and rare missense variants. We found marginal evidence that the combination of ATM protein truncating and splice junction variants contribute to breast cancer risk. In contrast, there was stronger evidence that a subset of rare, evolutionarily unlikely missense substitutions confer increased risk. On the basis of subset analyses, we hypothesize that rare missense substitutions falling in and around the FAT, kinase, and FATC domains of the protein may be disproportionately responsible for that risk, and that a subset of these may confer higher risk than do protein truncating variants.

## 1220/W/Poster Board #878

**Genome-wide copy number analysis of NF1-MPNSTs using a high resolution Affymetrix SNP6.0 platform.** *M. Upadhyaya[1], G. Spurlock[1], L. Kluwe[2], N. Thomas[1], A. Guha[3], V. Mautner[2], J. Yan[4].* 1) Medical Genetics, Cardiff University, Cardiff, Wales, United Kingdom; 2) Department of Neurosurgery, University Clinic Hamburg-Eppendorf, Hamburg, Germany; 3) The Arthur and Sonia Labatt Brain Tumor A Research Centre, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; 4) Almac Diagnostics, Durham, North Carolina USA.

Neurofibromatosis type 1 (NF1), a familial cancer syndrome affecting 1 in 3,500 individuals worldwide, is characterised by the development of benign and malignant peripheral nerve sheath tumours. NF1 patients have a 7-13% lifetime risk of developing an aggressive malignant peripheral nerve sheath tumour (MPNST) that results in significant morbidity and mortality. MPNST usually develop from either an existing benign plexiform neurofibroma, or a focal subcutaneous neurofibroma. MPNSTs are difficult to diagnose as the clinical symptoms of cancerous tumours overlap with benign neurofibromas. Surgical intervention is a potentially hazardous method of determining whether a deep-seated lesion is benign or malignant.. Genomic DNA analysis has identified copy number alterations in many solid tumours including MPNSTs, where such changes are predicted to confer a selective advantage for cell proliferation and clonal expansion, both essential aspects of tumorigenesis. This is the first study aimed at identifying diagnostic and prognostic tumour-specific markers associated with MPNST using high resolution Affymetrix SNP 6.0 microarray platform. DNA from 15 NF1 derived MPNST and 5 benign plexiform neurofibromas (PNF), all with paired blood samples, were analysed for loss-of-heterozygosity (LOH) and genomic copy number variants (CNV). Data were processed using Affymetrix Genotyping Console 2.1 and analyzed via Partek Genomics Suite 6.4 for quality control. Hidden Markov Model (HMM) and Genomic Segmentation algorithms were used to detect amplified and deleted genomic regions. The genomic aberrations identified in MPNST DNA showed specific CNV and LOH changes, with no similar aberrations evident in PNF DNA. This study confirmed previous genes associated with MPNST but also identified several additional genes: PDGFRB (5q31-q32), ITGB8 (7p15.3), PDGFA and RAC1 (7p22) and MMP12 (11q22.3). Biological pathway analysis was used to identify potential target genes of clinical significance and significantly 8 genes (ACTB, ACTG1, LIMK1, PRKCA, PTK2, RAC1, ROCK2, TRIO) all involved in cytoskeletal remodelling and cell adhesion were found to be significantly amplified in MPNST. This is thus the first study to highlight specific pathway in the development of MPNSTs. These genes and their products are potential therapeutic and prognostic targets in these malignant tumours, which currently lack an effective treatment or reliable prognostic and diagnostic markers.

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## 1221/W/Poster Board #879

**The secretory small GTPase Rab27B regulates invasive tumor growth and metastasis through extracellular HSP90α.** *W. Westbroek[1], D. Maynard[1], A. Hendrix[2], O. De Wever[3], M.C. Seabra[4], W.A. Gahl[1].* 1) Med Gen Branch, NHGRI/MGB/NIH, Bethesda, MD; 2) Dept Med Oncology, ghent University Hospital, Ghent, Belgium; 3) Lab of Exp Cancer Res, Ghent University Hospital, Ghent, Belgium; 4) Dept Mol Med, NHLI, Imperial College, London, UK.

Tumors achieve invasive growth by delivering factors into the tumor microenvironment; the molecular mechanisms for secretion of these pro-invasive factors remain largely unknown. One likely process involves vesicle exocytosis. Key players in exocytic and endocytic membrane trafficking include small Rab GTPases. Rabs are post-translationally modified by geranylgeranylation for membrane interaction and serve as molecular switches, oscillating between their GTP-bound active and GDP-bound inactive state. Our studies on various secretory Rab GTPases revealed that over-expression of Rab27B in several breast cancer cell lines promoted cell cycle progression, F-actin reorganization, and invasion into matrigel. By establishing MCF-7 cells that stably expressed wild type Rab27B, constitutively active Rab27B-Q78L (defective in GTP hydrolysis), dominant negative Rab27B-T23N (defective in GTP binding), and the geranylgeranyl mutant Rab27B-GER, we determined that Rab27B-mediated invasion was achieved when Rab27B was geranylated and in its active GTP-bound state. Our in vivo xenograft mouse model showed that nude mice injected with MCF-7 Rab27B or Rab27B-Q78L developed invasive xenografts that were 8-fold larger than the non-invasive MCF-7 Rab27B-T23N or Rab27B-GER xenografts. Proteomic analysis of Rab27B-positive purified vesicles and the secretome of MCF-7 Rab27B identified heat shock protein HSP90α as a key pro-invasive factor. By ELISA assays and western blotting, we showed that HSP90α secretion was upregulated in the conditioned media prepared from MCF-7 Rab27B and Rab27B-Q78L. In addition, treatment with an HSP90α neutralizing antibody reversed the in vitro invasive phenotype of MCF-7 Rab27B cells, while treatment with recombinant HSP90α of MCF-7 cells induced invasion in a dose-dependent manner. Finally, we examined endogenous Rab27B expression status in 60 different clinical samples derived from breast cancer patients by real time quantitative PCR and immune-histology. We showed that upregulation of endogenous Rab27B mRNA and protein correlated with lymph node metastasis, tumor grade and positive ER-status. In summary, we identified Rab27B as an important player in invasion and metastasis; Rab27B could be a critical marker in the signature of ER-positive breast cancers with poor prognosis. Considerable therapeutic potential may reside in efforts to control Rab27B levels and modulate Rab27B-regulated pathways through pharmacologic or genetic interventions.

## 1222/W/Poster Board #880

**Pathway Analysis of Genes with Copy Number Aberrations in Cancers.** *Q. Zhang[1], L. Ding[2], D. Larson[2], L. Lin[2], I. Borecki[1], M. Province[1].* 1) Divsion of Statistical Genomics, Washington University School of Medicine, St Louis, MO; 2) Genome Center, Washington University School of Medicine, St Louis, MO.

DNA copy number aberration (CNA) is a significant hallmark of genomic abnormality in tumor cells. Genes with CNAs in cancer patients may play important roles in oncogenesis. To understand the pathway distribution of genes in CNA regions, we conducted both gene-based and KEGG pathway-based analysis on DNA array data for 357 lung cancer (adenocarcinoma) patients and 193 brain cancer (glioblastoma) patients from the tumor sequencing project (TSP) and the cancer genome atlas (TCGA), respectively. We identified 3421 (out of 15427) genes and 3132 (out of 23100 genes) that have significant CNAs (FDR<0.05 and Frequency>0.05) in lung cancer patients and brain cancer patients, respectively. A pathway enrichment analysis indentified 25 significant CNA-enriched pathways (most related to metabolism and cellular processes) , including the pathways of Starch and Sucrose Metabolism, Fatty Acid Biosynthesis, Amino Acid Metabolism, Signaling Molecules and Interaction, Regulation of Autophagy, T Cell Receptor Signaling, Adipocytokine Signaling, Hematopoietic Cell Lineage, Cell Adhesion Molecules, Progesterone-mediated Oocyte Maturation, etc. The differences of pathway enrichment between cancer types, smoking status, genders, tumor grades and stages were also be investigated and reported.

## 1223/W/Poster Board #881

**Modeling the human 8p11-myeloproliferative syndrome in NOD/SCID mice.** *H. Ågerstam[1], M. Järås[1], A. Andersson[1], P. Johnels[1], C. Lassen[1], D. Gisselsson[1], T. Olofsson[2], J. Richter[2], X. Fan[3], M. Ehinger[4], T. Fioretos[1].* 1) Department of Clinical Genetics, Lund University, Lund, Sweden; 2) Department of Hematology, Lund University Hospital, Lund, Sweden; 3) The Rausing Laboratory, Lund University Hospital, Lund, Sweden; 4) Department of Pathology, Lund University Hospital, Lund, Sweden.

The 8p11 myeloproliferative syndrome (EMS), also referred to as stem cell leukemia/lymphoma, is a chronic myeloproliferative disorder clinically characterized by leukocytosis, eosinophilia, splenomegaly, and a short chronic phase that rapidly progresses into aggressive acute myeloid or lymphoblastic leukemia. At the molecular level, EMS is characterized by fusion of at least eight different 5' partner genes to the 3' part of the *FGFR1* gene, resulting in constitutive activation of the tyrosine kinases in FGFR1. The aim of this study was to address the functional consequences of ectopic *FGFR1* expression in the potentially most relevant cellular context, that of normal primary human hematopoietic cells. For this purpose we used normal human $CD34^+$ cells from umbilical-cord blood and retrovirally transduced them with *ZMYM2/FGFR1* (previously known as *ZNF198/FGFR1*) or *BCR/FGFR1*. The cells were grown in suspension culture and in methylcellulose medium or transplanted into NOD/SCID mice. The phenotype of the differentiating cells in vitro and in vivo was analyzed by flow cytometry. Bone marrow and spleen sections from transplanted mice were analyzed by histopathology and immunohistochemisty. Expression of *ZMYM2/FGFR1* or *BCR/FGFR1* in normal human $CD34^+$ cells lead to increased cellular proliferation and differentiation towards the erythroid lineage in vitro. In the xenotransplantation NOD/SCID assay, *BCR/FGFR1* induced several features of human EMS, including expansion of several myeloid cell lineages and accumulation of blasts in bone marrow. Moreover, bone marrow fibrosis together with increased extramedullary hematopoiesis was observed. This study suggests that *FGFR1* fusion oncogenes, by themselves, are capable of initiating an EMS-like disorder, and provides the first humanized model of a myeloproliferative disorder transforming into acute leukemia in mice. The established in-vivo EMS model provides a valuable tool for future studies of this disorder.

## 1224/W/Poster Board #882

**Soft tissue alterations of oral cavity in Neurofibromatosis type 1 (NF1).** *E-M. Jouhilahti[1], V. Visnapuu[1,2], T. Soukka[2], S. Peltonen[3], R-P. Happonen[2], J. Peltonen[1,3].* 1) Institute of Biomedicine, Department of Cell Biology and Anatomy, University of Turku, Kiinamyllynkatu 10, 20520 Turku, Finland; 2) Department of Oral Diseases, Turku University Central Hospital, Lemminkaisenkatu 2, 20520 Turku, Finland; 3) Department of Dermatology, University of Turku, Kiinamyllynkatu 8, 20520 Turku, Finland.

BACKGROUND: Type 1 neurofibromatosis (NF1) is the most common one gene syndrome. It is caused by a mutation in the NF1tumor suppressor gene. NF1 syndrome is characterized by benign cutaneous neurofibromas which consist mostly of Schwann cells and fibroblasts. OBJECTIVE: The aim of the study is to analyze the oral soft tissue growths and compare them with NF1-related cutaneous neurofibromas. SUBJECTS AND METHODS: A total of 110 Caucasian patients with neurofibromatosis type 1 were included in this study with clinical oral examination. The patients were recruited to the study among the patients attending the NF1 clinic at the Department of Dermatology and Department of Oral Diseases, Turku University Central Hospital, as well as among members of the Finnish NF patient organization. All patients fulfilled the NIH diagnostic criteria for NF1. Oral soft tissue tumors were removed from 8 patients. Three tissue samples represented plexiform neurofibromas and five samples were solitary nodules. The plexiform manifestations were located in lower surface of tongue (1), in the gingiva (1) and on the mucosa under the tongue. The solitary tumors were located in the right border of tongue (2), in the lower surface of tongue (1) in the palate (1) and in the gingiva (1). The tissue samples were fixed in 10 % neutral formalin and embedded in paraffin. Sections, 5 μm thick, were cut and stained with hematoxylin-eosin (HE) and Masson's trichrome. In addition, a panel of selected biomarkers was used for immunocytochemistry in which the avidin-biotin method was used. RESULTS: All tissue samples analyzed contained variable amounts of S100 positive Schwann cells and type IV collagen positive collagenous matrix. Nestin was expressed by spindle like cells in solitary tumors locating in the border of the tongue and in the gingiva. CONCLUSION: The soft tissue alterations of oral cavity of NF1 patients show characteristics of both cutaneous and plexiform neurofibromas. The solitary tumors locating in the border of the tongue and in the gingiva resembled cutaneous neurofibromas based on the immunohistochemical analysis.

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## 1225/W/Poster Board #883

**Absence of correlation between *COL1A1-PDGFB* molecular subtype and the clinico-histological features of *Dermatofibrosarcoma Protuberans*: analysis of 35 new cases and review of 137 cases from the literature.** *D. Giacchero[1,2,3], G. Maire[1,2], F. Berthier[4], N. Ebran[1,2], A. Carlotti[5], P. Celerier[6], JM. Coindre[7], E. Esteve[8], S. Fraitag[9], D. Ranchère-Vince[10], P. Saiag[11], P. Terrier[12], JP. Lacour[3], F. Pedeutour[1,2].* 1) Laboratory of Solid Tumor Genetics, Nice University Hospital, Nice, France; 2) CNRS, UMR 6543, Faculty of Medicine, Nice, France; 3) Dermatology, Nice University Hospital, Nice, France; 4) Department of Medical Information, Nice University Hospital, Nice, France; 5) Department of Pathology, Cochin University Hospital, Paris, France; 6) Department of Dermatology, Le Mans Regional Hospital, Le Mans, France; 7) Department of Pathology, Bergonié Institute, Bordeaux, France; 8) Department of Dermatology, Orleans Regional Hospital, Orléans, France; 9) Department of Pathology, Necker University Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France; 10) Department of Pathology, Centre Léon Bérard, Lyon, France; 11) Department of Dermatology, Ambroise Paré University Hospital, Boulogne, France; 12) Department of Pathology, Gustave Roussy Institute, Villejuif, France.

The translocation t(17;22) resulting in C*OL1A1-PDGFB* fusion gene is found in dermatofibrosarcoma protuberans (DFSP) and its clinico-pathological variants. The chimeric *COL1A1-PDGFB* transcript shows a great variability of the *COL1A1* breakpoint from exon 6 to exon 49, contrasting with the consistent involvement of the *PDGFB* exon 2. The clinical or histological variations have never been assessed in regards to the *COL1A1* breakpoint position. Using RT-PCR and sequence analysis, we molecularly characterized the *COL1A1-PDGFB* breakpoints of 35 novel DFSP. We also retrospectively reviewed 137 cases with clinical information and molecular data. In a total of 172 cases of DFSP with molecular data, 38 different exons of *COL1A1* were involved in the fusion gene. Exons 25, 29, 32, 40, 46 and 47 were the most frequently involved exons (n≥ 10). Eight exons were described in unique cases. Among the 35 new cases analyzed, we identified 3 novel *COL1A1* breakpoints involving exons 9, 14 and 39. This study, pooling 172 cases in total, analyzes the largest series of DFSP with molecular data. Analyzing all cases, and with respect to the small number of patients for each group of breakpoints, we have not found any significant correlation between the *COL1A1* breakpoint localization and any clinical or histological features. The knowledge of the *COL1A1* breakpoint position does not give insights for the patient's care management. In the context of molecular routine diagnosis test, the identification of the *COL1A1-PDGFB* rearrangement is therefore sufficient, and could be evaluated alternatively by fluorescence in-situ hybridization (FISH).

## 1226/W/Poster Board #884

**Comparison of clonal patterns of chromosomal aberrations between DCIS and invasive breast cancer lesions of the same patient using a panel of ten FISH probes.** *K. Heselmeyer-Haddad[1], L. Berroa[1], C. Ortiz-Melendez[1], S. Valentine[1], S. Tewelde[1], K. Calzone[1], R. Christensen[2], P. Soballe[3], A. Schaffer[4], S. Prindiville[5], T. Ried[1].* 1) Genetics Branch, CCR, NCI, NIH, Bethesda, MD; 2) Department of Pathology, National Naval Medical Center, Bethesda, MD; 3) Department of General Surgery, Naval Medical Center, San Diego, CA; 4) Computational Biology Branch, NCBI, NIH, Bethesda, MD; 5) Coordinating Center for Clinical Trials, Office of the Director, NCI, NIH, Bethesda, MD.

Ductal carcinoma *in situ* (DCIS) refers to lesions with malignant epithelial cells that have not invaded the surrounding stroma. These lesions are confined to the ducts of the breast. It is thought that DCIS represents a stage that precedes invasive ductal carcinoma (IDC). However the linkage between these two lesions is still obscure. In order to analyze the patterns of genetic alterations as an approach to understand the correlation between DCIS and IDC, we developed four panels of FISH probes. The panels contained 5 oncogenes (COX2, MYC, CCND1, HER2, ZNF217) and 3 tumor suppressor genes (DBC2, CDH1, p53) specific for breast tumorigenesis and two centromeric probes (CEP10, CEP4) as a control for the ploidy of the cells. Objectives: Determine gain and loss patterns of the gene probes within DCIS and IDC, compare similarities in the genetic make-up of the DCIS and the corresponding IDC lesion, characterize clonal evolution from the DCIS lesion to IDC.

The database of the "Susceptibility to Breast Cancer Study" conducted at the National Naval Medical Center and National Cancer Institute, was searched for cases of breast carcinoma with neighboring DCIS. Archived paraffin blocks were analyzed by a pathologist. Representative areas for invasive cancer and DCIS were micro-dissected and disintegrated. One cytospin for each lesion was hybridized with four FISH probe panels subsequently. A relocation software allowed for counting the same 150-200 nuclei for all four sets of probes. In collaboration with NCBI, algorithms were developed to compare clonal patterns. Preliminary data show that there are cases which exhibit the exact same major clone in both the DCIS lesion and the corresponding IDC lesion. However, many of the cases displayed differences in the major clones for DCIS and IDC. These findings suggest that the clonal evolution from DCIS to IDC differs from case to case, indicating that in some cases major clones present in the DCIS are also successful in the IDC while in others the major clone(s) of the DCIS is/are replaced by clones with a different oncogene and tumor suppressor gene combination in the IDC. This study will improve our understanding of the molecular events that are involved in the progression from pre-invasive to invasive cancer and may lead to the development of prognostic biomarkers that can predict this transition.

## 1227/W/Poster Board #885

**Genome-wide SNP-array and Mutational Analysis of Genes Involved in RAS-signaling in Chronic Myeloid Leukemia treated with Dasatinib and Rapidly Developing AML with Monosomy 7 in Philadelphia-negative cells.** *N. Larsson[1], M. Ekblom[2], H. Lilljebjörn[1], C. Lassen[1], J. Richter[2], T. Fioretos[1].* 1) Clinical Genetics, Lund University, Lund, Lund, Sweden; 2) Department of Hematology, Lund University Hospital, Lund Sweden.

Despite the recent success of tyrosine kinase inhibitors (TKI) in the treatment of chronic myeloid leukemia (CML), some patients (2-17%) develop clonal cytogenetic changes in the Philadelphia (Ph-) negative cell population. A fraction of these patients, in particular those displaying trisomy 8 or monosomy 7, are at risk of developing a myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). Consequently, there is a need to characterize the clinical features of such cases and to increase our understanding of the pathogenetic mechanisms underlying the emergence of clonal cytogenetic changes in Ph-negative cells. So far, most cases reported have received treatment with imatinib. Herein, we describe a case of CML who rapidly developed monosomy 7 in Ph-negative cells 14 months after the initiation of dasatinib, a second generation TKI. Two months later the patient developed a fatal AML. Retrospective investigations by fluorescent in situ hybridization revealed that the monosomy 7 clone appeared already two months after the initiation of dasatinib treatment. Genome-wide 500 K single nucleotide polymorphism (SNP) array analysis of the monosomy 7 clone, revealed no acquired submicroscopic copy number changes. Given the strong association between monosomy 7 and mutations of genes involved in the RAS pathway in juvenile myelomonocytic leukaemia, we also screened for pathogenetic variants in KRAS, NRAS, and PTPN11, but did not detect any changes.

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## 1228/W/Poster Board #886

**Homologous Recombination in Skin Fibroblasts.** *I. Tereshchenko, L. Serrano, N. Goldsmith, K. Patel, A. Shawki, X. Jiang, B. Zheng, A. Brooks, S. Buyske, J. Tischfield.* Rutgers, The State University of NJ, Piscataway, NJ.

Using an in vivo mouse assay we have demonstrated that the major mechanism of loss of heterozygosity (LOH) in somatic cells is mitotic recombination (MR). To categorize the subchromosomal regions that manifest different frequencies of mitotic recombination in mouse primary skin fibroblasts, we performed genotyping of both STS and SNP markers. MR on mouse chromosome 8 occurs non-randomly and demonstrates age-specific characteristics. The fraction of crossover events originating in the region between 119 Mb and 125 Mb was elevated two-fold in mice aged 7-9 months compared to those aged 2-3 months (0.87 vs 0.41, respectively; P<0.001), while the overall frequency of MR did not change significantly (mean frequencies of $4.3x10^{-5}$ and $6.4x10^{-5}$, respectively). Also, the activity of MR did not correlate with the distribution of fragile sites on mouse chromosome 8. Moreover, repression of MR in particular subchromosomal regions apparently has functional and structural relevance. For example, we did not observe MR activity in the evolutionarily conserved non-coding region positioned between two members of the cadherin gene family, *CDH11* and *CDH5*, on the chromosomal band boundary 8qD2/D3. Indeed, this 324.1 Kb region has also been considered a desert of meiotic recombination. We hypothesize that the specific sequence composition, chromatin structure, and nuclear localization of this subchromosomal region suppressed MR in mouse fibroblasts. In contrast, a mitotic recombination hotspot was mapped to a euchromatic region situated between 120.677 Mb and 124.082 Mb on 8qE1. We speculate that transcriptional co-regulation of the evolutionarily conserved gene clusters and the presence of extensive regions of subchromosomal sequence homology in the inbred parental mouse strains promotes homologous chromosome interaction and facilitates MR in the primary skin fibroblasts of the C57/129 F1 hybrids. Elevated MR may have acute biological consequences that lead to tumorigenicity. Indeed, regions that manifest a high degree of MR on chromosome 8 also demonstrate elevated LOH in mouse mammary carcinomas and, furthermore, LOH in syntenic human chromosomal regions is consistently associated with breast and prostate cancers.

## 1229/W/Poster Board #887

**A new way of maintaining telomere in CML: Alternative lengthening of telomere.** *O. Samassekou[1], J. Hébert[2,3], J. Yan[1].* 1) Dept Medical Genetics, Department of Pediatrics, Faculty of Medicine and Health Sciences, Universite de Sherbrooke,3001 12th Avenue North, Sherbrooke, J1H 5N4, Quebec, Canada; 2) Leukemia Cell Bank of Quebec and Division of Hematology, Maisonneuve-Rosemont Hospital,3001 12th Avenue North, Sherbrooke, J1H 5N4, Quebec, Canada; 3) Department of Medicine, Université de Montréal,Montreal, Quebec H3C 3J7, Canada.

Chronic myeloid leukemia (CML) is a neoplasia characterized by a proliferation of a myeloid cell lineage and a translocation t(9;22). As most of cancer, the telomere length in CML cells is shorter than normal blood cells. However, currently no data are available about the specific telomere length in CML. We studied the telomere length on each chromosome in 21 CML and 13 normal samples. After obtaining metaphases from CML and normal cells, in situ hybridization with peptide nucleic acid probes was performed. The fluorescence intensity of each specific telomere was converted in kb according to the telomere restriction fragment technique (TRF) result for each sample. The individual telomere lengths of CML samples were compared to those of normal ones. The individual telomere lengths in CML cells were ranging from 1.70 kb to 24.83 kb. We surprisingly noticed an elongation or maintenance of specific telomere on 10p, 14p, 17p, 18p and 19p. This lengthening was confirmed by TRF which showed specific distinguishable bands from the smear. Contrary, in CML cells, all individual telomeres on chromosome long arms were shortened. To understand the difference of individual telomere length maintenance in CML, we measured the telomerase activity by real time polymerase chain reaction. We found an increase of telomerase activity in 70% cases. However, this augmentation could not explain the huge difference between short and long telomeres on one hand and between the homologous chromosomes on the other hand. Moreover, the samples which had basal telomerase activity showed some individual long telomeres. To further understand the mechanism by which these telomeres were maintained or elongated, we studied the alternative lengthening of telomere (ALT). We found that in more than 50% of the samples the presence of t-circles by doing 2D gel and single strand telomere DNA which can be either TTAGGG or CCCTAA strands. These finding are considered to be some of ALT features. Besides the action of the telomerase to maintain telomere length, ALT is another mechanism to conserve telomere length in CML. ALT might be the first mechanism to maintain telomere length in CML before the reactivation of telomerase, and these two mechanism might synergistically act on individual telomeres. It is important to take into account the two mechanisms maintaining telomere length when targeting the telomerase by therapeutic agents because the telomerase activity can hide the ALT mechanism.

## 1230/W/Poster Board #888

**Strand-specific RNA sequencing of HepG2 cells identifies genes that are differentially expressed, alternatively spliced and allelically imbalanced in response to TGF-beta.** *B. Tuch[1], C. Barbacioru[1], O. Wallerman[2], R. Andersson[7], A. Moustakas[3], C.H. Heldin[3], N. Eriksson[6], S. Stanley[1], J. Gu[4], S. Kuersten[4], M. Barker[1], J. Komorowski[7,8], K. McKernan[5], F.M. De La Vega[1], C. Wadelius[2].* 1) Life Technologies, Foster City, CA; 2) Dept. of Genetics and Pathology, Uppsala University, Sweden; 3) Ludwig Institute for Cancer Research, Uppsala, Sweden; 4) Life Technologies, Austin, TX; 5) Life Technologies, Beverly, MA; 6) Uppsala Clinical Research Center, Uppsala University, Sweden; 7) Linnaeus Centre for Bioinformatics, Uppsala University, Sweden; 8) Interdisciplinary Centre for Mathematical and Computer Modelling, Warsaw University, Poland.

Transforming growth factor beta (TGF-beta) is a secreted protein that controls many complex behaviors of tumor cells. In early stage adenomas TGF-beta acts as a tumor suppressor, whereas in advanced carcinomas it promotes tumor cell invasiveness and metastasis. HepG2, a human hepatocellular carcinoma cell line, has lost responsiveness to the tumor suppressive action of TGF-beta and when treated with TGF-beta, these cells show enhanced motility and invasiveness. Thus, HepG2 cells provide a good model for advanced tumor cells. RNA sequencing (RNA-Seq) can provide a detailed view of the dynamic transcriptional landscape of cells experiencing environmental perturbation and was therefore applied here to study TGF-beta response. We serum starved HepG2 cells for 24 hours and then treated for 1 hour with 2.5 ng/ml TGF-beta1 or with vehicle control. Total RNA was isolated, depleted of rRNA, fragmented and then used to create strand-specific cDNA libraries with the SOLiD Whole Transcriptome Protocol. Approximately one billion 50 bp reads were sequenced with the SOLiD System, over half of which could be aligned to the human genome. The aligned transcriptomes cover roughly 4% of each strand of the genome and more than half of the reads align within the exonic regions of annotated transcripts. The expression level of each gene was defined and 349 genes were determined to be differentially expressed between the two conditions. These include both previously established and novel targets of the TGF-beta pathway. The 50 bp length of the sequenced fragments permitted us to accurately align reads to splice junctions. In all, ~5 million reads aligned to ~90,000 splice junctions in both the control and stimulated conditions, which allowed us to determine the effects of TGF-beta on alternative splicing in fine detail. Finally, by genotyping HepG2 cells, we have identified over 300,000 heterozygous SNP positions, which we have employed to examine allele-specific gene expression in our RNA-Seq data. We observed many differences in the balance of expressed alleles between TGF-beta treated and untreated cells, which could be explained by linked SNPs in cis-regulatory elements. We are now comparing these results to ChIP-Seq data gathered under the same conditions to understand the relationship between allele-specific binding of transcriptional regulators and allele-specific expression of nearby genes.

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## 1231/W/Poster Board #889

**Extracolonic tumor spectrum in 276 patients with MUTYH-associated polyposis (MAP): a widening phenotype.** *S. Aretz[1], N. Jones[2], D. Christian[1], C. Engel[3], M. Nielsen[4], A. Kaufmann[1], V. Steinke[1], H.F. Vasen[5], P. Propping[1], J.R. Sampson[2], F.J. Hes[4], S. Vogt[1].* 1) Institute of Human Genetics, University of Bonn, Bonn, Germany; 2) Institute of Medical Genetics, Cardiff University, Cardiff, UK; 3) Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany; 4) Centre for Human and Clinical Genetics, LUMC, Leiden, the Netherlands; 5) Department of Gastroenterology and Hepatology, LUMC, Leiden, The Netherlands.

Background: MUTYH-associated polyposis (MAP) is a recently described autosomal recessive condition characterized by the appearance of multiple colorectal adenomas and a lifetime risk of colorectal cancer of up to 100%. The attenuated or atypical form of familial adenomatous polyposis (FAP) is the most important differential diagnosis. To date, no systematic evaluation of extracolonic MAP manifestations has been reported. Methods: In a collaborative multicenter European study a large cohort of MAP patients (276 cases from 181 apparently unrelated families) was recruited. Based on medical records and anamnestic information the extracolonic tumor spectrum and incidence were evaluated to assess cumulative lifetime risks and compared with general population rates to obtain standardized incidence ratios (SIRs). Results: The median age at evaluation was 54 years. Duodenal polyposis occurred in 17%; the relative risk of duodenal cancer was very high (SIR 129; 95%CI 16-466), while the lifetime risk was 4%. The incidence of extraintestinal malignancies as a whole was almost doubled (SIR 1.9; 95%CI 1.4-2.5) with a lifetime risk of 38%. We found a low to moderate but significant increase in the incidence of ovarian, bladder and skin cancers (SIR 5.7, SIR 7.2, and SIR 2.8, respectively), and a trend suggesting an increased risk of breast cancer. Mean ages at onset were 52-60 years. In contrast to FAP no desmoid tumors were observed. Interestingly, sebaceous gland tumors (SGT) that are characteristic of the Muir-Torre syndrome, a phenotype variant of hereditary non-polyposis colon cancer (Lynch syndrome, HNPCC) occurred in five patients. No genotype-phenotype correlation was identified. Conclusions: The relative risks of a several extraintestinal malignancies were increased, however, no predominant lesion was observed. The spectrum of cancers and their relatively advanced age at onset suggest that specific intensive surveillance recommendations other than frequent gastrointestinal endoscopies are unlikely to be helpful in MAP. The tumor spectrum is wide and points to a phenotypic overlap with Lynch syndrome. SGTs might serve as diagnostic marker lesion in some cases. The study was supported by the German Cancer Aid (Deutsche Krebshilfe), the Dutch Digestive Diseases Foundation, and the Wales Gene Park and Cancer Research Wales.

## 1232/W/Poster Board #890

**Rare copy number variants at 17p13.1 are associated with developmental delay or the Li-Fraumeni cancer predisposition syndrome.** *A. Shlien[1,2], B. Baskin[3], M.I.W. Achatz[4], K.E. Nichols[5], L. Hudgins[6], C.F. Morel[7], M.P. Adam[8], N. Zhukova[1], L. Rotin[1], A. Novokmet[9], H. Druker[9], M. Shago[3], P.N. Ray[3,10], P. Hainaut[11], D. Malkin[1,2,9,12].* 1) Gen & Genome Biol, Hosp Sick Children, Toronto, ON, Canada; 2) Department of Medical Biophysics, University of Toronto, ON, Canada; 3) Division of Molecular Genetics, Department of Paediatric Laboratory Medicine, Hosp Sick Children, Toronto, ON, Canada; 4) Hospital do Câncer A.C. Camargo, São Paulo, Brazil; 5) Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA, USA; 6) Department of Pediatrics, Division of Medical Genetics, Stanford University School of Medicine, Stanford, CA, USA; 7) Department of Medicine, University Health Network, University of Toronto, Toronto, ON, Canada; 8) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA; 9) Division of Hematology/Oncology, Hosp Sick Children, Toronto, ON, Canada; 10) Department of Molecular Genetics, The University of Toronto, Toronto, ON, Canada; 11) Molecular Carcinogenesis and Biomarkers Group, International Agency for Research on Cancer, Lyon, France; 12) Department of Pediatrics, The University of Toronto, Toronto, ON, Canada.

Constitutional DNA copy number variations (CNVs) are a significant form of genetic variation which have been associated with a number of neuropsychiatric conditions, but have been less studied in cancer. Li-Fraumeni syndrome (LFS) is an autosomal dominant disorder, often associated with germline mutations of the TP53 tumor suppressor gene, which strongly predisposes to various cancers. In a previous genomewide profile of CNVs in DNA of peripheral blood of LFS families (Shlien A, et al 2008) we showed that the number of CNVs per genome is largely invariable in the healthy population, but increased in TP53 mutation carriers. Here, in a replication cohort analyzed on arrays with greater coverage (1.8 million probes), we show that: 1) CNV deletions are more frequent than duplications in mutation carriers (p=3.28 x 10-5); 2) the number of CNVs exceeds 300 in carriers (vs. 154 in controls); and 3) the difference in CNVs persists in these individuals' tumors. Having shown an excess of CNVs in LFS patients, we looked for evidence of pathogenic CNVs in patients lacking TP53 mutations. Our diagnostic labs screened ~500 patients with diverse diagnoses for DNA dosage changes using clinical CGH or MLPA. Eight probands were identified harboring microdeletions at TP53 (17p13.1) that were not detectable by karyotyping. Probands presented with a spectrum of phenotypes ranging from malignancies of the bone and adrenal gland to hypotonia, developmental delay and dysmorphic features. Remarkably, none of the four patients with developmental delay have presented with cancer, or any sign of cancer susceptibility, and none of the four TP53 deletion patients affected with cancer had any cognitive impairment or congenital anomalies. Therefore, while they overlap, 17p13.1 CNVs are associated with different phenotypes. To explore the biological basis of this difference we fine-mapped the breakpoints of these CNVs using a high-throughput qPCR assay. We note that breakpoints *within* TP53 are associated with cancer, while breakpoints *outside* TP53 (i.e. encompassing, but not limited to the gene) are associated with developmental delays. 17p13.1 deletions, which we determined arise by NHEJ, can be *de novo* or inherited and show no overt parent-of-origin bias. These results indicate that the TP53-associated cancer phenotype can be modified by the extent of the genomic alteration at this locus, and suggest novel mechanisms of genetic regulation of neoplastic and developmental phenotypes.

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## 1233/W/Poster Board #891

**Gastric Adenocarcinoma and Proximal Polyposis of the Stomach; a new syndrome.** *N. Wayte[1,2], D. Worthley[1,3], K. Phillips[4], N. Lindor[5], D. Huntsman[6], F. Carneiro[7], G. Chenevix-Trench[1], G. Suthers[4].* 1) The Queensland Institute of Medical Research, Brisbane, Australia; 2) School of Chemical and Molecular Biosciences, the University of Queensland, Brisbane, Australia; 3) RBWH Clinical Research Centre, Brisbane, Australia; 4) Familial Cancer Unit, SA Pathology, Adelaide, Australia; 5) Department of Medical Genetics, Mayo Clinic, Rochester, Minnesota, USA; 6) Department of Pathology, Genetic Pathology Evaluation Centre of the Prostate Research Centre, Vancouver General Hospital; 7) Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal.

Gastric cancer is a rare event in several well-characterised general cancer syndromes, such as Li-Fraumeni and Lynch syndromes, but most familial aggregation occurs in a range of gastric cancer-specific syndromes: hereditary diffuse gastric cancer (~30% due to CDH1 mutations), familial diffuse gastric cancer, and familial intestinal gastric cancer (<10% due to BRCA2 mutations). Fundic gland polyps are composed of normal gastric corpus/fundus--type epithelium arranged in a disorderly configuration, and occur only in the gastric body and fundus. Although usually benign, dysplastic changes can occur in fundic gland polyps, particularly in the context of Familial Adenomatous Polyposis. Long term use of proton pump inhibitors is associated with an increased frequency of fundic gland polyps. We have identified four families with a novel autosomal dominant syndrome of fundic gland polyposis and predisposition to gastric cancer of the intestinal type. The largest and first family to be characterised in detail was ascertained in Australia after referral of the proband following an episode of melaena, but with no known family history of gastroenterological disease. Upper gastrointestinal endoscopy revealed florid proximal gastric polyposis and histopathology showed predominantly fundic gland polyps with variable dysplasia including intramucosal carcinoma. Follow up of the rest of the family over four generations indicated that it has a novel autosomal dominant syndrome of gastric polyposis, and recently two young affected women developed intestinal gastric cancer. The key features of this novel syndrome are the presence of hundreds of fundic gland polyps carpeting the gastric body and fundus with complete sparing of the antrum, the dysplastic foci on the surface of many of these fundic gland polyps and the predisposition to intestinal gastric cancer. There are 16 affected family members; penetrance is incomplete and/or age related, and with variable age of onset. We have also identified three smaller families in North America with the same syndrome, but with a higher frequency of gastric cancer, probably reflecting ascertainment bias. We have called the syndrome Gastric Adenocarcinoma and Proximal Polyposis of the Stomach (GAPPS) in reference to the fundic gland polyposis in the proximal part of the stomach and high risk of gastric cancer.

## 1234/W/Poster Board #892

**Identification of copy number variable regions (CNVRs) associated with risk of prostate cancer in Mexican-Americans.** *A. Blackburn[1], J. Gelfond[2], H.H.H. Göring[3], J. Beuten[4,6], I. Thompson[5], R.J. Leach[1,6], D.M. Lehman[7].* 1) Department of Cellular and Structural Biology, Univ of Texas Health Science Center, San Antonio, TX; 2) Department of Epidemiology and Biostatistics, Univ of Texas Health Science Center, San Antonio, TX; 3) Southwest Foundation for Biomedical Science, Department of Genetics, San Antonio, TX; 4) Greehey Children's Cancer Research Institute, Univ of Texas Health Science Center, San Antonio, TX; 5) Department of Urology, Univ of Texas Health Science Center, San Antonio, TX; 6) Department of Pediatrics, Univ of Texas Health Science Center, San Antonio, TX; 7) Department of Medicine, Univ of Texas Health Science Center, San Antonio, TX.

The role of heritable structural variation in risk for prostate cancer is still unknown. We performed a genome-wide study in 100 prostate cancer cases and 67 age matched male controls of Mexican American origin using an array developed by deCODE Genetics and Illumina, surveying copy number variable regions (CNVRs) and complex regions that include segmental duplications, megasatellites, and regions lacking annotated SNPs. The prostate cancer cases were either participants of the San Antonio Biomarkers of Risk of Prostate Cancer (SABOR) cohort, or a parallel study of prevalent prostate cancer from the same metropolitan population recruited using the same means. Following normalization and batch correction of the microarray genotyping data, quality control tests were performed and duplicate samples were consistent for >98% of the markers. Association tests were conducted on the allele counts based on the normalized array intensities for each polymorphic marker using logistic regression analysis with age included as a covariate in the model. A likelihood ratio test was performed comparing the null hypothesis of no association to the two-sided alternative hypothesis of association. To correct for possible confounding by hidden population stratification due to admixture, a principle components methodology was used that is similar to the method implemented in EIGENSTRAT. All principle components (among the top 20) that were found to be significant predictors of prostate cancer status at the 0.05 significance level were selected for inclusion in the subsequent association analyses. The 26 closest genes to the most significantly associated variants were identified as a gene set. Next, we used a Robust Probe-level Linear Model to normalize Affymetrix HG-U95Av2 expression data from GEO for 16 disease-free prostate tissue samples, and 20 prostate tumor tissue samples and their adjacent normal prostate tissue samples, using affylmGUI in R. Using Gene Set Enrichment Analysis (GSEA) it was determined that this CNVR gene set showed enrichment in tumor tissue and in adjacent normal tissue compared to disease free normal tissue. The genes, including PTPRN2 and HINT1, that contribute to the core enrichment in both GSEA comparisons are currently being confirmed in the entire cohort of Mexican-American SABOR subjects using quantitative PCR assays. This study is one of the first to support evidence for CNVR differences in heritable risk for prostate cancer.

## 1235/W/Poster Board #893

**New common low penetrance genes predicting high naevus counts are associated with melanoma risk.** *M. Falchi[1, 2], N. Hayward[3], D. Duffy[3], J. Newton Bishop[4], T. Pastinen[5], A. Cervino[1], Z. Zhao[3], P. Deloukas[6], N. Soranzo[1, 6], D. Elder[7], J. Barrett[4], N. Martin[3], T. Bishop[4], G. Montgomery[5], T. Spector[1], V. Bataille[1, 8].* 1) Dept of Twin Res & Gen Epidem, King's College, London, United Kingdom; 2) Dept of Genomics Medicine, Imperial College London, UK; 3) Queensland Institute of Medical Research, Queensland, Australia; 4) Leeds Institute of Molecular Medicine, University of Leeds, UK; 5) Dept of Human and Medical Genetics, Mc Gill University, Montreal, Canada; 6) Human Genetics Dept, Wellcome Trust Sanger institute, Cambridge, UK; 7) Dept of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, USA; 8) Dermatology Dept, West Herts NHS Trust, Hertfordshire, UK.

Twin studies have shown that nevi numbers are heritable. We conducted a genome-wide association study for total body nevus count (all naevi above 2 mm in diameter counted by trained examiners) using 297,108 tag SNPs in 1,524 Caucasian female twins from the Twin's UK database. We identified strongly associated variants in MTAP, a gene adjacent to the familial melanoma susceptibility locus CDKN2A on 9p21 (lead SNP rs4636294, P = 3.4 x 10-15). We further identified PLA2G6 on 22q13.1 (lead SNP rs2284063, P = 3.4 x 10-8). Our results were validated in an independent cohort of 4,107 Australian adolescent twins of both sexes. Both loci were also associated with melanoma risk in 3,131 melanoma cases from two independent studies in the UK and Australia (allele specific odds ratio (OR) = 1.23 at rs10757257, P = 3.4 x 10-8 ; OR = 1.23 at rs132985, P = 2.6 x 10-7 ). About one subject in 11 is homozygous for the variant at both loci and show twice the number of nevi compared to those homozygous for the wild alleles and this is also associated with a two fold increase in melanoma risk. These data provide the first evidence for common, low penetrance melanoma alleles whose effect is mediated through nevus number and highlight the use of intermediate phenotypes in the discovery of disease genes.

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## 1236/W/Poster Board #894

**Pathways And Genes Associated With Ependymoma Location, Grade And Subgroups Reveal A New Ependymomal Subgroup Located In The Posterior Fossa Called Biphasic-Cerebriform Ependymomas.** *C. Godfraind[1], T. Palm[2], F. Chapon[3], I. Salmon[4], M. Coutelier[2], C. Lacroix[5], F. Gray[6], F. Scaravilli[7], C. Vandenbroecke[8], D. Ellison[9], D. Figarella-Branger[10], M. Vikkula[2].* 1) Div Pathology, Univ Catholique de Louvain, Brussels, Belgium; 2) Laboratory of Human Molecular Genetics, de Duve Institute, Université catholique de Louvain, Brussels, Belgium; 3) Laboratory of Pathology, CHU-Caen, France; 4) Laboratory of Pathology, Erasme University Hospital, Université Libre de Bruxelles, Brussels, Belgium; 5) Laboratory of Pathology, Hopital Kremlin-Bicêtre, Paris, France; 6) Department of Pathology, APHP Hôpital Lariboisière, Université Paris VII, Paris, France; 7) Institute of Neurology, London, United Kingdom; 8) Department of Pathological Anatomy, Ghent University Hospital, Ghent, Belgium; 9) Department of Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, USA; 10) Department of Pathology and Neuropathology, La Timone's Hospital, AP-HM, and EA3281 Université de la Mediterrannée, Marseille, France.

*Purpose of the study* : Unravelling specific signatures of ependymoma sub-groups and understanding molecular alterations involved in their tumorigenesis. *Materials and Methods*: A series of 34 frozen ependymomas were analysed using Affymetrix HG-U133 Plus 2.0 arrays. Obtained data were compared with histological features. *Results*: Spinal cord ependymomas demonstrated a HOX gene signature, whereas intra-cranial tumors had Notch, Hedghog and BMP pathways upregulation. High expression levels of the oncogenes SOX2, HDAC1, NPM1, YAP1 and JAG1 were observed in PF ependymomas when compared to normal brain. Grade II ependymomas were associated with up-regulation of dyneins, and grade III with activation of the Wnt and E2F1 pathway, cell cycle, adherens junction dysfunction, apoptosis and angiogenesis. Hierarchical clustering and Correspendance analysis established the presence of three molecular sub-groups of posterior fossa (PF) ependymomas, while WHO only recognizes two. One of them regrouped tumors with specific histology characterized by biphasic and cerebriform appearance, which is either WHO grade II or III. They also demonstrated activation of genes implicated in glycogene metabolism and central nervous system development. We suggest to name this newly identified sub-group of children PF ependymomas "biphasic-cerebriform ependymoma". *Conclusion*: These data illustrated heterogeneity of ependymomas and assigned gene signatures to tumor locations and grades. They reinforced the notion of these tumors to derive from cancer stem cells bearing radial glial cell phenotype. In this context, Hox, Notch and Hedghog pathways play a role in maintenance and renewal of cancer stem cells, and BMP in their differentiation. This, together with the genes up-regulated in posterior fossa ependymomas, pinpoints Notch pathway as a major player of ependymoma tumorigenesis, and Wnt of high grade oncogenesis. Identification of three molecular subgroups in PF ependymomas, among which, the newly identified biphasic-cerebriform pattern, may partly account for discordance in histological ependymoma grading.

## 1237/W/Poster Board #895

**Epigenetic annotation of the ABO blood group susceptibility locus identified by GWAS in pancreatic cancer.** *J. Jia, I. Collins, H. Parikh, A. White, R. Stolzenberg-Solomon, P. Hartge, R. Hoover, S. Chanock, L. Amundadottir, the PanScan Consortium.* Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Pancreatic cancer is a highly lethal cancer with a survival rate of less than 5% at 5 years. Few well established risk factors are known and improved diagnostic and therapeutic modalities are desperately needed. PanScan is an ongoing genome wide association study (GWAS) of pancreatic cancer conducted within the framework of the NCI-sponsored Cohort Consortium and the Pancreatic Cancer Case-Control Consortium (PANC4). It includes a total of 4,000 pancreatic cancer cases and 4,000 controls. Susceptibility loci from PanScan are currently being investigated in our laboratory to understand the biological basis of the association signals discovered. One of these loci is in the vicinity of the ABO gene on Chr9q34 where four SNPs (rs505922, rs495828, rs657152 and rs630014) have been associated with a significantly increased risk of pancreatic cancer but they are strongly correlated (r2=0.18-0.93). To investigate the epigenetic germ-line alterations observed in the ABO GWAS locus, we have conducted chromatin immunoprecipitation (ChIP) experiments followed by qPCR to tag histone modifications that mark promoters, enhancers and silencers (H3K4me1, H3K4me3 and H3K27me3) in six pancreatic cell lines derived from normal and malignant pancreatic tissues. Differential patterns of histone modifications marks have been observed in preliminary studies involving two well-characterized cell lines derived from a pancreatic cancer (Panc-1) and from normal pancreatic cells (hTERT-HPNE). In the ABO region, we observed strong silencing (H3K27me3) histone modification marks in the hTERT-HPNE cell line but strong activating (H3K4me1) signal in the Panc1 cell line. Interestingly, these correspond to the genomic area where the SNPs with the strongest association signal for pancreatic cancer risk are located. In ChIP-seq (ChIP sequencing) experiments, we have begun to comprehensively catalogue histone modifications marks on a genome wide level in pancreatic cell lines. Together with RNA-seq analysis, designed to interrogate the full transcriptome profile in a series of pancreatic cell lines, we have begun to see patterns that could provide plausibility for the association signal in this region and thereby, establish a first step in the characterization of the molecular phenotype of the susceptibility alleles in ABO for pancreatic cancer risk.

## 1238/W/Poster Board #896

**Genome-wide association study for colorectal cancer in German familial cases and replication of candidate markers in independent cohorts.** *J. Lascorz[1], A. Försti[1, 2], N. Kunkel[1], B. Chen[1], K. Hemminki[1, 2], German HNPCC Consortium, NGFN Plus CCN Group.* 1) Division of Molecular Genetic Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany; 2) Center for Family and Community Medicine, Karolinska Institute, Huddinge, Sweden.

Genetic susceptibility of colorectal cancer (CRC) accounts for ~30% of its aetiology. Rare, high-penetrance germline mutations in a few genes (mainly APC and DNA mismatch repair genes) account for less than 5% of CRC cases. Much of the remaining variation in genetic risk is supposed to be attributable to common susceptibility loci, each exerting a small influence on risk. We carried out a genome-wide scan (GWAS) in 371 German familial CRC cases and 1263 healthy controls using the Affymetrix Genome Wide Human SNP 6.0 Array. Polymorphisms with MAF <0.05, call rate <95%, or Hardy-Weinberg equilibrium exact p-value <10-5 in the control group, as well as samples with call rate <90%, were excluded. A total of 875 SNPs showed at least one p-value (allele, genotype, dominant, recessive or trend) <10-4, and careful visual inspection of genotype cluster plots reduced this number to 523 reliable genotyped markers. Out of them, we selected polymorphisms for replication using additionally the following criteria: several SNPs within/close to a gene, location in candidate genes or in already known risk loci for CRC, and coding polymorphisms. In order to select the best candidate markers, results of the initial 875 SNPs were also compared with another unpublished GWAS performed in 400 CRC cases and 400 controls of German origin. So far, genotyping of 47 selected SNPs has been completed in a German replication cohort of 655 familial CRC cases and 760 controls using KASPar assays. Six SNPs have shown association in this replication cohort, two of them located in the known risk loci on 8q24.21 and 11q23, the other four in so far undescribed loci, with allele ORs ranging between 1.2 and 1.3. Further replication of these four markers in two additional cohorts of German origin is currently ongoing.

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## 1239/W/Poster Board #897

**Three loci identified as testicular germ cell tumour susceptibility loci by genome wide association study; further investigation examining maternal and imprinting effects.** *E.A. Rapley[1], J. Nsengimana[5], C. Turnbull[1], A. Al Olama[2], E. Dermitzakis[3], R. Linger[1], R. Huddart[4], A. Renwick[1], D. Hughes[1], S. Hines[1], S. Seal[1], J. Morrison[2], P. Deloukas[3], N. Rahman[1], D.F. Easton[2], D.T. Bishop[5], M.R. Stratton[1,2], The UK Testicular Cancer Collaboration.* 1) Section Cancer Genetics, Inst Cancer Research, Sutton, Surrey, United Kingdom; 2) Cancer Research UK, Genetic Epidemiology Unit, Strangeways Research Laboratory, Cambridge CB1 8RN, UK; 3) The Wellcome Trust Sanger Institute, Hinxton, CB10 1SA, UK; 4) Academic Radiotherapy Unit, Institute of Cancer Research, Sutton, Surrey, SM2 5PT, UK; 5) Section of Epidemiology & Biostatistics, Leeds Institute of Molecular Medicine, Leeds, LS9 7TF, UK.

Testicular Germ Cell Tumour (TGCT) is the most common cancer in men ages 15-45 years. Family history is a major risk factor with the estimated the risk to brothers to be 8-10 fold, much higher than the familial risks for most other cancer classes which are generally ~2-fold. We recently conducted a genome-wide association (GWA) study in TGCT. 307,666 SNPs were genotyped in 730 cases and 1,435 controls from the UK; associations were replicated in a further 571 cases and 1,806 controls. We found strong evidence for susceptibility loci on chromosome 5(per allele OR=1.37(95% CI 1.19-1.58), p=$3x10^{-13}$), chromosome 6 (OR=1.50(95% CI 1.28-1.75), p= $10^{-13}$) and chromosome 12 (OR=2.55(95% CI 2.05-3.19), p=$10^{-31}$). The gene responsible for the association on chromosome 12 may be KITLG, important in the biology of germ cells. The other loci implicate the SPRY4 gene on chromosome 5 and BAK1 on chromosome 6. Using a family-based dataset from Leeds we evaluated possible maternal and imprinting effects at these loci. The dataset consisted of 147 family trios, 86 incomplete trios and 285 unrelated controls. A family based test (using a log-linear method, LEM software) was used to test the affect from alleles carried by cases (main/child effect), maternal and imprinting effects. Further assessment of main/child effect and maternal effect was made using a non-family approach (using an unconditional logistic regression model and ignoring family information, STATA software) Main/child effects were confirmed on all the loci tested (p=.0006 on chr5, p=.009 on chr6 and p=$9.9x10^{-8}$ on chr12) in keeping with the GWA data. The imprinting effect was not significant for these SNPs. Maternal effects at the chromosome 12 loci were marginally significant or close to significance when using the family (p=.06) or the non-family tests (p=.04), or the combined data(p=.06). We identified three susceptibility loci for TGCT. The strongest association was demonstrated for SNPs within the KITLG, the other loci are in the vicinity of genes which are activated or repressed by this pathway. There is no evidence of imprinting or maternal effects on chromosomes 5 or 6 but there may be a maternal effect for the loci on chromosome 12. The three loci account for 7%; of the sibling risk and 10%;of the risk to offspring of cases of TGCT. Our results suggest that there are other common variants with similar effects to those identified on chromosomes 5 and 6 to be detected by additional association studies.

## 1240/W/Poster Board #898

**Evaluation of reported GWAS hits in a large, population-based series of contralateral breast cancer.** *S.N. Teraoka[1], A.S. Reiner[2], X. Liang[2], J.L. Bernstein[2], R.W. Haile[3], L. Bernstein[4], P. Concannon[1], The WECARE Study Consortium.* 1) Dept Biochem & Molec Gen, University of Virginia, Charlottesville, VA; 2) Dept Epidemiology & Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY; 3) Dept Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA; 4) Dept Cancer Etiology, City of Hope National Medical Center, Duarte, CA.

Genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) in several genomic regions that are associated with breast cancer. To assess the role of these common variants in risk of developing second primary breast cancer, we genotyped 24 SNPs that were significantly associated with breast cancer in final or earlier stages of published GWAS, in 2103 subjects in the WECARE Study. The WECARE Study is a multi-center, population-based, case-control study designed to examine gene-environment interactions and risk of contralateral breast cancer (CBC). The study population included 708 women with CBC (cases), and 1395 women with unilateral breast cancer (UBC) (matched controls), all of whom were ascertained through five population-based cancer registries in the U.S. and in Denmark. Controls were matched 2:1 on birth year, year of breast cancer diagnosis, registry, race, and counter-matched on registry-reported radiation exposure. Among SNPs representing 19 genomic regions screened, rs2981582 (in *FGFR2*) was significantly associated with CBC; per allele rate ratio (RR) = 1.3, 95% confidence interval (CI) = 1.1-1.7, homozygous RR = 1.4, 95% CI = 1.1-2.0), as was rs7313833 (near *PTHLH*); per allele rate ratio (RR) = 1.3, 95% confidence interval (CI) = 1.1-1.5, homozygous RR = 1.6, 95% CI = 1.2-2.3. Additionally, homozygosity for the minor alleles at SNPs rs13281615 (8q24) (RR = 1.5, 95% CI = 1.1-2.0), rs3803662 (near *TNRC9 (TOX3)* (RR = 1.4, 95% CI = 1.0-2.0), rs13387042 (2q35) (RR = 1.4, 95% CI = 1.0-1.8), and rs11235127 (near *TMEM135*) (RR = 2.3, 95% CI = 1.3-4.0) were associated with increased CBC. These preliminary findings demonstrate that common risk variants identified in GWAS studies also contribute to risk of CBC. Candidate genes in the vicinity of these identified risk loci include *PTHLH*, encoding a hormone involved in breast morphogenesis and breast cancer cell invasiveness, and *TMEM135*, reported to be regulated in response to Tamoxifen treatment in the mouse. Previously we showed that Tamoxifen use in the WECARE Study reduced the risk of CBC (multi-variable adjusted RR = 0.7, 95% CI = 0.5-0.9). In the current study, our results suggest that the effect of Tamoxifen is modified by the *TMEM135* SNP, such that the protective effect of Tamoxifen appears to be predominately among those carrying the common GG genotype (RR = 0.6, 95% CI = 0.4-0.8).

## 1241/W/Poster Board #899

**LOHAS: Loss-Of-Heterozygosity Analysis Suite.** *H.C. Yang[1], L.C. Chang[1], R. Huggins[2], C.H. Chen[1].* 1) Institute of Statistical Science Academia Sinica, No 128, Academia Road, Nankang, Taipei, Taiwan; 2) Department of Mathematics and Statistics, The University of Melbourne, Melbourne, VIC 3010, Australia.

Detection of loss of heterozygosity (LOH) plays an important role in genetic, genomic and cancer researches. We develop statistical methods to estimate LOH intensity, identify samples with unusual genome structure, and map the genomic segments involving LOH or chromosomal aberrations based on single-nucleotide-polymorphism (SNP) data. The methods are also applied to study long contiguous stretches of homozygosity (LCSH) in general populations. We analyze genome-wide SNP data from a leukaemia study and the HapMap Project. Important leukaemia-associated LOH regions and population-specific LCSH regions are successfully identified. Sub-types of the disease and sub-populations of the study samples can be un-supervisedly classified using our methods. The developed algorithms are packaged into software Loss-Of-Heterozygosity Analysis Suite (LOHAS).

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## 1242/W/Poster Board #900

**Pathway analysis of breast cancer genome wide association study highlights two canonical signaling cascades.** *I. Menashe, D. Maeder, S. Bhattacharjee, M. Rotunno, P.S. Rosenberg, N. Chatterjee.* Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Rockville, MD.

Genome-wide association studies (GWAS) have identified genetic variants associated with breast cancer susceptibility, however these studies focus on relatively few highly significant loci while less attention is given to other genotyped markers. Employing pathway analysis to existing GWAS data may shed light on biological processes involved in breast cancer, and illuminate new candidate genes. Therefore, we employed a pathway-based approach to the breast cancer GWAS data of the National Cancer Institute (NCI) Cancer Genetic Markers of Susceptibility (CGEMS) that includes 1145 cases and 1142 controls. Pathways were retrieved from three resources: the KEGG pathway database, BioCarta, and the NCI Protein Interaction Database (PID). We restricted our analysis to pathways with 10-100 genes and excluded *FGFR2* that is highly associated with breast cancer in this GWAS. Association between single nucleotide polymorphisms (SNPs) and breast cancer was assessed using the Cochran-Armitage test for trend. Each gene was represented by its most strongly associated SNP. Finally, we used a weighted Kolmogorov-Smirnov (KS) procedure to identify pathways enriched with gene-based association signals.

A total of 478 pathways containing 4142 genes, (157/2956, 221/1357, and 104/1418 pathways/genes for KEGG, BioCarta, and PID respectively) were included in our study. Interestingly, the most strongly associated pathways ($P_{KS} < 0.05$) shared overlapping genes. To further explore this phenomenon, we used a clustering algorithm to identify pathways with similar gene content and assess the excess of association signals within each cluster. Consequently, pathways containing the signaling cascade of the genes GRB2*, SOS1, HRAS, RAF1, MAP2K1* and *MAPK3* and pathways containing the signaling cascade of the genes *WNT1, FZD1,DVL1, GSK3B, APC* and *CTNNB1*, were significantly more likely to be enriched with association signals than expected by chance ($P = 0.0051$ and $P = 0.0365$ respectively). These results suggest that genetic variations associated with these two canonical signaling cascades may modulate breast cancer susceptibility. Additional studies are needed to replicate our findings and investigate the details of these associations.

## 1243/W/Poster Board #901

**Comparison of the repair kinetics of the UVA- and UVB-induced cyclobutane pyrimidine dimers in the genome of human keratinocytes and its relation to expression of DNA repair genes.** *M. Karbaschi, M.S. Cooke, M.D. Evans.* Dept. Cancer Studies and Molecular Medicine. Robert Kilpatrick Clinical Sciences Building. University of Leicester. LE2 7LX, UK.

Solar ultraviolet radiation (UVR) by inducing DNA photo-lesions, has become the prime cause of most skin cancers. These cancers could be prevented if we protect ourselves from UVR. UVR comprises three main regions: UVC is absorbed by the ozone layer and does not affect the skin. UVB is directly absorbed by DNA and induces different forms of lesions like cyclobutane pyrimidine dimers (CPDs). In contrast with UVB, UVA is indirectly absorbed by DNA. It has long been considered that the UVA component of solar UVR carries a minimal risk for skin carcinogenesis, as it does not lead to the formation of CPD, not least because DNA does not absorb UVA. However, more recently, UVA-induced formation of CPD has been demonstrated, thought to be via a photosensiter-based mechanism. UVA is suspected to play a key role in induction of skin tumors and may be even more important than UVB in mutagenesis. Most sunscreens filter out UVB absorption, but they cannot block most of UVA, so they do not help to prevent skin cancer. A better assessment of the routes by which UVA and UVB induce CPDs, may lead to prevention of skin cancer. This study focuses upon the differential formation and, importantly, repairs of CPD induced by UVA versus UVB. Firstly, formation of UVA-induced CPDs versus UVB-induced CPDs in genome of human keratinocytes (HaCaT cell line) was compared by use of the highly sensitive single cell gel electrophoresis (T4endoV modified comet assay) in HaCaT keratinocyte cell line. Then, the repair rate of CPDs induced by UVA and UVB was also compared by comet assay. Finally, before and after irradiation with UVA and UVB, a comparison of the expression levels of genes coding for components of the nuclotide excision repair pathway, by Q-PCR, was done. The data suggests that, following irradiation, levels of CPD and ALS were significantly higher in the cells irradiated with UVB, compared to UVA. However, for the first time, we noted the rate of repair for UVA-induced CPD to be much faster than the rate of UVB-induced CPD.

## 1244/W/Poster Board #902

**Overexpressed Cyclophilin B Protects cancer cells from ROS-Mediated Cell Death.** *K. Kim, S. Kim, W. Choe.* Department of Biochemistry and Molecular Biology, Medical Science and Engineering Research Center for Bioreaction to Reactive Oxygen Species, Biomedical Science Institute, School of Medicine, Kyunghee University, Seoul 130-701, Korea.

Cyclophilins (Cyps) were identified as cellular binding proteins for the immunosuppressive drug cyclosporine A (CsA) and are constitutively expressed in most tissues. Cyclophilins are multifunctional proteins that are involved in protein folding, mitochondrial functions, interaction with CD147, the immune system, and cancers . One of these family, Cyclophilin B is found mainly in the ER lumen, and it has peptidyl-prolyl cis-trans isomerase (PPIase) activity that catalyzes protein folding reactions in cells. Reactive oxygen species (ROS) are generated as by-products of the mitochondrial respiratory process. They have emerged as an important signaling molecule based on their unique biochemical properties. It consists of various radicals, which might exert different effects on cellular signaling. Low levels of ROS regulate cellular signaling and play and important role in normal cell proliferation. In cancer cell biology, it has been appreciated for a number of years that ROS production is increased in cancer cells. Also ROS are thought to play multiple roles in tumor initiation, progression and maintenance. In contrast to their role in opposite site, ROS appear to activate and modulate cancer cell death. ROS levels are increased in cells exposed to various stress agent, including anticancer drug, and they promote cell death by stimulation pro apoptotic signaling molecules. In this study, we determined the potential role of role of Cyclophilin B as a protector cancer cells from oxidative stress. With two cancer cell lines, Huh7 (Human Hepatoma) and MCF7 (Human Breast Cancer cell), we performed immunoblotting and MTT Assay after treatment with most common but strong oxidative stress chemical, $H_2O_2$ for 24 Hours. According to our results, overexpressed CypB increased cell viability though activation of ERK pathways. Also overexpressed CypB reduced ROS generation.

## 1245/W/Poster Board #903

**The Clinical Implications of Breast Cancer Risk Polymorphisms in FGFR2, TNRC9 and MAPKKK1 in the Context of Family History.** *A.J. Martin[1], S. White[1], A. Onen[1], J. Gale[1], H. Talbot[1], P. Quinlan[2], L. Baker[2], L. Jordan[3], C. Purdie[3], A. Ashfield[2], A. Thompson[2], J. Dunlop[4], J.N Berg[1].* 1) Human Genetics, Centre for Oncology and Molecular Medicine, University of Dundee, United Kingdom DD1 9SY; 2) Department of Surgery and Molecular Oncology, University of Dundee; 3) Department of Pathology, Tayside University Hospitals Trust, Dundee; 4) East of Scotland Genetics Service,Tayside University Hospitals Trust, Dundee.

The majority of familial predisposition to breast cancer is inherited in a multifactorial fashion, rather than caused by known high penetrance genes such as BRCA1 or BRCA2. However, it is uncertain how testing for multifactorial genetic polymorphisms can be used to improve risk assessment for unaffected women with a family history of breast cancer. United Kingdom guidelines, set by the National Institute of Clinical Excellence stratify such women into 3 risk groups, using family history alone; Group 1, or population risk comprising those with less than 3% 10-year-risk at age 40 of developing breast cancer; Group 2 or moderate risk comprising those with a 10-year-risk of developing breast cancer between 3% and 8%; and Group 3 (high risk) who are those with a 10 year risk of greater than 8%, and who are likely to carry a mutation in a high penetrance gene. The 3 most significant single nucleotide polymorphisms (SNPs) that have been identified to date as risk factors for breast cancer are rs2981582 in FGFR2, rs3803662 near TNRC9 and rs889312 near MAPKKK1. Individually these SNPs confer a small increase in risk in breast cancer. To understand whether these SNPs are relevant in clinical practice, we calculated genotype relative risks for combined genotypes using data from the Tayside Breast cancer patient cohort. Relative risks varied from 0.52 for patients with no risk alleles at these loci to 3.08 for those with 5 or 6 risk alleles. We used the BOADICEA risk assessment tool to determine 10 year and lifetime risks of breast cancer for 160 unaffected women attending the clinic for a family history of breast cancer. Assuming a multiplicative interaction between family history and genotype in this patient cohort, and by taking a weighted average by genotype, we estimated the proportion of women who would have a significant change in risk group. Using the 3 locus genotype in addition to family history, 29% of women in the clinic would be assigned to a different category compared to using family history alone. 17% would be assigned a higher risk and 12% a lower risk category. We conclude that the amount of risk modification offered by genotyping at currently known multifactorial loci is sufficient to modify risk when combined with other risk parameters. Testing for SNPs in FGFR2, TNRC9 and MAPKKK1 would lead to better targeting of treatment and screening for patients with a family history of breast cancer. (This work is supported by the EU FP7 project ‘HAMAM’).

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## 1246/W/Poster Board #904

***XRCC1* Arg399Gln polymorphism in acute lymphoblastic leukemia.** *J.P. Meza-Espinoza[1], E. Leal Ugarte[1], V. Peralta Leal[1], D. Ruiz Diaz[1], M. Gutierrez Angulo[2], N. Macias Gomez[3], P. Barros Nuñez[4], J. Duran Gonzalez[5].* 1) Universidad Autónoma de Tamaulipas, Matamoros, Tamps. Mexico; 2) Centro Universitario de los Altos, Universidad de Guadalajara; Tepatitlan, Jalisco, Mexico; 3) Centro Universitario del Sur, Universidad de Guadalajara; Cd. Guzman, Jalisco, Mexico; 4) Centro de Investigacion Biomedica de Occidente del Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico; 5) University of Texas at Brownsville; Brownsville TX, USA.

**Background.** The genome integrity is of vital importance for the correct functionality of the cell, and the DNA repair systems have key roles in its maintenance. Deficient capacity reparation of DNA has been associated with cancer and birth defects. Polymorphisms in DNA repair genes are very common and may result in interindividual variation in DNA repair capacity, therefore them could play a role for the susceptibility to cancer. The X-ray repair cross-complementing group 1 (XRCC1) gene plays a role in the DNA repair. This gene carries polymorphisms that produce amino acids substitutions, for instance Arg399Gln, therefore has been associated with risk to several neoplasias, including acute lymphoblastic leukemia (ALL). **Objective.** Determine the influence of the *XRCC1* Arg399Gln polymorphism on the development of childhood ALL in Mexican patients. **Subjects and methods.** We studied 120 Mexican children with ALL and a control group composed by 120 healthy subjects. Both groups were mestizos of the Mexican West. All of them were genotyped for the *XRCC1* Arg399Gln polymorphism by means of polymerase chain reaction and restriction fragment length polymorphism using the endonuclease *Hpa* II. Comparison of allele and genotype frequencies between patients and controls was realized by Chi-square test. Hardy-Weinberg equilibrium was determined by Fisher exact test. **Results.** The allele frequencies in the control group was 73.7% and 26.3% for Arg-allele and Gln-allele, respectively; whereas in patients were 68.3% for Arg and 31.7% for Gln. The genotype frequencies in the control group were 54.2% for wild homozygous, 39.2% for heterozygous, and 6.6% for polymorphic homozygous; whereas in the ALL patients were 47.5%, 42.5%, and 10%, respectively. **Conclusion.** The allele frequencies were consistent with Hardy-Weinberg equilibrium (p=1.0; Fisher exact test). No significant differences were observed in allele frequencies between patients and controls (p=0.23; Chi square); moreover, we did not find association for ALL childhood with heterozygous or polymorphic homozygous genotypes. Our findings suggest that Arg/Gln or Gln/Gln variants do not increase the risk for childhood ALL in Mexican mestizos patients, which is discordant of some reports in other populations; namely India, Thailand, and China. Perhaps these differences could be fortuitous; however, such discordances may rather be due to the diverse genetic backgrounds.

## 1247/W/Poster Board #905

**Loss of Heterozygosity Analysis in Wilms Tumor.** *R. Padilla[1], M. Haruta[2], S. Berosik[1], A. Chhibber[1], C. Davidson[1], A. Felton[1], R. Fish[1], S.C. Hung[1], B. Johnson[1], M. Kondo[1], J. Lee[1], A. Pradhan[1], L. Joe[1].* 1) Life Technologies, Foster City, CA; 2) Saitama Cancer Center Research Institute, Ina, Saitama, Japan.

Loss of heterozygosity (LOH) is used to help elucidate the molecular mechanisms underlying the development of pediatric tumors such as Wilms tumor, hepatoblastoma, and neuroblastoma. LOH is the loss of the second of two alleles when the first allele may be inactivated, sometimes the result of an inherited germ-line mutation. WT1, a tumor-suppressor gene, encodes proteins critical to protecting kidney cells from cancer during kidney development. In many Wilms tumor cases, the first allele is inactivated by a mutation within the WT1 gene itself, whereas the second allele is subject to inactivation by LOH at one or more nearby polymorphic markers§. To investigate the LOH of contiguous genes implicated in Wilms' tumorigenesis, DNA samples from 26 subjects with suspected Wilms tumor were analyzed. Specific assays have been developed whereby the regions of interest are amplified using one fluorescently-labeled and one unlabeled PCR primer for each locus interrogated. Samples of normal tissue were obtained from either peripheral blood or normal renal tissue adjacent to the tumor from the same individual. We present data from LOH assays performed on Wilms tumor samples using a new capillary electrophoresis workflow. Peak height ratios of healthy and tumor samples were compared to successfully identify and flag LOH candidate samples. This methodology would also be useful to a single researcher or a consortium of investigators with multiple instruments who require consistent and comparable results from each instrument.

## 1248/W/Poster Board #906

**Prostate cancer genome anatomy dissected from the tumors of Caucasian and African-American men reveals known and novel risk genes that may account for the health disparity.** *A. Pearlman[1], C. Campbell[1], S. Shajahan[1], L. Hao[1,2], J. Melamed[1], H. Ostrer[1].* 1) Human Genetics, NYU Langone Medical Center, New York, NY; 2) Center of Genome Informatics, University of Medicine and Dentistry in New Jersey, Newark, NJ.

A well known health disparity exists between the prostate cancer cases of Caucasian and African-American men. Men of African descent have an increased incidence, earlier onset, and more aggressive form of disease as compared with men of predominantly European origin. As part of our efforts to identify somatic alterations that are predictive of ethnic differences or prognosis and to aid the identification of tumor suppressor genes we conducted an array CGH study using Affymetrix SNP Array 6.0, on paired normal and tumor tissue from 9 African American (AA) and 20 Caucasian American (CA) men. The ethnicities of the samples were verified through principal component analysis of the SNP genotypes relative to the HapMap populations. The groups were matched for Gleason score (range 6-9) and stage (T2b-T4), however, the AA group had a marginally lower age at prostatectomy (AA = 59±6 vs. CA = 65±6, p = 0.05). Each patient's normal sample was used as a reference for the paired tumor and copy-number alterations were scored. A ranked list of genes was generated by mapping each probe on the array to the nearest up/downstream gene, and combining the copy number scores across all associated probes for each gene. For the combined set of tumors, the top ranked gene, CSMD1 (deleted in 19/29 samples, Z=137), has been identified as a tumor suppressor gene linked to carcinoma and neoplasm metastasis. SGCZ, our 2nd ranked gene (deleted in 19/29 samples, Z=125) has been proposed to be involved in cytoskeleton organization. The 3rd ranked gene (deleted in 19/29 samples, Z=84), CDH13, was recently, identified in a Genome Wide Association Study and shown to be hyper-methylated, with transcript down-regulated in a variety of cancers. Ranked 5 (deleted in 16/29 samples, Z=79) is the putative prostate cancer tumor suppressor MSR1. The AA subset showed a number of differences when compared to the CA group, including significant deletions from chromosome 8p11-22, and 6q14.2-22.31. In addition, the CA group showed a deletion covering chromosome 21q22.2, containing the DSCAM gene, whereas the AA group did not show this effect. Overall, the total number of copy-number events was similar in the two subgroups (AA 8.2% and CA 8.4%, p = 0.70). There were no significant differences when examining the number of amplifications or deletions separately. Thus, differences in disease between the AA and CA groups may be accounted for by the locations, rather than quantity, of events.

## 1249/W/Poster Board #907

**Whole transcriptome sequencing of three oral cancers.** *D. Smith[1], B. Tuch[2], R. Laborde[1], M. Barker[2], F. de la Vega[2].* 1) Lab Med & Path/Exper Path, Mayo Clinic, Rochester, MN; 2) Life Technologies, Foster City, CA.

Cancers of the head and neck comprise 6 percent of all human malignancies and the majority of these are squamous cell carcinomas. Previous studies of gene expression in these cancers used microarray analysis to identify a number of aberrantly expressed genes. A powerful new approach to analyze the entire transcriptome of samples is based upon massively parallel sequencing to generate gibabases of sequence data. We utilized the SOLiD/AB next generation DNA sequencer to analyze and compare the entire transcriptome of three oral tongue cancers to normal oral tongue tissue from the same patients. Using the novel SHREK protocol, which preserves transcript strandedness across the entire gene length, for the preparation of libraries from ribo-RNA depleted samples we obtained 100 Gbs of whole transcriptome sequence. We demonstrate that this RNA-seq protocol is a powerful tool for analyzing changes across the entire transcriptome during cancer development. We have demonstrated that gene expression profiling results obtained by sequencing are largely concordant with microarray data using identical samples. These data indicate advantages of RNA-Seq, and its' ability to preserve transcript strandedness thus it was very useful in identifying differentially regulated, antisense transcripts. MMP1 is an example of a secreted matrix metalloproteinase that breaks down the interstitial collagen and exists as an anti-sense transcript that was detected as dysregulated by sequencing but was not by our microarray experiment. Additionally, sequencing has proven to be much more effective at identifying differential expression of genes expressed at low levels in tissue. One example is the oncogene HMGA2, with an increase in expression of only 1.5-fold detected by microarray as compared to a 206-fold increase detected by sequencing. RNA-Seq also readily identifies differential expression of transcript isoforms. This depth of sequencing will not only identify differential expression of transcripts, but also allow for detection of mutations within many of the detected transcripts, allowing the detection of mutations that may be used to screen for the development of specific cancers. Finally, this data also can be analyzed to detect novel transcripts including many potential non-coding transcripts. This technology can transform the way that cancer is analyzed and may offer important insights into the specific alterations that underly the development of cancers of the oral.

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## 1250/W/Poster Board #908

**Analysis of the BRCA1 Promotor Polymorphism, rs11655505, as a Breast Cancer Risk Factor in the Tayside Breast Cancer Cohort.** 
*H.R. Talbot[1], L.B. Jordan[2], C.A. Purdie[2], A.M. Ashfield[3], P.R. Quinlan[3], A.J. Martin[1], L. Baker[3], C. Palmer[4], R. Tavendale[4], A.M. Thompson[3], J.N. Berg[1].* 1) Human Genetics, Centre for Oncology and Molecular Medicine, University of Dundee, United Kingdom DD1 9SY; 2) Department of Pathology, Tayside University Hospitals Trust, Dundee; 3) Department of Surgery and Molecular Oncology, University of Dundee; 4) Biomedical Research Institute, University of Dundee.

Single nucleotide polymorphisms (SNPs) in the BRCA1 gene may contribute to a multifactorial predisposition to breast cancer. SNP rs11655505, which lies within the BRCA1 promoter region has been shown to increase BRCA1 promoter activity. One association study has suggested that the rare 'T' allele protects against breast cancer in Chinese women. We analysed rs11655505 in a cohort of population breast cancer patients recruited from the Tayside region of Scotland. We genotyped 426 breast cancer patients, taken from the Tayside Breast Cancer Cohort, and 399 control subjects using Applied Biosystems allelic discrimination assays. No significant association between rs11655505 genotype and breast cancer was identified, (chi-squared test for trend =1.63, p=0.2020), odds ratios, comparing each genotype to wild-type (C/C) homozygote frequencies were non-significant, 1.31 (95% confidence interval 0.981 to 1.75) for C/T heterozygotes and 1.136 (95% confidence interval 0.680 to 1.898) for T/T homozygotes. No correlation between clinical features or tumour pathology and rs11655505 genotype was found. This included analysis for tumour grade, oestrogen receptor status, triple negative vs. non-triple negative tumour status, nodal metastasis, tumour size and age of onset. These findings suggest that rs11655505 does not protect against breast cancer development within the Scottish population. This may reflect a genuine difference between the Scottish and Chinese populations, with rs11655505 occurring within different haplotypes in the two populations. In this case rs11655505 would be expected to tag a different SNP that is responsible for the protective effect in the Chinese population. Alternatively rs11655505 may have a more modest effect than estimated in the first study and this would require a much larger case-control study to identify any effect. In either case, there is no evidence that analysis of rs11655505 in the Scottish population would contribute to estimating a patient's risk of developing breast cancer. (This work was undertaken in Collaboration with the Tayside Tissue Bank and was supported by Breast Cancer Research Scotland).

## 1251/W/Poster Board #909

**Electronic Medical Records: Opportunity for Genetic Patient Ascertainment.** 
*E. Parkhurst, S. Abboy.* Dept Genetics, Kaiser Permanente, West Los Angeles, CA.

BACKGROUND: About 5-10% of breast, ovarian, and colorectal cancer is due to hereditary cancer syndromes. Previous studies have shown that a minority of patients with risk factors for hereditary cancer syndromes are referred for genetic counseling and testing; about 20-30% of eligible colon cancer patients and about 45% for early onset breast cancer. PURPOSE: A pilot study was conducted to determine how many patients with early onset cancer diagnoses are being referred to our genetics clinic and if emailing those at-risk patient's providers could be an effective way to increase referrals of appropriate patients. METHODS: We ran queries of the relational database for the Kaiser Permanente electronic medical records system. Search criteria were by age and ICD9 code; ≤ age 45 for breast cancer, male breast cancer at any age, and ≤ age 50 for colon and uterine cancers. Messages were sent by email to the primary care provider and oncologist of living, eligible patients who had not yet been referred to genetics clinic. Emails regarding 96 different patients were sent to 62 different providers. RESULTS: The database search found 178 patients with early onset cancer; 125 with breast cancer, 34 with colon cancer, 10 with uterine cancer and 9 with male breast cancer. Of those, 50 (40%) breast cancer patients had been previously referred for genetic counseling, but only 5 (15%) colon cancer patients, 1 (11%) male breast cancer case, and 0 uterine cancer patients. Of the 32 referrals sent in response to our emails, 5 were sent by the patient's oncologist, and 27(84%) were from the patient's primary care provider, either internal medicine or family medicine. CONCLUSION: Our hospital's genetic cancer referral rates are similar to what other studies have shown. Primary care providers were more responsive to outreach than oncologists. Providers were more likely to refer for early onset breast cancer than other cancers and future provider training should focus on more than just breast cancer indications for referral. With a response rate of 33% we concluded that an email to the patient's provider is not sufficient to ensure referral of appropriate patients. As more healthcare organizations move towards electronic medical records, database searches may become a powerful tool for case finding or improving referrals in other genetics clinics.

## 1252/W/Poster Board #910

**Biallelic mutations in *FANCD1/BRCA2* identified in the course of genetic counseling for hereditary breast and ovarian cancer.** 
*E. Schalles, C. Cullinane.* Todd Cancer Institute, Long Beach Memorial Medical Center, Long Beach, CA.

Monoallelic and biallelic *BRCA2* mutations are associated with distinctive phenotypes and increased risk of malignancy. In 1995 monoallelic *BRCA2* mutations were linked to hereditary breast and ovarian cancer (HBOC) and in 2002 biallelic *BRCA2* mutations were discovered in patients identified with Fanconi anemia complementation group FA-D1. Since then, fewer than 30 individuals with biallelic mutations in *FANCD1/BRCA2* have been reported in the literature. We present a family with biallelic mutations in *FANCD1/BRCA2* ascertained through genetic counseling for HBOC. A 44 year old Hispanic female diagnosed with unilateral invasive ductal breast carcinoma at age 43 was identified as a carrier of the deleterious *BRCA2* mutation, 886delGT and was subsequently referred for cancer genetic counseling. During the consultation a four-generation pedigree was obtained. The patient reported that her daughter had been diagnosed with Wilms' tumor at 3 1/2 years old and passed away at age 11 from leukemia. We consequently provided cancer genetic counseling to her 47 year old Hispanic husband who proceeded with genetic testing and was found to carry the deleterious *BRCA2* mutation, 2971del5. Medical records confirmed that in 1996 their daughter underwent a right radical nephrectomy due to Wilms' tumor and in September 2003 was diagnosed with acute myelogenous leukemia; she passed away in November 2003 from multi-organ system failure. Records also indicate that she had an imperforate anus, microcephaly and multiple skin hyperpigmentations, depigmentations and café-au-lait spots. The *BRCA2* mutation, 886delGT was not identified in our patient's mother. There was limited knowledge of her paternal health history. Her husband reported that his mother was diagnosed with breast cancer at age 59 and passed away at age 64 and his maternal grandmother had a history of breast cancer and passed away at age 65. He also had limited knowledge of his paternal health history. While a rare occurrence the identification of biallelic mutations in *FANCD1/BRCA2* is obviously a possibility within an HBOC genetic counseling situation. Further identification and reporting of individuals who are carriers of biallelic mutations in *FANCD1/BRCA2* will contribute to the characterization of the phenotypic spectrum associated with this cancer syndrome. In addition, counseling practices may be modified depending on how individuals are ascertained, either directly with a FA-D1 diagnosis or through HBOC families.

## 1253/W/Poster Board #911

**Expanding the spectrum of mutations associated with the PTEN promoter.** 
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The phosphatase and tensin homolog (*PTEN*) gene is a tumor suppressor gene located on the long arm of chromosome 10. The *PTEN* gene encodes a lipid phosphatase that negatively regulates the phosphatidylinositol 3-kinase (PI3K)/AKT (protein kinase B) pathway. Mutations within the *PTEN* gene are associated with PTEN Hamartoma Tumor syndrome (PHTS), an autosomal dominant disorder characterized by hamartomatous tumors. Clinically PHTS includes Cowden syndrome (CS, MIM 158350), Bannayan-Riley-Ruvalcaba syndrome (BRRS, MIM 153480), Proteus syndrome (PS, MIM 176920) and Proteus-like syndrome. Mutations in the *PTEN* gene have also been identified in children with autism spectrum disorders (ASDs) and macrocephaly. In addition to sequence variations in the coding exons and flanking intronic regions, mutations have also been reported in the 600 base pair promoter region of the *PTEN* gene and account for approximately 10% of *PTEN* mutations in individuals with CS. There have been a limited number of reports describing *PTEN* promoter mutations in individuals with ASDs and macrocephaly. Currently, our laboratory's molecular testing strategy for the analysis of the *PTEN* gene includes; sequence analysis of the nine coding exons, intron-exon boundaries and promoter region and dosage analysis using multiplex ligation dependent probe amplification (MLPA). Among the 112 individuals referred to our laboratory for molecular testing of the *PTEN* gene, we identified three previously reported intragenic mutations and four heterozygous promoter alterations. Two of the promoter variants, -903 G→A and -1026 C→A, identified in seven individuals, have been previously reported as polymorphisms. The remaining two novel promoter variants, -975 G→A and -1059 C→G, were identified in two patients referred to our laboratory for autism with macrocephaly and CS, respectively. Further clinical and genetic analysis of the two probands with novel *PTEN* promoter mutations and family members will provide insight regarding the wide clinical spectrum associated with mutations within the *PTEN* gene. These findings also highlight the importance of performing promoter mutation analysis to improve the detection rate of PHTS and other clinical disorders associated with the *PTEN* gene.

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### 1254/W/Poster Board #912

**Screening of BRCA1 and BRCA2 mutations in 300 patients by qPCR-HRM, a combined method for point mutation and large rearrangement detection.** *F. Coulet[1], M. Eyries[1], F. Pires[1], E. Rouleau[2], C. Lefol[2], C. Colas[1], A. Hardouin[3], R. Lidereau[2], F. Soubrier[1].* 1) Oncogenetic laboratory, APHP-GH Pitié Salpêtrière, Paris, Ile de France, France; 2) Oncogenetic laboratory, Centre René Huguenin, INSERM U735, Saint Cloud, Ile de France, France; 3) Laboratory of Clinical and Oncological Biology, Centre François Baclesse, Caen, Normandie, France.

Screening of the two major breast and ovarian cancer susceptibility genes BRCA1 and BRCA2 are time-consuming and must include exploration of genomic rearrangements that represent 5 to 15% of the alterations observed in these genes. We evaluated a method combining both point mutations detection by High Resolution Melting (HRM) which monitors the fluorescence of double strand DNA with saturating dye and large gene rearrangements detection by allelic quantification with semi-quantitative analysis (qPCR). The two analyses could be done on the same thermocycler run (qPCR-HRM). We evaluated the sensitivity of the qPCR-HRM technology by analysing 201 known point variants scattered over all amplicons of BRCA1 and BRCA2 genes and seven large gene rearrangements involving one or several exons of BRCA1 or BRCA2; 100% of the variants were detected. Furthermore, a retrospective study was done with 44 patients previously BRCA1 tested by DHPLC, all the variants were detected by HRM analysis and 4 supplementary homozygous polymorphisms were identified by HRM analysis. Finally, a prospective study was done with 300 patients allowing 27 deleterious mutations, 33 unclassified variants and three genomic rearrangements to be detected. qPCR-HRM is a simple, sensitive and rapid closed tube method that does not require modified PCR primers. The major advantages of this method is the simultaneous detection of point mutations, large scale gene rearrangement and absence of amplification of one allele, a combined approach which is critical in genetic diagnosis.

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## 1255/W/Poster Board #913

**Fusion of the *FUS* and *CREB3L2* genes in a ring chromosome in low-grade fibromyxoid sarcoma.** *H. Bartuma[1], M. Isaksson[1], A. Collin[1], H. Domanski[2], N. Mandahl[1], F. Mertens[1].* 1) Department of Clinical Genetics, Lund University Hostpial, Lund, Sweden; 2) Departments of Cytology and Pathology, Lund University Hospital, Lund, Sweden.

Low-grade fibromyxoid sarcoma (LGFMS) is a rare, low-grade malignant soft tissue tumor that often is mistaken for either more benign or malignant tumor types. Commonly, this tumor affects young adults and typically arises in the deep proximal extremities or trunk with frequent recurrences and can metastasize to the lungs many years later. A recurrent balanced translocation between chromosomes 7 and 16, t(7;16)(q32-34;p11), has been identified and has been shown to lead to the expression of a *FUS/CREB3L2* fusion gene in almost all reported cases. Furthermore, this gene fusion is so far specific for LGFMS. Previously, supernumerary ring chromosomes have been identified in LGFMS, but it has not yet been formally demonstrated that ring chromosomes harbor the *FUS/CREB3L2* fusion gene. Here, we report the genetic findings in an LGFMS from the lung from a 77-year-old man. Banding analysis revealed a ring chromosome, and further studies with fluorescence in situ hybridization and RT-PCR showed that the ring contained material from chromosomes 7 and 16, that the FUS gene was present in two rearranged copies, and that it expressed the *FUS/CREB3L2* fusion gene.

## 1256/W/Poster Board #914

**Comparative evaluation of CNV analysis in breast cancer using different population references.** *A. Hidalgo[1], S. Rodriguez-Cuevas[2], L. Uribe-Figueroa[1], F. Guisa-Hohenstein[2], C. Rangel-Escareno[1], G. Ortiz-Ramos[1], R. Mojica-Espinosa[1], V. Quintanar-Jurado[1], S. Romero-Cordoba[1], R. Rebollar-Vega[1], S. March-Mifsut[1], G. Jimenez-Sanchez[1].* 1) National Institute of Genomic Medicine (INMEGEN), Mexico City, Mexico; 2) Fundación Mexicana de Fomento Educativo para la Prevención y Detección Oportuna del Cáncer de Mama (FUCAM), Mexico City, Mexico.

Copy Number Variation (CNV) analysis is a powerful strategy to discover cancer related genes. A specific pattern of DNA gains and losses has been described for breast cancer. However, this information is very limited in women from Mexican or Hispanic origin. The use of high-resolution platforms for CNV analysis poses several challenges, including false copy number calls due to normal CNV, particularly when a diploid control from a different population is used. We analyzed 50 tumor tissues with >70% tumor cells and peripheral blood pairs from Mexican women without prior treatment, using the Affymetrix 6.0 Gene Chip to describe CNV alterations in these tumors (Partek Genomics Suite). To evaluate the influence of different population references in the analysis, we compared results from these tumors with the following references: 1) normal paired tissue from the same individuals, 2) CNV data from the HapMap samples (n=270), and 3) CNV data from 240 Mexican Mestizo population controls. Our first comparison, tumor/normal paired analysis, identified amplifications in the 8q24.11-q24.12 region in 25 (50%) samples and deletions in 13q14.2 and 16q21 in 20 (40%) of the samples as the most common alterations. To evaluate the efficiency of other potential references, we performed two additional comparative analyses. The second comparison included peripheral blood DNA against data from the HapMap samples and identified 8,059 CNV 1kb in size with less than 1 or more than 3 copies. Of these, 1,899 were not in the Database of Genomic Variants (DGV), 250 of these regions were present in >50% of the normal samples. Our third scenario compared peripheral blood samples with the Mexican population reference. We found 15,478 CNV regions, of which 3,176 were not present in the DGV. Of these, 350 were present in >50% of the normal samples. When the tumors were compared against the Mexican or the HapMap reference samples, we found an increased number of CNV present in all tumors indicating that the difference correspond to normal CNV changes present in the reference DNA samples. Our results indicate that CNV analysis is sensitive to the population reference used being tumor/normal paired the closest reference, followed by a reference with a similar population background, since a multiethnic reference generates a significant number of false positives, as opposed to real somatic changes in the tumors.

## 1257/W/Poster Board #915

**Whole-genome array-CGH analysis in MDS/AML patients: Characterization of chromosomal abnormalities and detection of cryptic aberrations.** *P. Li, R. Bajaj, K. Wilcox, F. Xu.* Dept Gen, Yale Univ Sch Med, New Haven, CT.

Whole genome oligonucleotide array comparative genomic hybridization (array-CGH) using Agilent's 44K oligonucleotide array (CGH4410B) has been used in clinical diagnosis of constitutional chromosomal and genomic imbalances with an average resolution of 300-500 Kb (5-7 contiguous probes) and 99% specificity and sensitivity. To evaluate the clinical validity and analytical outcome of this genomic approach for detecting acquired somatic abnormalities, we have applied array-CGH on 30 MDS and AML cases with recognized chromosomal abnormalities in >50% of analyzed metaphase cells. This genomic analysis detected all gains of chromosomes 1, 4, 5, 6, 7, 8, 11, 13, 14, 19 and 20, as well as losses of chromosomes Y, 7, 8 and 18. A total of 103 deletions (43 Kb to 135 Mb, average size: 19.143 Mb), 28 duplications (48 Kb to 118 Mb, average size: 21.883 Mb), seven triplications (average size: 9.432 Mb), two dmin amplifications (3-4 Mb) and six marker chromosomes were further characterized with defined breakpoints and genomic contents. Clinical significant recurrent or overlap deletions at 5q (involving the RPS14 gene), 12p12.3 (ETV6 gene), 13q14.3 (miR-15a/16-1), 17p13 (TP53 gene), 17q11.2 (NF1 gene) and 20q were noted in 10 cases (10/30, 33%), seven cases (23%), four cases (13%), four cases (13%), four cases (13%) and three cases (10%), respectively. All three deletions at 17q11.2 were undetectable by routine chromosome analysis and were confirmed by FISH using BAC clones for the NF1 gene. With the exception for one case with a secondary 9p deletion, all chromosomal imbalances in 29 cases (29/30, 97%) and additional complex or cryptic genomic aberrations in 23 cases (77%) were detected by array-CGH analysis. These results indicated that array-CGH could be a valuable tool for cancer cytogenetic and genomic diagnostics.

## 1258/W/Poster Board #916

**A Novel Chromosomal Alteration in A Patient of Unclassifiable Myelodysplastic/Myeloproliferative Neoplasm with Thrombocytosis.** *J. Lin[1,2,3,4], M. Guo[1], R. Girard[4], R. Nagwekar[1], A. Pigal[1], H.O. Shah[1,2].* 1) Dept Pathology, Nassau Univ Medical Ctr, East Meadow, NY; 2) Health Sciences Center, Stony Brook, SUNY; 3) New York College of Osteopathic Medicine, NY; 4) American University of the Caribbean, Netherlands Antilles/ Coral Gables, FL.

Cytogenetic studies have a major role in the evaluation of patients with myelodysplastic syndrome (MDS). About 50% of MDS cases have clonal cytogenetic abnormalities, including +8, -5, del(5q), -7, del(7q) and del (20q). There is no cytogenetic or molecular genetic finding specific for myeloproliferative neoplasm, unclassified(MDS/MPN,U). We report a case of MDS/ MPN,U with thrombocytosis. A 71 year old woman with a past medical history of anemia was admitted as severe anemia. At the time of admission, the patient had complains of weakness and headache. The complete blood count at admission revealed the complete blood count showed hypochromicnormocytic anemia, leukopenia, dysgranulocytopoiesis, and an important finding of thrombocytosis with WBC 2.5 k/mm3, N 28%, L 50%, M 9%, 4 % blast; RBC 1.4 million/mm3, Hgb 3.7 g/dL, Hct 11.5%, MCV 80.4fL, RDW 16.7%, platelet 1237 k/mm3 . A CT scan of abdomen revealed hepatomegaly. Bone marrow biopsy showed a cellularity of 60%, demonstrated 16% blasts and revealed granulocytic series dysplastic change. The megakaryocytes were very prominent with dysplasia showed up to 37 cells/HPF. Cytogenic analysis of bone marrow cells revealed a specific alteration has not yet been reported. The composite karyotype of the bone marrow cells disclosed: abnormal female karyotype 46,XX,add(13)(p13)?c[17]. Final diagnosis for this woman was Myelodysplastic Syndrome / Myeloproliferative Neoplasm, Unclassifiable. This is a case of rare proportions. Although the precise mechanisms for pathogenesis is not yet clear, it is important to recognize this disorder because dysplastic and proliferative features may both need to be considered to achieve the best regimen for therapy.

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## 1259/W/Poster Board #917

**Primary Ewing Sarcoma/Primitive Neuroectodermal Tumor of Soft Tissue in the Perinephric Region.** *H.O. Shah[1, 3], G. Fan[1], N. Chen[1], L. Freedman[1,3], J.H. Lin[1,2,3], No.* 1) Dept Pathology, Cytogenetics, Nassau Univ Med Ctr, East Meadow, NY; 2) New York College of Osteopathic Medicine, Old Westbury, NY; 3) Department of Pathology, State University of New York at Stony Brook, Stony Brook, NY.

Primary Ewing's sarcoma/primitive neuroectodermal tumor (PNET) of soft tissue is a rare entity. We describe a primary Ewing's sarcoma/PNET of soft tissue in the perinephric region in a 36 year old man who was admitted as acute pyelonephritis. CT scan revealed left renal enlargement with multiple hilar masses and a posterior left perinephric nodule. CT-guided fine-needle aspiration biopsy of the posterior left perinephric nodule showed characteristic cytological and histological appearances of a small round blue cell tumor with focal Homer-Wright rosette formation. The tumor cells showed diffuse positivity with CD99, vimentin, BCL2, NSE (neuron specific enolase), and synaptophysin but were negative for muscle markers (desmin, myogenin, and smooth muscle actin), melanoma markers (S-100, HMB 45, Mart-1, and tyrosinase), epithelial markers (pankeratin, epithelial membrane antigen, CK7 and CK117), and lymphoid markers (CD45, CD3, and CD20). Fluorescence in situ hybridization analysis using dual-color DNA probes for the Ewing Sarcoma breakpoint region 1 (EWSR1) on chromosome 22q12 revealed a rearrangement of EWSR1 locus, confirming the diagnosis of Ewing's sarcoma/PNET. To our knowledge this is the first intact documentation of primary Ewing's sarcoma/PNET of soft tissue in the perinephric region with an immunohistochemical penal and cytogenetic analysis.

## 1260/W/Poster Board #918

**Copy number of hTERC (3q26) in pediatric patients with acute lymphoblastic leukemia at diagnosis detected by FISH.** *A. Corona-Rivera[1,2], H.J. Pimentel-Gutiérrez[1], C. Ortega de la Torre[1], V. Soto-Chávez[2], F. Sánchez-Zubieta[2], L. Bobadilla-Morales[1,2].* 1) Lab de Citogenetica Genotoxicidad y Biomonitoreo, Instituto "Dr. Enrique Corona Rivera", Universidad de Guadalajara, CUCS, Guadalajara, Jalisco, Mexico; 2) Unidad de Citogenética, Servicio de Hematología Oncología Pediátrica, División de Pediatría, Nuevo Hospital Civil "Dr. Juan I. Menchaca", Guadalajara, Jalisco, México.

Telomeres are the ending portion of chromosomes which limit them. Telomerase gene is located in 3q26 (Nowak, et al., 2006). Telomerase maintain the telomere length and is associated with cell cycle. Short telomeres and a high telomerase activity (ALL, AML, CML and Non Hodgkin lymphoma) is related to a severe hematologic neoplasia like, this suggest that telomere length and telomerase activity may be useful for monitoring disease. In ALL increase in telomerase activity related to *hTERC* amplification has been observed with FISH (Nowak, 2006). However SNP Microarrays studies and DNA sequencing did not find 3q gain in 225 ALL patients (Mullinhan et al., 2007). The aim of this study is to characterize copy number of *hTERC* gen (3q26) in pediatric patients with ALL. We included patient with ALL at diagnosis. Bone marrow sample was obtained to perform routine karyotype and *hTERC* FISH. We scored cells on 65 patients most of them with ALL diagnosis. Observations were done on 1000 cells per patient. The FISH pattern observed in patient samples was 2 Green (3cep)/2 Red (3q26 *hTERC*), that indicated no amplification. In one patient the most common pattern was 2G/3R, indicating a gain in 3q26. Triploidy and tetraploidy were observed in 4 patients, which was concordant with the Karyotype. It seems that there is no hTERC gain at diagnosis in pediatric ALL patients.

## 1261/W/Poster Board #919

**Recurrent Aberrations in High Grade B-Cell Lymphomas with Concurrent 8q24/ MYC Rearrangements and t(14;18).** *R. Garcia, N. Uddin, I. Villalobos, S. Holdridge, C. Chastain, J. Doolittle, C. Tirado.* UT Southwestern, Dallas , TX.

Simultaneous 8q24/ MYC rearrangements of Burkitt's lymphoma (BL) and t(14;18) of Follicular lymphoma (FL) occur rarely in high grade B-cell lymphomas, and are associated with an aggressive clinical outcome. These dual translocations or double hit event (DH) in HGLs have been reported in transformed FL, BL, acute lymphoblastic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL) and plasmablastic myeloma. The objective of this study was to determine frequent chromosome alterations with concurrent 8q24/ MYC and t(14;18) rearrangements. Simultaneous 8q24 alterations and t(14;18) with specific morphological patterns (BL/ALL, FL and DLBCL) were searched from the Mitelman database (from 1981-2007). We identified a total of 25, 35 and 39 cases for FL, BL/ALL and DLBCL respectively. Each morphologic entity was first evaluated for frequent alterations, and also for paired aberrations that happen more frequently together than expected from their probability of random distribution. The strength of association of paired aberrations with the five highest chi square values are reported here. Results were then compared with the Fisher Exact Test and chi square to determine differences in different morphologic groups. In FL, the 8 most frequent alterations were +7, +12, t(2;8), +X, +21, -13, -X and +Y. Statistically significant paired aberrations within the FL dataset included: -X/+7, -X/+12, +21/-13, +12/+7 and -X/ t(2;8). In DLBCL, the 9 most recurrent alterations included +7, t(8;22), +12, -15, +11, +21, +X, -X, add(8)(q24) and the statistically significant paired aberrations with were +11/-15, +12/+X, t(8;22)/-15, -X/-15, -X/+7. Similarly, t(8;22), +7, +20, +8, +X, +12, t(2;8), +11 were the 8 most frequent aberrations in the BL group; and the main pairs with statistical significance within this group were +20/+8, +11/+20, +8/+7, +20/+7 and +X/+20. Comparison between morphologic groups showed a higher frequency for t(8;22) in both BL and DLBCL than FL (18 and 15 vs 4 events respectively); however, only BL showed a statistically significant association ($p < .05$). Other findings included a significantly higher frequency for pair rearrangement +8/+20 in BL compared to both DLBCL and FL (4, 0, 0 respectively; $p < .05$). Taken together, findings in this study help to outline frequent alterations, statistically significant pairs of aberrations within specific morphologic groups and identify significant differences between DH high grade B-cell lymphomas.

## 1262/W/Poster Board #920

**A Case of Triple Hit Lymphoma.** *K. Geiersbach[1], M. Sennett[2], S. Shetty[1].* 1) Department of Pathology and ARUP Laboratories, Salt Lake City, UT; 2) Department of Pathology, Northwest Texas Hospital, Amarillo, TX.

**Clinical Background**: A 49 year old female with a 23 pack-year history of smoking and COPD presented with fever, pneumonia, and cranial nerve palsy. CT scan showed bilateral pneumonia and significant mediastinal lymphadenopathy. Mediastinoscopy was performed and a biopsy of the left lower paratracheal node was obtained. **Laboratory Findings**: Analysis by flow cytometry showed a clonal population of neoplastic cells. Cells coexpressed CD19, CD20, and CD10, showed surface lambda light chain restriction, and partial positivity for FMC-7 and CD23; CD5 was negative. By histopathology and immunohistochemistry, the neoplasm consisted of medium sized B lymphocytes which were strongly BCL2 positive and weakly BCL6 positive. Proliferative index by Ki-67 was 90%. Bone marrow was hypercellular (99%); blood and bone marrow were infiltrated with malignant lymphocytes. Pathology diagnosis was rendered as high grade B cell lymphoma of germinal center origin. Subsequent cytogenetic analysis of the peripheral blood revealed a complex karyotype containing 3 translocations: t(2;18)(p12;q21), t(3;?)(q27;?), and t(8;14)(q24;q32), as well as deletions of 13q and 16q. By FISH, the 8;14 translocation was shown to result in disruption of the MYC locus on 8q24 and the IGH@ locus on 14q32, consistent with an IGH/MYC fusion. FISH also showed rearrangements of BCL6 (3q27) and BCL2 (18q21). **Discussion**: The findings are consistent with a very rare entity, triple hit lymphoma, an IGH-rearranged lymphoma with simultaneous rearrangements of the MYC, BCL6, and BCL2 loci. This entity is grouped with double hit lymphoma, within the WHO category of "B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma." This case further clarifies the laboratory findings in triple hit lymphoma and may contribute to knowledge of its prognostic significance.

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## 1263/W/Poster Board #921

**A case of congenital leukemia that probably arose from acquired double trisomy, 48,XX,+18,+21.** *N. Itoh[1], H. Kuniba[1], H. Kawara[2], O. Shimokawa[2], N. Harada[2], M. Egashira[1], M. Tagawa[1], Y. Funakoshi[1], M. Okada[1], T. Kondoh[1], H. Moriuchi[1].* 1) Pediatrics, Nagasaki University, Nagasaki, Japan; 2) Kyushu Medical Science, Nagasaki, Japan.

Congenital leukemia is very rare and occasionally associated with a variety of chromosomal abnormalities. 11q23/MLL rearrangement or AML1/RUNX1 (21q22.3) gene mutation is found in some patients; however, molecular mechanisms of in utero leukemogenesis remain obscure. We here report a newborn patient with congenital leukemia that probably arose from acquired double trisomy of chromosomes 18 and 21. A 24-year-old woman was admitted for hydrops fetalis. She was on 33 weeks + 0 day of her second pregnancy. She had a healthy 3-year-old boy and no miscarriage. Immediately she underwent emergency cesarean section, giving birth to an infant weighing 2542 g with asphyxia. After cardiopulmonary resuscitation, she was transferred to the NICU. Physical examinations showed generalized edema, cyanosis and normal female genitalia, but no malformation. The initial white blood cell (WBC) count was 188,100/¨μl with 92% blast cells (CD34+/CD19-/CD10-/CD2-/CD3-; PO negative). Bone marrow examination could not be performed due to severe hydrops. Complete blood exchange was performed twice, which reduced WBC count to 11,000/μl temporally; however, she died on day 11 after birth from multiple organ failure. Autopsy studies revealed massive leukemic infiltration into liver, the small intestine, spleen, kidneys and lungs and no malformation or visceral anomaly. G-banded chromosomal analysis without PHA stimulation showed 48,XX,+18,+21 in all peripheral blood (PB) cells (20 metaphases) tested, which was confirmed by FISH analysis of 300 interphase nuclei. Whole genome array comparative genomic hybridization analysis of the DNAs extracted from autopsied liver and bone tissue revealed the karyotypes of arr18p11.32q23(18,339-76,089,909)x3,21q11.2q22.3(14,859,742-46,932,616)x3 and arr(1-22,X)x2, respectively. 11q23/MLL rearrangement was not found in any of PB cells tested (100 metaphases) by FISH analysis. Direct sequencing of AML1/RUNX1 gene revealed no mutation in the patient, and real-time PCR excluded the possibility of AML1/RUNX1 gene copy number change. Based on the above data, we speculate that the double trisomy arose from somatic non-disjunction, not from germinal cells, and contributed significantly to leukemogenesis in this patient; however, we don't know yet how it happened. It also remains unknown if all blood cells or possibly all mesoderm-derived cells exhibited the double trisomy and if it arose directly from normal karyotype or went through either single trisomy.

## 1264/W/Poster Board #922

**Identification of Genomic Alteration Events and Associated Gene Expression Profiles for MSS and CIMP-negative Colon Tumors by High Resolution Genome-Wide SNP and Gene Expression Arrays.** *L. Loo[1], I. Cheng[1], M. Tiirikainen[1], G. Okimoto[1], A. Lum-Jones[1], A. Seifried[1], S. Gallinger[2], S. Thibodeau[3], G. Casey[4], L. Le Marchand[1].* 1) Cancer Research Center of Hawaii, Honolulu, HI; 2) Mount Sinai Hospital, University of Toronto, Toronto, Ontario Canada; 3) Mayo Clinic College of Medicine, Rochester, MN; 4) University of Southern California, Los Angeles, CA.

**Background:** Colon cancer is the result of a multi-step process involving the accumulation of genetic and epigenetic alterations, leading to the transformation of normal colon epithelium to adenocarcinoma. Recent advances in microarray technology have lead to higher resolution scans for detecting specific regions of allelic gains and losses by single nucleotide polymorphism (SNP) arrays. In addition, when combined with high resolution gene expression arrays, these genomic alterations can be correlated with alterations in gene expression. **Objective:** To identify frequent copy number alterations (CNA) and associated gene expression profiles in 40 paired colon adenocarcinomas and normal tissue, utilizing high-density SNP and gene expression arrays. **Methods:** DNA and RNA were extracted from 40 fresh frozen paired colon tumors and normal tissue collected by the Colon Cancer Family Registry. We focused on microsatellite stable (MSS) and CpG island methylator phenotype (CIMP)-negative colon tumors, the most common molecular subtype. Genomic profiles were identified by genotyping both tumor and normal tissue with the Affymetrix Genome-Wide Human SNP 6.0 array, containing >1.8 million genetic markers. Gene expression profiles in tumors were identified by utilizing the Affymetrix GeneChip Human Exon 1.0 ST array, interrogating >1 million exons. Preliminary genomic alteration and gene expression analysis was performed with the Partek Genomics Suite software. **Results:** We identified broad regions of CNAs that occur at high-frequency (>50%) in several chromosomal regions: 7p, 7q, 13q, 20p, 20q, Xp, Xq (gains) and 8p, 17p, 18p, 18q (losses). In addition, CNAs occurring in 20-50% of the tumors were observed at 8q (gain) and 14q (loss) and focal events at 1q (gain), and 5q and 16p (loss). We also detected allelic imbalance associated with a subset of these sites of frequent CNA events. Preliminary results from the integration of CNA and gene expression data indicate that a subset of these frequent CNA events is associated with disruptions to gene expression. **Conclusions:** Our high-resolution analysis has identified specific molecular alterations in this common subtype of colon cancer. This genome-wide characterization of both genomic alterations and gene expression may help identify key genes and pathways involved in colon cancer. We will present detailed results from this integrated and comprehensive genetic profiling of MSS and CIMP-negative colon cancers.

## 1265/W/Poster Board #923

**Frequent Aberrations of Chromosomes 1 and 12 in Childhood and Adolescent Germ Cell Tumors.** *X. Lu, Y. Zhao, Y. Wang, M. Folsom, R. Naeem, R. Egler, A. Pappo, J. Hicks, P. Rao.* Texas Children's Hospital Cancer Ctr, Department of Pediatrics, Baylor College of Medicine, Houston, TX.

Pediatric germ cell tumors are heterogeneous and relatively rare tumors that account for approximately 3% of pediatric malignant tumors. To understand the genetic basis of these tumors, we performed G-banding, fluorescence in situ hybridization (FISH) and multicolor spectral karyotyping (SKY) analyses on 39 patients comprised of 18 benign teratomas, 10 malignant mixed germ cell tumors, nine yolk sac tumors, and one dysgerminoma and one seminoma enrolled at Texas Children's Hospital Cancer Center between 2003 to 2009. Frequent chromosomal aberrations involving chromosomes 1 (1p deletion/1q gain) and 12 (isochromosome 12p) were found in 13 (33%) cases including nine males and four females. Near triploidy/tetraploidy, gain of isochromosome 12p and loss of chromosomes 1p, 4, 5, 9, 10, 11, 13, 14, 18 and 19 were detected in nine (90%) malignant mixed germ cell tumors. In nine yolk sac tumors analyzed, chromosome abnormalities were detected in seven patients that include near triploidy/tetraploidy with recurrent 1p deletion, gains of 1q, 2, 7, 9, 12, 14, and isochromosome 12p (N=2). Twelve of 18 benign teratomas of infants and children were chromosomally normal and only six patients showed abnormal karyotypes without isochromosome 12p. Cytogenetic aberrations rate is significantly higher in adolescent patients (87%) compared to pediatric patients (42%). The gain of an isochromosome 12p and 1p deletion/1q gain were more frequent in the tumors from adolescent boys. Clinical correlation studies are warranted to better assess tumor aggressiveness and chemotherapy treatment in childhood or adolescent germ cell tumors.

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## 1266/W/Poster Board #924

**Patient with APL had PML/RARA fusion by FISH but no t(15;17) translocation by G-banding mid-way through treatment.** *J. Mangel[1], I. Chin-Yee[1], K. Howson-Jan[1], S. Collins[1], J. Xu[2].* 1) Hematology; 2) Cytogenetics; London Health Sciences Centre and University of Western Ontario, Canada.

A 37 year old man previously treated for testicular carcinoma presented with easy bruising, shortness of breath, fever and cough. He was found to be pancytopenic (WBC 0.6, neut 0.2, Hb 102, plat 23), and his bone marrow (BM) aspirate and biopsy were consistent with a diagnosis of acute promyelocytic leukemia (APL), microgranular variant. At diagnosis, BM cytogenetics showed the diagnostic translocation t(15;17)(q22;q21) by G-banding in 75% (12/16) of the metaphase cells. The patient underwent standard induction chemotherapy with all-trans retinoic acid (ATRA) 45 mg/m2, daunorubicin 60 mg/m2 x 3days, and cytarabine 100 mg/m2 x 7 days. ATRA was discontinued prematurely because the patient developed severe oral ulcerations rendering him unable to swallow pills. By day 28, blood counts had recovered, but repeat BM aspirate still suggested ongoing leukemia. Cytogenetics identified t(15;17) by G-banding in 60% (15/25) of the metaphase cells, and PML/RARA fusion by FISH was found in 86% (172/200) of interphase cells. Interestingly, in the absence of any further interim treatment, a repeat BM aspirate one week later showed fewer blasts, a lower percentage of FISH gene fusion positivity (52.5%, 105/200, of the interphase cells), but no t(15;17) by G-banding in 25 metaphase cells. The patient resumed treatment with ATRA, and repeat BM aspirate performed 5 weeks later confirmed morphological remission, with both negative FISH and G-banding on that sample. The failure to detect t(15;17) by G-banding in the 3rd BM while FISH testing remained positive is puzzling. It is unlikely to be due to random sampling error, given that such a high proportion (52.5%) of the cells were positive by FISH. We hypothesize that the t(15;17) tumor cells failed to proliferate in this patient due to suppression of mitosis in response to treatment. Further investigation into the effects drugs might play on mitosis of tumor cells would be of interest. Both FISH and cytogenetics are usually performed at diagnosis in patients with suspected APL, but this case highlights the need for performing FISH analysis for PML-RARA gene fusion in the follow-up setting in patients with APL, even in patients with negative findings by G-banding.

## 1267/W/Poster Board #925

**CYTOGENETIC ANALYSIS OF THE L5178-Y MOUSE LYMPHOMA CELL LINE.** *J. Peregrina[1,2], J.M. Moreno-Ortiz[3], M.L. Ayala-Madrigal[3], M. Gutiérrez-Angulo[3,4], M. Partida-Pérez[3], R. Ramírez-Ramírez[3], E. Flores-Torales[1].* 1) Biología Celular y Molecular, Universidad de Guadalajara, Zapopan, Jalisco, Mexico; 2) Hospital Civil Fray Antonio Alcalde; 3) Instituto de Genética Humana, UdeG; 4) CUALTOS, UdeG.

The cell line L5178Y is derived from a thymic tumor induced in a DBA/2 mouse by methylcholanthrene and adapted to ascitic form. It is kept by serial intraperitoneal transplantation in ascitic phase in mice with H-2d/d haplotype. In this process, the cell line acquires a variety of numerical and structural aberrations that modifying their tumorigenic properties probably with phenotypes more aggressive. Although the karyotype of original cell line is unknown, it has been described in three sublines L5178Y-S, L5178Y-R and L5178Y TK+/-3.7.2C with different results that shown the independent chromosomal evolution and genetic instability of the original cell line. In this work we realized the chromosomal analysis in the cell line L5178Y. The lymphoma cells (cryopreserved) were cultured in RPMI-1640 supplemented with 20% fetal bovine serum at 37°C with CO2 to 5% during 24 hours. The harvesting protocol was as follows: colcemid 0.05 μg/ml for 40 min, KCl 0.075 M for 15 min, both incubated at 37°C, and the cells were washing with cold carnoy's solution. The chromosomes were spread on slides and kept at 37°C for chromosome aging. The comparative analysis was done with normal chromosomes obtained from mouse balb-c bone marrow cells, cultured and harvested with the same conditions. The chromosomes were stained with Giemsa-Trypsin protocol. The comparison of banding patterns between BALB-C mouse and L5178 cell line chromosomes showed a modal number of 40 and 56 chromosomes respectively. The chromosomal aberrations were the follows: the 1, 6, 7, 10, 12, 13, 14, 16, and 18 chromosomes were apparently unchanged. The numerical abnormalities were seen for 8, 9, 11, 15, 17 and 19 chromosomes. The chromosomes 2, 4, 9 showed an additional segment and the 5 chromosome with deletion of distal segment. Moreover, ten markers chromosomes were found. The increased genetic instability in malignant cell of tumor maintained within in vivo systems are due mainly to continuous exchange of microenvironments. Given this, expansive techniques by means of in vitro harvests and cryopreservation have been used to prevent the generation of different clones.

## 1268/W/Poster Board #926

**Deletions involving Chromosomal Band 9p in Acute Lymphoblastic Leukemia.** *S.N.J. Sait[1], A.W Block[1], M.L. Brecher[2], E.W. Wang[3], M. Wetzler[3], G. Deeb[4].* 1) Clinical Cytogenetics Lab, Roswell Park Cancer Inst., Buffalo, NY; 2) Department of Pediatrics, Roswell Park Cancer Inst., Buffalo, NY; 3) Department of Medicine, Roswell Park Cancer Inst., Buffalo, NY; 4) Department of Pathology, Roswell Park Cancer Inst., Buffalo, NY.

Deletions involving the chromosomal band 9p21 [del(9p)] have been reported in a subset of acute lymphoblastic leukemia (ALL). Although del(9p) has not been designated as a separate diagnostic and prognostic subclass in the current WHO classification (2008); it has been shown that patients with these abnormalities might fall into a separate prognostic group in adult and pediatric B-cell ALL. The del(9p) is often missed by conventional cytogenetics (CC) and represent a heterogeneous group with deletions of variable sizes and additional chromosome abnormalities. We report our experience with a cohort of 15 patients seen at Roswell Park Cancer Institute. Deletions seen were both mono- and bi-allelic and confirmed by fluorescence in situ hybridization (FISH) using the Vysis/Abbott Molecular LSI p16/CEP 9 dual color probe set. The del(9p) was seen in both pediatric (5/15) and adult (10/15) ALL patients with a male:female ratio of 9:6. Based on flow cytometry analyses; 3/15 patients had a T and 12/15 a B-lineage with typical immunophenotypes except for aberrant dim CD13/CD33 myeloid antigen expression in 2 cases. In 7 patients, the del(9p) was the sole abnormality and in 5, these deletions could not be detected by conventional cytogenetics. In 2 patients this abnormality was initially identified by array comparative genomic hybridization (aCGH). Since similar abnormalities have been seen in both B and T lineage ALL, they do not represent a specific lineage marker. The prognostic significance of this abnormality is still controversial and is classified as standard risk, however, classifying them as normal karyotype might be misleading. As this is a subtle abnormality that could be missed by conventional cytogenetics, adjunct molecular cytogenetic technologies should be used identify the presence of the del(9p) especially in normal karyotype ALL.

## 1269/W/Poster Board #927

**Genome-wide allelic imbalance in prostate cancer: Comparison of aggressive and non-aggressive disease.** *Y. Tai[1], I. Cheng[2], G. Chen[3], S. Plummer[3], C. Neslund-Dudas[4], G. Casey[3], B. Rybicki[4], J. Witte[1].* 1) UCSF, San Francisco, CA; 2) University of Hawaii, Honolulu HI; 3) University of Southern California, Los Angeles, CA; 4) Henry Ford Health System, Detroit MI.

Introduction: Prostate cancer is a complex, heterogeneous disease that may be asymptomatic and latent among some men and more aggressive among others. The underlying biological mechanisms driving these differences in disease sub-groups are largely unknown. Thus, to characterize genomic alterations among prostate tumors and compare differences by disease aggressiveness, we conducted a genome-wide survey of allelic imbalance of prostate tumors, using the Illumina 1M SNP array. Methods: DNA was extracted from 64 freshly frozen prostate tumors and paired blood samples (32 aggressive and 32 non-aggressive disease) from prostate cancer patients undergoing radical prostatectomy treatment at the Henry Ford Health System. Illumina 1M-Duo bead arrays comprised of ~1.2 million markers were genotyped for each patient. To identify losses/gains and allelic imbalance (AI), we used the software BAFsegmentation. We compared the percentages of losses/gains and AI for each chromosomal arm to those from the remaining arms, and compared the percentage of the chromosomal arm consisting of losses/gains and AI regions between aggressive and non-aggressive samples. Results: Eleven of the 44 chromosome arms showed significantly higher percentages of regions with loss and AI than the rest of the genome. Chromosome arms 2p, 3q, 5q, 7q, 8p, 8q and 12p showed significantly (p value less than 0.05) higher percentages of regions that were both gain and AI, compared to the rest of the genome. Furthermore, chromosome arms 1p, 2p, 2q, 3p, 4q, 15q and 16q showed significantly higher percentages of loss and AI regions among aggressive tumors in comparison with non-aggressive tumors. There were no such differences observed for gain and AI. Conclusions: Higher percentages of regions with AI and loss were observed in many chromosomal arms compared to the rest of genome. Regions with AI and loss on 7 chromosomal arms may be related to prostate cancer aggressiveness. Future work will localize AI among subregions in order to pinpoint loci potentially involved with tumor aggressiveness.

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## 1270/W/Poster Board #928

**The detection rate of chromosome abnormalities in CLL can be improved by performing both conventional metaphase analysis and FISH.** *E. Van Assche[1], P. De Schouwer[2], P. Zachee[3], K. Vermeulen[4], Z. Berneman[4], K. Bryssinck[1], E. Devos[1], J. Wauters[1], B. Blaumeiser[1].* 1) Dept Medical Genetics, Univ Hospital Antwerp, Wilrijk, Belgium; 2) Clinical Laboratory, ZNA Stuivenberg, 2060 Antwerp, Belgium; 3) Hematology, ZNA Stuivenberg, 2060 Antwerp, Belgium; 4) Hematology and Hemostasis, Univ Hospital Antwerp, Wilrijk, Belgium.

B-cell chronic lymphocytic leukemia (B-CLL) is the commonest leukemia of the adult population in Western countries. CLL has a variable prognosis, which is associated with various parameters including genetic parameters. Conventional metaphase analysis (CMA) of bone marrow cells of B-CLL patients is often hampered by low mitotic activity of malignant B-cells in culture. Consequently, clonal aberrations detected by CMA, are only found in 40-50% of patients. Therefore, fluorescence in situ hybridization (FISH) on interphase nuclei is often used as this technique does not require proliferating cells. Using FISH, clonal aberrations are detected in more than 80% of CLL patients, depending on the number of chromosomal targets interrogated. Recent studies suggest that a complex karyotype, including chromosome translocations, is also an important marker of poor prognosis in CLL. According to Mayr et al. (2006) the mitotic activity of CLL cultures can be increased considerably by adding CpG-oligodeoxynucleotides (CpG-ODN) and IL-2 to the culture medium. Last year we introduced this new culture technique in our lab. Initially, cultures of bone marrow and blood samples of CLL patients were set up with our conventional bone marrow medium on the one side and with the addition of CpG-ODN and IL-2 in the culture medium, on the other side. Since the failure rate of the cultures with CpG-ODN and IL-2 dropped spectacularly (from 46 to 10%) we considered that it was justified to use only this culture medium for the CLL samples. In our unselected patient population, 46 of 51 cases could be successfully stimulated for metaphase generation. Of 46 cases, 33 showed chromosomal aberrations after CMA. FISH analysis was successful in all 51 cases and 34 were abnormal for the 4 regions (11q22.3, 13q14.3, CEP12 and 17p13.1) we interrogated. The most frequent abnormality was 13q14.3 deletion, which was observed in 19 (37%) patients. In 16/19 cases the cryptic deletion, not visible by standard G-banding, was detected by FISH analysis only. In 27 patients additional aberrations, including structural, and complex aberrations, were detected by CMA and not by FISH. Our results, demonstrate an increased detection rate of abnormalities in CLL by using CMA. FISH analysis can further increase the detection rate of cryptic abnormalities. However neither CMA nor FISH can detect all the abnormalities, demonstrating the complementary nature of these 2 techniques and the necessity to perform both.

## 1271/W/Poster Board #929

**New culture methods for cytogenetic investigations of chronic lymphocytic leukaemia.** *C.E. Wren[1], H. Moriarty[2], K.A. Marsden[1], E.M. Tegg[1].* 1) Royal Hobart Hospital, Hobart, Tasmania, Australia; 2) Charles Sturt University, Wagga Wagga, NSW, Australia.

Aim: To determine which culture method (24 hour unstimulated; 72 hour with additional fetal calf serum; 72 hour with IL-4, 72 hour with LPS; 72 hour with TPA; 72 hour with CpG-oligonucleotide DSP30+IL-2) yielded the highest culture success rate, mitotic index, banding resolution and abnormality rate in patients with chronic lymphocytic leukaemia (CLL). Methods: This is a blind retrospective study that included patients presenting to the haematology department of the Royal Hobart Hospital from the 1st of January 2008 to 30th of September 2008. A peripheral blood EDTA sample was used as the study sample for cytogenetic analysis. Results and Discussion: 24 patients (consisting of 11 newly diagnosed and 13 known patients were included in this study), yielding a total of 45 samples. Overall the conventional cytogenetic (CC) culture success rate was 100.0% with an abnormality rate of 62.5% (n=24). This study also compared CC abnormality rates with fluorescence in-situ hybridization (FISH) results using probes for CLL (LSI D13S319 / 13q34 / CEP 12: LSI ATM / p53). FISH detected an abnormality rate of 75.0% (n=24). The combined CC and FISH abnormality rate was 87.5% (n=24). The cytogenetic abnormalities in the newly diagnosed patients were also correlated to CD38 expression, ZAP70 expression and clinical stage but no correlation was found. Conclusion: EDTA peripheral blood samples are an excellent source of material for CC culture in patients with CLL. The use of a combination of 3 cultures types for each patient (72 hour with TPA; 72 hour with CpG-oligonucleotide DSP30+IL-2; 72 hour with TPA and CpG-oligonucleotide DSP30+IL-2) resulted in a 100% culture success rate, high mitotic index and high abnormality rate (62.5%).

## 1272/W/Poster Board #930

**Case with a complex karyotype including t(8;14) and t(14;18) and preservation of follicular pattern in a transformed lymphoma.** *J. Xu[1], L. Minuk[2], K. Howson-Jan[2], K. Rizkalla[3].* 1) Cytogenetics; 2) Hematology; 3) Pathology; London Health Sciences Centre and University of Western Ontario, Canada.

A 48-year-old male presented with abdominal pain and had diffuse intra-abdominal, mediastinal and cervical adenopathy. His bone marrow (BM) showed 80% infiltration with neoplastic lymphocytes positive for Bcl-2 and Ki-67 (90%) and expressing CD19/dim CD10, CD20, and bright kappa light chains. Biopsy of a right sided neck node showed follicular lymphoma (FL), predominantly grade III/III with transformation, associated with diffuse areas. He was given 1 cycle of CHOP-R chemotherapy and managed for tumor lysis syndrome with rasburicase and temporary hemodialysis. After finding the t(8;14), treatment was changed to modified Magrath protocol with intensified intrathecal chemotherapy for CNS involvement. He had up to cycle 2A of treatment and developed progressive neurologic abnormalities secondary to leptomeningeal spread of lymphoma. Craniospinal radiation therapy was given but discontinued after 12 fractions due to progressive asthenia and pancytopenia. He died of progressive lymphoma 6 months after original diagnosis. G-banding showed a complex karyotype in both lymph node (LN) and BM. The LN had +X,+5, t(5;9)(q13;p13), der(8)t(6;8)(p11.2;p11.2), t(8;14)(q24.1;q32), add(11)(q21), add(12)(q13),del(13)(q32),t(14;18)(q32;q21). The BM had the same aberrations as in the LN and additionally, dup(1)(q21q42). All aberrations but dup(1) and t(5;9) were seen in every abnormal cell and thus they are likely primary events in the tumorigenesis. Both t(8;14) and t(14;18), "a double-hit", are seen in 100% (25/25) of the LN cells and 58% (15/26) of the BM cells. FISH of the BM showed fusion of CMYC/IGH and IGH/BCL2. t(8;14) is associated with Burkitt's lymphoma (BL) whereas t(14;18), with FL or diffuse large B-cell lymphoma (DLBCL). Notably t(5;9) and breakpoints 5q13 and 6p11.2 seen in this case are not common findings in BL, DLBCL or FL. A double-hit is reportedly associated with poor prognosis. The BM morphology is consistent with leukemic infiltrate and the LN shows residual FL in a background of diffuse lymphoma with minimal Burkitt's features. This case has follicular component (not all diffuse) and with this component, the proliferation rate is not that high. This finding raises 2 possibilities: 1. FL with blastic transformation associated with CMYC translocation; or less likely 2. composite FL and BL. We hypothesize that in addition to the double hit, other 8 aberrations observed might have also played a role in the histopathologic features in our case.

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## 1273/W/Poster Board #931

**GSVD comparison of genome-wide array CGH data from patient-matched normal and tumor TCGA samples reveals known and novel copy number alterations in GBM.** *C. Lee[1], O. Alter[1,2,3].* 1) Department of Biomedical Engineering, The University of Texas at Austin, Austin, TX; 2) Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX; 3) Institute for Computational Engineering and Sciences, The University of Texas at Austin, Austin, TX.

Glioblastoma multiforme (GBM) is the most common type of primary brain tumor in adults. GBM is characterized by aggressive growth and treatment resistance, with median survival times of approximately one year after initial diagnosis. As do other cancers, GBM tumors exhibit a range of copy number alterations (CNAs), many of which play roles in the pathogenesis and development of the disease. Here, we describe the use of the generalized singular value decomposition (GSVD) [1,2] to identify copy number alterations (CNAs) in GBM using array CGH data from patient-matched normal and tumor samples provided by The Cancer Genome Atlas (TCGA). The GSVD computation simultaneously separates the two (i.e., normal and tumor) datasets of genome-wide copy number into one set of "probelets" and two corresponding sets of "arraylets." Each probelet describes a mathematically decoupled pattern of CNAs across the patients, and the two corresponding arraylets describe the corresponding, mathematically decorrelated, patterns of CNAs across probes in the normal and tumor genomes, respectively. Analysis of the arraylets that correspond to probelets of almost negligible significance in the normal dataset allows identification of specific genomic regions containing CNAs prevalent in tumors. The analysis of the corresponding probelets may reveal specific tumor subtypes or clinical phenotypes, that these patterns of CNA characterize. Unlike other copy number analysis methods, GSVD is not limited to analyzing each sample individually and allows identification of CNAs without first having to perform computationally-expensive segmentation and breakpoint identification in each sample. We show that the GSVD of TCGA array data identifies known CNA regions important in GBM, including regions of 4q12 (containing PDGFRA), 7p11.12 (containing EGFR), 9p21.3 (containing CDKN2A), and 12q14.1 (containing CDK4). Additionally, previously unknown CNA regions are revealed, including a segment of 2p24.3 containing genes whose overexpression is known to play roles in the onset of other brain cancers, and segments of 14q32.33 and 19q12 containing genes responsible for regulating apoptosis and cell-cycle progression. These results demonstrate the utility of the GSVD for comparative analyses of genome-wide copy number data in disease-related contexts. [1] Golub and Van Loan, Matrix Computations (Johns Hopkins University Press, Baltimore, MD, 1996). [2] Alter, Brown and Botstein, PNAS 100, 3351 (2003).

## 1274/W/Poster Board #932

**ABL Amplification in a Patient with Precursor T-Lymphoblastic Lymphoma.** *G. Sun[1], J. Katzel[2], A. Mazumder[2].* 1) Cytogenetics, Genzyme Gen, New York, NY; 2) St. Vincent Comprehensive Cancer Center, New York, NY.

Precursor T-cell lymphoblastic leukemia/lymphoma (precursor T-LBL) accounts for approximately 2% of all adult NHL. For the majority of patients conventional cytogenetic analysis is unable to detect clonal chromosomal abnormalities. However, the addition of fluorescence in situ hybridization (FISH) testing has identified several new genetic aberrations. One recently identified FISH abnormality is the episomal amplification of the NUP214-ABL1 fusion oncogene. Our patient is a 39 woman who presented with night sweats and superior vena cava syndrome. CT scan revealed a large mass in the anterior mediastinum causing compression of the SVC as well as bilateral pleural effusions. The hemogram and smear revealed only an elevated WBC count of 21k with 70% neutrophils. LDH was slightly elevated. Thoracentesis was diagnostic for precursor T-LBL. She responded to steroids and radiation therapy. Once stabilized, a bone marrow biopsy was performed that confirmed the diagnosis. Cytogenetics revealed a normal female 46,XX karyotype. However, FISH testing from the bone marrow aspirate, using a panel of probes for T cell receptor alpha (TRA) at 14q11.2, p16 at 9p21, TP53 at 17p13.1, MYC at 8q24, MLL at 11q23 and BCR/ABL at 9q34/22q11.2 revealed a positive result for TRA (44% of cells). This represented a homologous deletion of the p16 gene (43%) and for episomes (6-30 copies in 42% of cells) by the BCR/ABL probe. The latter was confirmed to be NUP214-ABL1 fusion amplification by an NUP214-ABL1 probe. The patient was started on chemotherapy according to the HYPER CVAD regimen. After 1 cycle of therapy she had no radiographic evidence of disease. Dasatanib was also added to the regimen when the results of NUP214-ABL testing became available. Since FISH testing for the TRA was positive indicating a translocation involving this gene, the original cytogenetic slides were re-analyzed and found two of thirty metaphases showed clonal abnormalities:46,X,t(X;3)(q28;p21),t(10;14) (q24;q11.2). The t(10;14)/TLX1-TRA is the most commonly seen translocation in adult T-ALL and often associated with NUP214-ABL1 episomal amplification. This case is the first reported patient with amplification of the NUP214-ABL1 fusion gene presenting clinically as a lymphoma. Our results also confirm the value of FISH testing for patients with T-ALL/precursor T-LBL, providing valuable information diagnostic as well as therapeutic information.

## 1275/W/Poster Board #933

**Analysis of overdispersion patterns in a study into the monoclonal origin of multiple sporadic basal cell carcinomas.** *F. Quehenberger[1], E. Heitzer[2], P. Wolf[3].* 1) Institue of Medical Informatics, Statistics and Documentation, Medical University of Graz, Graz, Austria; 2) Department of Internal Medicine, Medical University of Graz, Graz, Austria; 3) Department of Dermatology, Medical University of Graz, Graz, Austria.

INTRODUCTION: There have been reports that not only basal cell carcinomas are of monoclonal origin, but also multiple lesions within a patient (Shulman et al. 2006). They found identical X-chromosome inactivation patterns and identical LOH patterns near the PTCH gene. In our study, however, by comparing the mutation patterns and LOH at the PTCH gene, we found no case which would have suggested monoclonal origin of lesions (Heitzer et al, 2009). Here we present the conclusions from the analysis of overdispersion from that study. METHODS: Tissue from three basal cell carcinomas and normal skin from each of six patients was genotyped at six SNPs near the PTCH gene. Overdispersion or clustering is observed if LOH occurs more often within the same patient than by chance alone or, if LOH has occurred, the same allele is lost more often than expected by chance alone. The permutation test based on score statistic of Tarone (1979) was used to test for overdispersion. The significance level was 0.05. The minimum over SNPs of the test statistic was used in order to correct for multiplicity. RESULTS: There was no significant overdispersion in the occurrence of LOH, but significant overdispersion in the allele that was lost. CONCLUSIONS: Although the study included only a small number of subjects, a small number of lesions per subject and not all of the SNPs were informative, statistically significant overdispersion could be established. If a part of a chromosome is lost, there is a strong tendency that it occurs at the same chromosome on different lesions. Loss of the same allele does not imply monoclonal origin of multiple basal cell carcinoma.

## 1276/W/Poster Board #934

**Integrated analysis of copy number alteration and RNA expression profiles of cancer using a high-resolution whole-genome oligonucleotide array.** *S. Jung[1,2], S. Seung-Hun[1,2], Y. Seon-Hee[2], C. Hye-Sun[1,2], L. Sug-Hyung[3], C. Yeun-Jun[1,2].* 1) Department of Microbiology, The Catholic University of Korea, Seoul, Korea; 2) Integrated Research Center for Genome Polymorphism, The Catholic University of Korea, Seoul, Korea; 3) Department of Pathology, The Catholic University of Korea, Seoul, Korea.

Recently, microarray-based comparative genomic hybridization (array-CGH) has emerged as a very efficient technology with higher resolution for the genome-wide identification of copy number alterations (CNA). Although CNAs are thought to affect gene expression, there is no platform currently available for the integrated CNA-expression analysis. To achieve highresolution copy number analysis integrated with expression profiles, we established human 30K oligoarray-based genome-wide copy number analysis system and explored the applicability of this system for integrated genome and transcriptome analysis using MDAMB-231 cell line. We compared the CNAs detected by the oligoarray with those detected by the 3K BAC array for validation. The oligoarray identified the single copy difference more accurately and sensitively than the BAC array. Seventeen CNAs detected by both platforms in MDA-MB-231 such as gains of 5p15.33-13.1, 8q11.22-8q21.13, 17p11.2, and losses of 1p32.3, 8p23.3-8p11.21, and 9p21 were consistently identified in previous studies on breast cancer. There were 122 other small CNAs (mean size 1.79 Mb) that were detected by oligoarray only, not by BAC-array. We performed genomic qPCR targeting 7 CNA regions, detected by oligoarray only, and one non-CNA region to validate the oligoarray CNA detection. All qPCR results were consistent with the oligoarray-CGH results. When we explored the possibility of combined interpretation of both DNA copy number and RNA expression profiles, mean DNA copy number and RNA expression levels showed a significant correlation. In conclusion, this 30K oligoarray-CGH system can be a reasonable choice for analyzing whole genome CNAs and RNA expression profiles at a lower cost.

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## 1277/W/Poster Board #935

**Integrative molecular profiling combined with in silico pathway analyses identify 6p amplification as a driver of tumorigenesis in osteosarcoma.** *J. Squire[1,2], M. Yoshimoto[1], J. Martin[1], B. Sadikovic[3], S. Chilton-MacNeill[3], P. Thorner[3], P. Nuin[1,4], M. Zielenska[3].* 1) Department of Pathology and Molecular Medicine, Richardson Labs, Queen's University, Kingston, Canada; 2) NCIC Clinical Trials Group, Kingston, ON, Canada; 3) Department of Pediatric Laboratory Medicine, the Hospital for Sick Children, Toronto, Canada; 4) Ontario Cancer Biomarker Network, Toronto, ON, Canada.

An unusually high frequency of chromosomal rearrangements characterizes osteosarcoma (OS) oncogenesis, and the resulting biological heterogeneity is thought to confer adaptability to aggressive treatment regimens. As the molecular basis of genomic rearrangements in cancer is highly clinically relevant, but poorly understood, our aims were to enhance understanding of its role in OS and to identify a distinct genomic region uniquely subject to such rearrangements and associated with OS tumorigenesis. Our recent progress has identified a number of distinct genomic regions that are uniquely subject to chromosomal instability, and are associated with OS-tumour progression and varying clinical response. These conclusions were based on our recent integrative microarray analysis of epigenomic, genomic, and gene expression profiles in OS. We have prioritized the genomic 6p12-21 amplicons for subsequent in silico experiments based on their known tumour biology and role in human and mouse OS. Copy number changes demonstrating gain within the 6p12-21 region were validated on formalin-fixed paraffin-embedded OS specimens using interphase fluorescence in situ hybridization with locus-specific BAC probes spanning both the RUNX2 locus as well as regions flanking the RUNX2 locus. Based on the aCGH and gene expression findings, the RUNX2 and CDC5L genes were suggested to be the target of the 6p amplicon. Interestingly, previous conditional mouse OS data sets showed overexpression of murine runx2, and bioinformatics approaches demonstrated homology between human 6p and mouse chromosome 17 segments, which contain the murine gene runx2. In silico analysis identified paired intrachromosomal segmental duplications and genomic variations surrounding 6p amplicons, suggesting that defects in homology-dependent repair pathways in OS cells may be the reason for the variation in breakpoints at 6p12-21 if the segmental duplications are involved in error-prone recombinational repair events. Overall, our observations highlight the plasticity of the human genome and the potential impact of segmental duplications on genomic rearrangement susceptibility. These findings provide an entry point for the development of targeted therapeutic strategies based on the genomics and key molecular pathways of genes such as RUNX2 that may be driving osteosarcoma progression.

## 1278/W/Poster Board #936

**Formation of secondary structure contributes to chromosome instability at fragile site FRA16B.** *A. Burrow, A. Marullo, L. Holder, Y.H. Wang.* Wake Forest University School of Medicine, Winston-Salem, NC.

Fragile sites are specific chromosomal loci that are especially susceptible to DNA breakage following conditions of partial replication stress. Rare fragile sites, which are present in less than 5% of the population, are inherited in a Mendelian manner and are associated with human genetic disorders. In contrast, common fragile sites have been observed in all individuals and are frequent sites of rearrangements in cancer cells. While the molecular basis underlying fragile site fragility remains elusive, most fragile DNAs examined thus far contain frequent, AT-rich flexibility islands capable of forming highly stable secondary structures. To understand the mechanism of breakage at fragile sites, the cause of instability at fragile site FRA16B has been examined. FRA16B contains a 33 bp AT-rich minisatellite element, and its expression can be observed following treatment with chemicals that induce both types of fragile sites. Furthermore, the repeat is predicted to form a stable hairpin structure. To date, there is no physical evidence of alternative DNA structures formed at AT-rich fragile sites, which comprises the majority of fragile sites, emphasizing the importance of determining whether fragile DNAs are able to form secondary structures *in vitro*, and to investigate the contribution of secondary structure formation on chromosome instability. The ability of FRA16B to form a secondary structure *in vitro* was examined in this study, and we found that upon reduplexing, FRA16B forms alternative structures, characterized by bands of reduced electrophoretic mobility in polyacrylamide gels. Furthermore, the effect of secondary structure formation on replication efficiency and instability of FRA16B plasmids was examined in mammalian cells using a SV40 replication system. Depending on both the replication orientation and distance from the origin, FRA16B constructs replicated less efficiently compared to the control, and demonstrated an increased number of instability events, including large base pair deletions, based on a mutation assay performed in *E. coli*. Comparison of the deletion sites with predicted secondary structures revealed that the deleted regions occurred at sites of extensive secondary structure, including multiple hairpins. These results strongly suggest that the secondary structure-forming potential of FRA16B contributes to its fragility by stalling DNA replication, and this mechanism may be shared among other fragile DNAs.

## 1279/W/Poster Board #937

**Telomere length in Neuroblastoma -- a novel prognostic factor?** *G.M. Lundberg[1], I. Øra[2], D. Gisselsson Nord[1, 3].* 1) Clinical Gentetics, Lund University Hospital, Lund, Sweden; 2) Department of Pediatric oncology, Lund University Hospital; 3) Department of Pathology, Lund University Hospital.

Aim: To assess possible correlations between telomere lengths in neuroblastoma (NB) and clinical outcome. Background: NBs are tumours of the sympathetic nervous system, occurring predominantly in early childhood and accounting for 8-10% of all paediatric cancers. Multiple studies have shown that NB has a heterogeneous pattern of somatic chromosome changes. Previously we have found abnormally short telomeres in five NB cell lines, concurrent with chromosome segregation disturbances such as anaphase bridging and mitotic multipolarity. Materials and Methods: Quantitative fluorescence in situ hybridisation was carried out on 34 NB-biopsies. The fluorescence intensity in NB cell nuclei was compared to that of non-neoplastic cell nuclei, mainly endothelium, to obtain a relative measure of telomere length. The results were correlated with histopathological data, cytogenetic abnormalities, relapses and survival. Result: Of the 34 tumours, 10 had longer, 14 shorter and 10 had the same median telomere length as that of the endothelium. Patients whose tumours had longer telomeres showed a higher frequency (60 % vs. 35 %) of stage 4 disease and a worse clinical outcome (20 % disease free survival vs. 80 %) than those having tumours with shorter telomeres (P= 0.02 log-rank test). Unchanged telomere length did not influence survival. Neither 1-p, 11-q status nor MYCN amplification correlated to disease free survival in the present study. Conclusion: Tumor telomere length could be a future prognostic parameter in NB patients, being a better predictor of disease-free survival than MYCN status in the present study. Studies of the relation between telomere length and the expression of telomere-regulating molecules and neuroblastic differentiation markers are ongoing.

## 1280/W/Poster Board #938

**Characterization of myelodysplastic syndromes and acute myeloid leukemia with isodicentric X chromosome.** *K. Paulsson[1], C. Haferlach[2], C. Fonatsch[3], A. Hagemeijer[4], M.K. Andersen[5], M. Slovak[6], B. Johansson[1] on behalf of the MDS Foundation.* 1) Department of Clinical Genetics, Lund University Hospital, Lund, Sweden; 2) Munich Leukemia Laboratory, Munich, Germany; 3) Department of Medical Genetics, Medical University of Vienna, Vienna, Austria; 4) Centre for Human Genetics, University of Leuven, Leuven, Belgium; 5) The Cytogenetic Laboratory, The University Hospital Rigshospitalet, Copenhagen, Denmark; 6) Department of Cytogenetics, City of Hope Comprehensive Cancer Center, Duarte, CA.

An isodicentric X chromosome [idic(X)] with breakpoints in Xq13 is a rare but recurrent finding in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) in elderly women. The molecular mechanism leading to formation of idic(X) is not known, but may involve either crossing over between sister chromatids at the G2 phase of the cell cycle or initial trisomy X with subsequent translocation between two of the three homologues. It is also unclear whether the active or the inactive X chromosome is involved in the aberration, although published data from two cases suggest the latter. We have utilized the Illumina 1M-duo single nucleotide polymorphism (SNP) array platform, comprising 1,000,000 SNPs genome-wide, to investigate the Xq13 breakpoints and identify additional clonal abnormalities in 14 cases of idic(X)-positive MDS or AML. The idic(X) breakpoints could be mapped in 12 of the cases, showing that the breakpoints clustered in two different Xq13 regions at approximately 70.9 and 72.1 Mb, respectively. Interestingly, both of these regions contain segmental duplications, suggesting a possible mechanism for sister chromatid recombination. Furthermore, methylation analyses of the *AR* gene at Xq12 revealed that six cases had gain of the inactive X chromosome and three had gain of the active X chromosome, corresponding to the isodicentric X. This suggests that idic(X) may be formed either from the active or the inactive X chromosome. In none of the cases was the breakpoint within a gene, showing that no fusion gene results from the rearrangement. The SNP array analyses also detected two recurrent regions of partial uniparental isodisomy, i.e., copy-neutral loss of heterozygosity; a phenomenon frequently associated with gene mutations. Taken together, our findings show that isodicentric X chromosomes in MDS and AML most likely arise by a crossing over between sister chromatids facilitated by the presence of segmental duplications, that there is no preference for the active or inactive X chromosome when the idic(X) is formed, that no fusion gene results from the rearrangement, and that additional gene mutations may be present.

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## 1281/W/Poster Board #939

**The spatial dynamics of ATM at sites of DNA damage differ from those of other DNA damage response proteins.** *P.S. Bradshaw[1,2], M.S. Meyn[1,2,3].* 1) Program in Genetics & Genome Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Dept of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 3) Dept of Paediatrics, University of Toronto, Toronto, ON, Canada.

ATM, the protein kinase defective in the cancer predisposition syndrome Ataxia-Telangiectasia, is critical for activating cellular responses to DNA Double Strand Breaks (DSBs). Accumulation of ATM at DSB sites, along with ATM phosphorylation of nearby target proteins, is thought to promote DSB repair by establishing a megabase region of modified chromatin that serves to recruit ancillary repair proteins. To better understand the role played by ATM in this process we have studied ATM behavior in human fibroblasts following induction of localized DNA damage by a laser microbeam. We find endogenous ATM forms foci at sites of DNA damage within 1 minute after photo-induction of DNA damage equivalent to ~10 Gy of γ-radiation. While maximal ATM accumulation occurs between 10 and 30 minutes post damage, some foci remain at 4 hours. Damage-associated ATM foci are small, discrete, and, unlike foci of the DNA damage response proteins Mre11, γH2AX, 53BP1 and BRCA1, do not enlarge and diffuse with time. Like ATM, the homologous recombination (HR) proteins RPA, Rad51 and FANCD2 form small, discrete damage-induced foci. However, at 1-hour post damage ATM foci do not colocalize with these HR protein foci. Consistent with our observations on endogenous ATM, FRAP data indicates that a GFP-tagged ATM stably associates with photo-induced DNA damage sites. Accumulation of both endogenous ATM and GFP-ATM at sites of photo-induced damage is dependent upon the MRN complex. In contrast, mutating the ATM 1981 phosphorylation site from serine to alanine impairs but does not completely block accumulation of GFP-ATM. Over-expression of the telomeric protein TRF2, an ATM kinase inhibitor whose binding encompasses the S1981 residue, also attenuates GFP-ATM accumulation at sites of DNA damage, while over-expression of the related protein, TRF1, does not.

Our data indicates that ATM rapidly associates with photo-induced DNA damage in an MRN-dependent dependent manner; whilst optimal accumulation requires ATM S1981 phosphorylation and may be regulated by TRF2. The spatial dynamics of ATM foci suggest that ATM is not a major component of the megabase protein platform domain. Our results are consistent with a model in which ATM rapidly accumulates at DNA break sites, then is displaced as breaks are either repaired by non-homologous end joining or processed for subsequently repair by homologous recombination.

## 1282/W/Poster Board #940

**Characterization of different *MLL* fusion genes in 40 leukemias and molecular studies of some rare *MLL* fusions.** *H. Chaker, J. Hébert.* Quebec Leukemia Cell Bank and Hematology-Oncology Division, Maisonneuve-Rosemont Hospital, Montreal, Quebec, Canada.

The mixed-lineage leukemia gene *MLL*, located at chromosomal band 11q23, is one of the most common genes involved in chromosomal abnormalities in leukemia. *MLL* rearrangements are associated with pediatric, adult and therapy-related acute leukemias of both myeloid and lymphoid lineages. In these different fusions, the N-terminal part of the MLL protein is fused to one of more than 50 partner proteins. A limited number of studies with frequent MLL fusions including MLL-MLLT1, MLL-MLLT3 and MLL-EEN, have shown a critical contribution of the partner protein to leukemogenesis. We have analyzed leukemic samples obtained from 40 adults with *MLL* rearrangements collected at the Quebec Leukemia Cell Bank, associated with different types of acute leukemia (myeloid, lymphoid, biphenotypic, therapy-related) and with chronic myelomonocytic leukemia (CMML). Cytogenetic findings were correlated with morphological, immunophenotypic and clinical data. We have investigated MLL fusions by conventional cytogenetics and FISH (Fluorescence in situ hybridization) using *MLL* and BACs (Bacterial Artificial Chromosomes) probes. Spectral karyotyping was used to define complex chromosomal rearrangements. Translocation breakpoints were studied by sequencing chimeric transcripts in less frequent and complex *MLL* fusions. Our results showed that 71.5% of analyzed *MLL* cases account for the most frequent partner genes: *MLLT3*(*AF9*), *AFF1*(*AF4*), *MLLT4*(*AF6*), *MLLT1*(*ENL*), *ELL* whereas 14.5% involve less common *MLL* fusion partners including *MLLT6*(*AF17*), *MLLT10*(*AF10*) and *SEPT9*. Other *MLL* fusions (14%) involved rare partners, such as *GAS7* and *CASC5*(*AF15q14*). Sequence analysis of a complex translocation involving chromosomes 11, 14 and 19 confirmed a *MLL-ELL* fusion in CMML. To date, only one case of CMML with a *MLL-ELL* has been reported. The two *MLL-GAS7* fusion transcripts detected in our case fused *MLL* exon 7 and exon 8 to *GAS7* exon 2. A rearrangement of the *CASC5* gene was detected by FISH using BAC clone RP11-451F21 covering the entire gene. One *MLL-CASC5* fusion transcript was confirmed by reverse transcriptase PCR using primers specific for *MLL* exon 10 and *CASC5* exon 11. Molecular characterization of rare *MLL* translocations may offer some insights regarding the role of the different *MLL* partner genes in these fusions and lead to a better understanding of their clinical impact.

## 1283/W/Poster Board #941

**Identification of cryptic and novel *RUNX1* fusions in acute leukemia.** *A. Giguère, J. Hébert.* Quebec Leukemia Cell Bank and Hematology-Oncology Division, Maisonneuve-Rosemont Hospital, Montreal, Quebec, Canada.

Cytogenetic analysis is an essential tool in the management of patients with acute leukemia. Chromosomal translocations are an important prognostic factor in this disease and often disrupt genes encoding transcription factors such as *RUNX1*. The *RUNX1* gene, located at chromosomal band 21q22, is involved in the establishment of definitive hematopoiesis and is a key regulator of myeloid differentiation. *RUNX1* is one of the most frequent rearranged genes in leukemia and is fused to more than 40 partner genes in different chromosomal translocations. *RUNX1T1* and *ETV6* are common partners of *RUNX1*, but a significant number of partners remains to be identified. In this study, we present the molecular cloning of the recurrent t(1;21)(p22;q22) translocation and the characterization of two cryptic *RUNX1* translocations using fluorescent in situ hybridization (FISH). A reciprocal translocation t(1;21) was detected in an adult patient with acute myeloid leukemia (AML) in relapse. FISH analysis with bacterial artificial chromosome (BAC) clone RP11-299D9 confirmed a rearrangement of *RUNX1* with chromosomal band 1p22. Using BACs targeting candidate genes on chromosome 1, the breakpoint was identified in a 100 kb region of band 1p22.3. Reverse transcriptase PCR and sequencing allowed the identification of the new *RUNX1* partner gene in this recurrent translocation and revealed at least three alternative *RUNX1* fusion transcripts but no reciprocal fusion. We also discovered a novel cryptic t(15;21)(q26;q22) in an adult case of biphenotypic acute leukemia. Using FISH analysis, a split signal was obtained with BAC clone RP11-17O20, suggesting that the breakpoint is within *RUNX1* intron 1. This specific breakpoint is highly associated with the t(12;21) in precursor B-cell acute lymphoblastic leukemia. Using BAC clone RP11-357H12 located at chromosomal band 15q26.1, a breakpoint region distal to the *POLG* gene was also defined. Finally, we describe a second case of adult AML with the cryptic t(9;21)(q34;q22). *RUNX1* rearrangement with chromosomal band 9q34 was confirmed by FISH using BAC clone RP11-299D9. Unfortunately, lack of sufficient material did not allow us to further identify the *RUNX1* partner gene in this case. Characterization of different *RUNX1* translocations is essential to better understand the oncogenic mechanisms associated with these rearrangements. In addition, these studies might help to identify new genes that are potential therapeutic targets in acute leukemia.

## 1284/W/Poster Board #942

**Human Multiple Myeloma Cell Lines: Molecular characterization by genome-wide profiling identifies alterations involving key enzymatic proteins.** *P.E. Leone[1], C. Elosua[1], P. Catalina[1], C. Palma[1], B.A. Walker[2], N.J. Dickens[2], A. Avramidou[2], L. Brito[2], E. Davenport[2], M.W. Jenner[2], D. Gonzalez[2], F.E. Davies[2], G.J. Morgan[2].* 1) Cytogenetics and Molecular Biology, Andalusian Stem Cell Bank, Granada, Spain; 2) Section of Haemato-Oncology, The Institute of Cancer Research, London, United Kingdom.

Multiple Myeloma (MM) is a malignancy depicted by clonal expansion of plasma cells in the bone marrow. There are two broad genetic subtypes of MM as defined as hyperdiploid MM, and nonhyperdiploid MM. These two subtypes of MM have different molecular pathogenesis. In order to contribute to the understanding of this malignancy and to unveil the different molecular pathogenesis, our interest is focused on Human MM Cell lines (HMCLs) as a model, and on a broad but specific group of enzymatic proteins: the Kinases. Kinase hyperactivity or lack of it often results in deregulation of cellular pathways involved in proliferation and survival. In our study, we describe the patterns of genetic lesions and molecular pathogenesis of 12 HMCLs with SNP-based mapping arrays from Affymetrix Human Mapping 500K array set. This technique allows the examination and identification of copy number changes, bi-allelic deletions (DAB) and the identification of loss of heterozygosity (LOH) due to loss and uniparental disomy (UPD), as well as gene localization and identification. The 12 HMCLs utilized are characterized for their structural alterations and not by hyperdiploidy. In addition, so as to fulfill the selection criteria, a minimum of 3 cell lines must present the alterations cited below. The most frequently identified alterations were located as follows: gains were observed in 1q, 4q, 7q, 8, 11q, 18, 19, and 20q.The DAB were ascertained on 3p. Similarly, we identified the regions of hemizygotic deletions on 1, 2q, 6q, 8q, 9p, 11q, 12, 13q, 14q, 17p, and 20p. In addition, regions of homozygous deletions were detected on 1p, 2q, 3, 4q, 6q, 8p, 9, 10q, 12p, 13q, 16q, 20p and 22q. Finally, the UPD obtained were traced on 1q, 4q, 8q, 10q, and 22q. These identified alterations are affecting a series of enzymatic genes belonging to targeted pathways: (1) PI3K/AKT pathway, which affect to a number of intracellular and extracellular myeloma growth cytokines; (2) Cyclin-Dependent Kinases, critical regulators of cell cycle progression and RNA transcription; (3) NFKB pathway, key regulator of the immune response; and (4) Aurora and related kinases, as another family of cell cycle regulators and often aberrantly activated in human tumor cells. These altered kinases may be potential targets for therapeutics. Our data demonstrates the genomic complexity of MM, enhancing the understanding of the molecular pathogenesis of the disease and the importance of the HMCLs as a model.

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## 1285/W/Poster Board #943

**Frequency and Complexity of 10;11 Chromosomal Rearrangements in Pediatric and Adult AML.** *B.J. Dave, M.L. Wiggins, C.M. Higgins, W.G. Sanger.* Human Genetics Laboratory, Munroe Meyer Institute for Genetics and Rehabilitation, University of Nebraska Medical Center, Omaha, NE.

Acute myeloid leukemia (AML) is the most common type of acute leukemia in adults, accounting for nearly 80% of all cases; however, AML represents only 20% of all pediatric acute leukemia cases. Chromosomal rearrangements of 11q23, involving the *MLL* gene region, are common in AML and its frequency peaks in infancy. Aberrations of 11q23 exhibit high heterogeneity and are associated with poor prognosis. Multiple translocation partners are involved in 11q23 rearrangements; one of these is chromosome 10. The 10;11 abnormality is often a consequence of a complex rearrangement including inversions, insertions, and translocations with a diversity of breakpoints on 10p. The majority of the 10;11 abnormalities result in the *MLL-MLLT10* (*AF10*) fusion, however, two other fusion transcripts, the *CALM-MLLT10* and the *MLL-ABI1*, have also been described. We investigated the frequency and complexity of chromosome 10;11 rearrangements in pediatric and adult AML cases diagnosed during 2000-2008 at the University of Nebraska Medical Center. Of the 34 histologically confirmed AML (20 pediatric and 14 adult) cases containing 11q23 (*MLL*) rearrangements, 10 cases (10/34; 29%) revealed a translocation of 10p with 11q23. We present cytogenetic and fluorescence in situ hybridization (FISH) studies in these 10 AML cases (4 pediatric and 6 adult). Interphase and metaphase FISH studies using a dual color break-apart probe confirmed a disruption of the *MLL* (11q23) gene region in each of these cases. Six cases (1 pediatric; 5 adult) revealed a t(10;11)(p12;q23), three cases (2 pediatric; 1 adult) had a t(10;11)(p11.2;q23) and one case (pediatric) had a t(10;11)(p13;q23). In 4 cases (4/10; 40%) the t(10;11) appeared as the sole abnormality. Three cases (3/10; 30%) contained trisomy 8 in addition to the t(10;11), and three other cases revealed complex rearrangements of chromosomes 10 and 11 which were resolved using multiple metaphase FISH studies. Thus, a high frequency of 10p;11q abnormalities were evident among both pediatric and adult AML cases with 11q23 (*MLL*) abnormalities. The rearrangement involving *MLLT10* (*AF10*) at 10p12 was predominantly seen in adults while the rearrangement of *ABI1* at 10p11.2 was more frequent in pediatric cases. Metaphase FISH studies are imperative to resolve the many complexities noted in the chromosome 10;11 abnormality and to determine the exact breakpoints and the genes disrupted as a consequence of the rearrangements.

## 1286/W/Poster Board #944

**ArrayCGH as a Tool for Interrogating Recurrent Genomic Rearrangements in Leukemias: Proof of Concept Study.** *F.B. David[1], W. Tang[1,3], D.F. Saxe[2], K. Mann[2], S. Sunay[3], H.J. Khoury[3], B. Leyland-Jones[1], M.M. Bouzyk[1,4].* 1) Winship Cancer Institute, Emory University, Atlanta, GA; 2) Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA; 3) Department of Hematology and Medical Oncology, Emory University, Atlanta, GA; 4) Department of Human Genetics, Emory University, Atlanta, GA.

The Philadelphia chromosome [Ph, t(9;22)] is a hallmark of chronic myelogenous leukemia (CML) and is present in up to 30% of adult B-lymphoblastic leukemia (ALL). This translocation is detected clinically by chromosome analysis, FISH and RT-PCR. These methods, however, do not have sufficient resolution to identify the precise base pair location of breakpoints. In addition, both FISH and RT-PCR will not detect additional abnormalities many of which are clinically significant. We report results of a proof-of-concept study using a custom 385K mixed density array comparative genomic hybridization chip (aCGH) from NimbleGen to detect the presence of the Ph chromosome with precise location of breakpoints as well as identifying other known genomic abnormalities in leukemias. The BCR-ABL regions of the breakpoint sites of the Ph chromosome translocation were tiled at an average of 1 probe every 2bp. Other key leukemia-related regions were tiled less densely at 100 to 2500bp. Samples analyzed were from 3 reference cell lines (2 CML and 1 ALL) and 5 patients [2 CML, 1 Ph+ ALL, 1 Ph-negative (Ph-) myeloproliferative neoplasm, 1 Ph- ALL] and 1 control. The M-BCR breakpoint of the two CML cell lines, K-562 and Meg-01, were found in introns 14-15 and 13-14, respectively. This agreed with published results, and was further confirmed by RT-PCR. The breakpoints of the two CML cell lines were characterized by relatively large deletions/duplications. The candidate breakpoint for Sup-B15 on chromosome 22 was characterized by microdeletions. In addition, our custom aCGH clearly differentiated between Ph+ CML and Ph+ ALL by resolving deletions in the IKZF1 region for both Ph+ ALL cell line and patient. Some of the other regions, including CDKN2A, PAX5, 1p36, ERCC8 (5q12), and 17q12, were found to have possible duplications or deletions in some of the leukemia patients and may have clinical significance. Although the aCGH data on the patient t(9;22) breakpoints were inconclusive, our results illustrate the feasibility of utilizing aCGH as a tool in screening for not just one but for multiple types of recurrent leukemic abnormalities on one chip. *F.B. David and W. Tang contributed equally.

## 1287/W/Poster Board #945

**Application of array Comparative Genomic Hybridization to the cytogenetic analysis of pediatric Acute Lymphoblastic Leukemia.** *A. Dawson[1,2,3,5,8], R. Yanofsky[3,5,7,8], S. Mai[6,7,2,4,8].* 1) Cytogenetics Laboratory, Diagnostic Services Manitoba; 2) Dept. Biochemistry and Medical Genetics; 3) Dept. Pediatrics and Child Health; 4) Dept. of Cell Biology & Physiology; 5) Health Sciences Centre; 6) Genomic Centre for Cancer Research and Diagnostics; 7) Cancer Care Manitoba, Winnipeg, MB, Canada; 8) University of Manitoba, Winnipeg, MB, Canada.

Current detection of cytogenetic abnormalities in leukemias is based primarily on karyotype and/or FISH analysis; whereas array comparative genomic hybridization (aCGH), or cytogenetic microarray, has revolutionized conventional cytogenetics because of its increased resolution and independance from cell culture. It is well known that karyotypic analysis of haematological malignancies, although genome wide, is limited due to altered cell kinetics (mitotic rate), a propensity of leukemic blasts to apoptose in culture, overgrowth by normal cells, and poor quality chromosomes of the abnormal clone. Although FISH can be used on interphase cells upon culture failure or normal karyotype, FISH is not genome wide and is limited to analysis of specific loci of the abnormal clone. Acute lymphoblastic leukemia (ALL) is the most common and amongst the most curable of pediatric cancers. Today, 70-80% of children with newly diagnosed ALL treated with contemporary intensive state-of-the-art protocols can be cured. Clonal chromosomal abnormalities can be identified in approximately 90% of cases of childhood ALL and many have association with specific clinical, biological, and prognostic features. However, ~ 30% of pediatric (and 50% of adult ALL) do not have cytogenetic abnormalities of clinical significance. Despite significant improvements in outcome for pediatric ALL, ~ 25% of patients fail therapy, and often these failures occur unpredictably in patients with a favorable prognosis and 'good' cytogenetics at diagnosis. Thus far, aCGH has not been extensively used in haematological malignancies to look for acquired chromosome abnormalities as research has concentrated on gene expression microarrays to search for novel oncogenic pathways, prognostic factors, or mechanisms of drug resistance. In the present study, BAC and/or oligonucleotide aCGH (HemeScan, Combimatrix Molecular Diagnostics) was applied to 10 retrospective cases of pediatric ALL with a poor clinical outcome and either no chromosome abnormality identified or a chromosome abnormality not generally associated with a poor outcome. The HemeScan arrays both detected previously unidentified chromosome abnormalities and corrected previous conventional karyotypes. The use of aCGH in the clinical study of leukemias is recommended, as this will allow for more sensitive and accurate analysis of the underlying genetic profiles, which will ultimately lead to improved prognosis and treatment.

## 1288/W/Poster Board #946

**Breakpoint Mapping with Chromosome Microdissection-Derived Microarray Probes.** *T. Dennis[1], M. Bittner[2].* 1) Genetics Department, Shodair Children's Hospital, Helena, MT; 2) Computational Biology Division, The Translational Genomics Research Institute, Phoenix, AZ.

Molecular mapping of breakpoints involved in chromosomal rearrangements is problematic for many clinical tumor samples. Typically, insufficient numbers of metaphases are available for multiple FISH probe analyses. Chromosome microdissection has proven to be a valuable tool for the elucidation of cryptic chromosome rearrangements and for translocation breakpoint analysis at cytogenetic resolution. Microdissection involves physically scraping off any part of a chromosome from G-banded metaphase preparations using microneedles. Recovered DNA can then be DOP-PCR amplified and subsequently labeled for FISH or used for other molecular genetic applications. Microdissected DNA can be successfully used as a direct probe for microarray analysis providing breakpoint characterization at array resolution in a single hybridization. The analysis is based on two expectations that are met with these probes. First, the probes complementary to the region of microdissected chromosome have a strong signal. Second, the coverage of probes complementary to the microdissected chromosome region are high. Thus, microdissected regions are indicated by sets of probes that have strong signals and are found on probes close to each other on chromosomal regions.

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## 1289/W/Poster Board #947

**Primary Large B-Cell Lymphoma Rearrangement of the *c-MYC* Gene.** *D. Juroske, P. Hu, M. Littlejohn, W. Im, C. Yin, P. Lin, A. Lennon.* Molecular Genetic Technology, School of Health Professions and Department of Hematopathology, University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030.

Diffuse large B-cell lymphoma (DLBCL) is the most common Non-Hodgkin's lymphoma and is a heterogeneous disease with variable genetic aberrations. Rearrangements of c-MYC (at chromosome 8q24) are reported in approximately 16% of DLBCL in the western population, and have been associated with a poor clinical outcome. Using fluorescence in situ hybridization (FISH) and a break-apart probe, we examined c-MYC rearrangements in 63 DLBCL cases from China. The DLBCL were subclassified as germinal center B-cell (GCB) type (1/8, 12.5%) and non-GCB type (7/19, 36.8%), respectively, based on immunohistochemical studies using antibodies specific for CD10, BCL-6 and MUM-1 on a tissue microarray. Seven of 34 (20.5%) cases analyzable by FISH were positive for c-MYC rearrangements (Fig. 3). The percentage of cells with c-MYC rearrangements per positive sample ranged from 19-84%. When these cases were subdivided into GCB and non-GCB types, c-MYC rearrangements were found in 1 of 8 (12.5%) of GCB cases and 7 of 19 (36.8%) of non-GCB cases. We conclude that c-MYC rearrangements occur at a similar frequency in Chinese and western populations. More cases need to be assessed to determine a significant difference in c-MYC rearrangement between GCB and non-GCB types. To our knowledge, this is one of the largest studies to assess the occurrence of c-MYC rearrangements in different types of DLBCL from China.

## 1290/W/Poster Board #948

**Comparison of bone marrow cytogenetics, FISH and Agilent 244K microarray results on 25 patients with AML, MDS or MPD; evidence for the utility of each methodology for the detection of unique genetic abnormalities. Rhett P. Ketterling, Troy J. Gliem, Gary A. Hicks, Anne E. Wiktor, Margot A. Cousin, Cindy Williamson, Erik C. Thorland. Department of Laboratory Medicine and Pathology, Division of Laboratory Genetics, Mayo Clinic, Rochester, MN, 55905.** *R.P. Ketterling, T.J. Gliem, G.A. Hicks, A.E. Wiktor, M.A. Cousin, C. Williamson, E.C. Thorland.* Dept Lab & Med, Mayo Clinic, Rochester, MN.

Introduction: Primary bone marrow (e.g., myeloid) malignancies encompass the spectrum of chronic myeloid neoplasms, both proliferative (MPD) and dysplastic (MDS) and the aggressive acute myeloid leukemias (AML). Interrogation of the tumor genome in myeloid cells has historically been performed by conventional chromosome studies or FISH. The advent of array comparative genomic hybridization (aCGH) testing should allow a new level of exploration into the myeloid tumor genome and has the potential to identify many novel and presently undetected genetic abnormalities associated with these neoplasms. Materials and Methods: Following IRB approval, bone marrow specimens from 25 patients diagnosed at Mayo Clinic with a myeloid malignancy were evaluated by conventional chromosome studies and Agilent 244K aCGH studies. Specific FISH studies were performed on a subset of cases. Results: The 25 patients included 8 patients with AML, 5 with MDS and 12 with MPD. Chromosome studies were abnormal in 23 of 25 cases with 25-100% abnormal metaphases demonstrating either single clonal chromosome abnormalities or complex clonal karyotypes. FISH studies verified the presence of classic translocations and inversions associated with myeloid leukemias in 5 patients, including inv(16), t(10;11), t(8;21), t(9;22) and t(3;21). aCGH identified 955 chromosomal gains or losses, approximately 40 per patient, each involving at least 4 consecutive probes. Of these 955 abnormalities, at least 591 (62%) were deemed to be benign copy number changes. Excluding all aCGH abnormalities <1 MB in size, the comparison of array results with chromosome studies indicates at least 26 abnormalities were identified only by aCGH while up to 29 were rearrangements identified only by chromosome studies, including the five classic translocations or inversions also verified by FISH testing. Conclusions: The combined data indicate each method offers the potential for identification of novel genetic abnormalities in myeloid malignancies. The integration of aCGH into the clinical genetic algorithm for myeloid malignancies is yet to be established, although the additional abnormalities uncovered by this powerful methodology have the potential to identify new genomic regions with both diagnostic and prognostic implications.

## 1291/W/Poster Board #949

**An unusual case of ovarian undifferentiated carcinoma.** *M.J. Macera[1,2], W. Thelmo[3], A. Abdu[3], F. Zarghami[4], F. Cohen[1,2], J. Breshin[1,2], P. Chandra[2], A. Babu[1,2].* 1) Div Molecular Medicine & Genetics; 2) Dept of Medicine; 3) Dept of Pathology; 4) Dept of OB/GYN, Wyckoff Heights Medical Center, Brooklyn, NY.

A 50 year old woman came to the hospital complaining of abdominal pain and diarrhea. A CAT scan revealed a pelvic mass that was obscuring her ovaries and uterus. She also had a pleural effusion on the right side that was drained and showed negative cytology. EMA was positive and she underwent surgery. Both ovaries and the uterine wall were infiltrated by undifferentiated carcinoma. The tumor invaded omental adipose tissue and mesoappendix. Lymph nodes also demonstrated extensive metastases. A segment of sigmoid/rectal colon was densely infiltrated by tumor. Clinically the tumor was well confined within the abdomen. Cytogenetic analysis of tumor cells showed normal cells and a near tetraploid cell line ranging from 90 to 104 chromosomes. Ovarian cancers with chromosome involvement are grouped into two categories: those near diploid tumors having the lowest numerical changes with numerous structural changes and those near tetraploid tumors that possess abnormal centromeres with fewer structural rearrangements. This case had near tetraploid numbers and numerous structural rearrangements with at least 16 marker chromosomes. Based on GTG banding results, FISH analysis was performed with various probes to help elucidate the rearrangements and marker chromosomes. The results are cep1[Sat 11/111]x8 (1q12), D11Z1x4 (11p11.1q11), CCND1x4 (11q13), ATMx4 (11q22.3) MLLx4 (11q23), TELx2 (12q13), D12Z3x5 (12p11.1-q11), IGHx5 (14q32), p53x3 (17p13), D1721x5 (17p11.1-q11.1), HER2/neux6 (17q11.21-12), BCL2x4 (18q21), AML1x2 (21q21). The most distinctive marker, seen in every abnormal cell, was a chromosome approximately twice the size of chromosome 1. This derivative chromosome by FISH analysis was (cep1x1, wcp12+, TEL-, D12Z3x1). G-banding suggested one breakpoint at 1p31, while loss of TEL suggests a second break point at 12p13. Abnormalities frequently associated with ovarian cancers include additional copies of 1q, 3q and 20q, with loss of 4p, 4q, 18p, 18q and 19q. This case has eight copies of 1q12, including four copies of structurally normal 1's, one highly rearranged marker involving at least two additional chromosomes and three markers that appear to contain at least the entire 1q. There are four copies of BCL2 in two isochromosomes 18q, with no normal 18. Tumors with diploid and near diploid numbers contribute to a better prognosis and greater survival over those that are near tetraploid.

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## 1292/W/Poster Board #950

**Intracystic papillary carcinoma of breast harbors significant genomic alteration compared with intracystic papilloma: Genome-wide copy number and LOH analysis using high-density single-nucleotide polymorphism arrays.** *M. Oikawa[1,2], T. Nagayasu[1], H. Yano[1], T. Hayashi[3], K. Abe[3], N. Niikawa[4,5], K. Yoshiura[2,5]*. 1) Surgical Oncology, Nagasaki University Graduate School, Nagasaki, Japan; 2) Human Genetics, Nagasaki University Graduate School, Nagasaki, Japan; 3) Department of Pathology, Nagasaki University Hospital, Nagasaki, Japan; 4) Research Institute of Personalized Health Sciences, Health Sciences University of Hokkaido, Tobetsu, Japan; 5) Solution Oriented Research for Science and Technology (SORST), Japan Science and Technology Agency (JST), Tokyo, Japan.

**Purpose**: Intracystic papillary tumors of breast consist of benign papilloma, carcinoma in situ and carcinoma with invasion, which account for approximately 10% of benign breast tumors and less than 3% of malignant. The problem for the clinical management of these lesions is difficult to discern benign lesions from malignant preoperatively. Although the observation from genome-wide copy number and LOH analysis in these lesions may be helpful for resolving this problem, this kind of study has been scarcely conducted. By using high-density single-nucleotide polymorphism arrays, this study aimed to reveal the profile of genomic alteration in these lesions and search for the possibility of novel diagnostic method. **Method**: Eight samples of intracystic papillary tumors from Nagasaki University Hospital pathology archives that contained three pailloma (Pap), three papillary carcinoma in situ (PC) and two papillary carcinoma with invasion (IDC) were identified. Areas of tumor and normal tissue, which were identified by guide slide stained with hematoxylin and eosin, were microdissected from same formalin fixed paraffin embedded (FFPE) blocks respectively followed by three days of proteinase K treatment and DNA extraction with Qiagen DNA Mini Kit. Using probe intensity and genotype data from GeneChip Genome-wide Human 5.0 (Affymetrix), paired copy number and LOH analysis was performed by Partek Genomic Suite Software. Statistical analyses were calculated by R (version 2.8.0).**Result**: QC call rate, which is one of the indices measuring the quality of SNP microarray's experiment, were from 70.75% to 86.5%, mean 78.4%. The mean of total genomic alteration, which expressed as percent of the genome that is aberrant (sum of gains, losses and copy-neutral LOH), were 0.85%, 15.4% and 35.3% in Pap, PC and IDC respectively. Compared with benign tumors (Pap), malignant tumors (PC + IDC) had significantly more changes in their genome (Wilcoxon's rank sum test, p=0.035). Though they had a tendency to increase genomic alteration along with malignant phenotype, it wasn't statically significant among three diagnostic category (Kruskal-Wallis chi-squared test, p=0.062). **Conclusion**: Among intracystic papillary tumors of breast, malignant tumors even in non-invasive harbor significant genomic alteration, albeit with difficulty in diagnosis by histopathological method. Our finding may be helpful for clinical management of them.

## 1293/W/Poster Board #951

**Novel translocations (2;13)(p21;q14.11) and (6;17)(q23.3;p13.3) associated with genomic disturbance in two patients with myeloid neoplasms.** *J.L. Poitras[1], D. Costa[2], M.J. Kluk[3,5], P.C. Amrein[3,5], R.M. Stone[4,5], C. Lee[1,5], P. Dal Cin[1,5], C.C. Morton[1,2,5]*. 1) Pathology, Brigham and Women's Hospital, Boston, MA; 2) Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Boston, MA; 3) Hematology/Oncology Unit, Massachusetts General Hospital, Boston, MA; 4) Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; 5) Harvard Medical School, Boston, MA.

Gross chromosomal rearrangements, particularly translocations, have proven a valuable tool in patient management through use in prognosis, diagnosis, and therapeutic intervention. Although many cancer genes have been illuminated by cytogenetic aberrations, hundreds of chromosomal rearrangements remain to be explored. The Tumor Genome Anatomy Project (TGAP) seeks to enhance annotation of the cancer genome through exploiting novel, uncharacterized rearrangements in diagnostic samples referred to the clinical cytogenetics laboratory. Using a systematic approach including FISH mapping, RT-PCR and sequencing, we have identified several candidate genes and novel gene fusions. Breakpoint mapping of two apparently balanced translocations, t(2;13)(p21;q14.11) and t(6;17)(q23.3;p13.3) in patients with myeloid neoplasms, detected concomitant deletions at or adjacent to all breakpoints. Array CGH on the 244K Agilent platform refined the deletion boundaries, revealing a 195 kb deletion at 2p21, a 1.4 Mb deletion distal to the 13q breakpoint at 13q14.3, a 1.7 Mb deletion directly adjacent to the 6q23.3 breakpoint, and a 562 kb deletion at 17p13.3. Additionally, a 133 kb deletion within the breakpoint region at 13q14.11 and a 265 kb deletion proximal to the breakpoint were discovered, neither of which were detected by FISH. While no obvious gene fusion resulting from either novel rearrangement can be determined from these data, possible formation of a fusion transcript cannot be ruled out because the levels of resolution of techniques used compromises delineation of the precise location of the breakpoints. Although the clinical relevance of these focal imbalances remains to be evaluated, cases presented here support high resolution evaluation of presumably balanced rearrangements in neoplasms. Such imbalances may portend important hitherto unrecognized prognostic and diagnostic categories.

## 1294/W/Poster Board #952

**High-resolution array-based comparative genomic hybridization detection of copy number changes in desmoplastic melanoma and malignant peripheral nerve sheath tumor.** *J.G. Pryor[1], B.A. Brown-Kipphut[1], G.A. Scott[2], M.A. Iqbal[1]*. 1) Pathology, University of Rochester Medical Center, Rochester, NY; 2) Dermatology, University of Rochester Medical Center, Rochester, NY.

Desmoplastic melanoma and malignant peripheral nerve sheath tumor (MPNST) can appear morphologically similar, posing diagnostic difficulties even with use of immunohistochemical stains. We attempted to determine whether whole genome microarray comparative genomic hybridization (aCGH) analysis could be used to detect copy number differences between these two tumors and potentially aid in diagnosis. Immunohistochemistry with antibodies directed against the S-100 protein was performed on 5 cases of desmoplastic melanoma, 8 cases of MPNST, and 1 normal lymph node control using formalin-fixed, paraffin-embedded archival specimens. Genomic DNA was extracted from microdissected tumor cells and lymphocytes. To obtain adequate quantities of DNA for performance of aCGH, whole genome amplification (WGA) was required for 5/5 desmoplastic melanoma cases and 6/8 MPNST cases. A portion of DNA from the lymph node control was also amplified to assess for amplification bias. A gel-based 5-primer multiplex PCR assay was used to determine the quality of the DNA samples, with the amplified and unamplified lymph node control, 5/5 desmoplastic melanomas and 5/8 MPNSTs (3 amplified and 2 unamplified) considered of adequate quality for aCGH. DNA samples were fluorometrically quantitated then run on PerkinElmer's SpectralChip 2600 BAC array platform, scanned on a GenePix 4000B scanner, then analyzed using Spectralware BAC array analysis software. Cytogenetic abnormalities present in the amplified but not the unamplified lymph node control were considered amplification bias and not reported for the test samples. Desmoplastic melanoma and MPNST exhibited karyotypic complexity and shared a number of cytogenetic abnormalities, including gains of 4p11-4q11, 4q32, 7p23-pter, 7q31-32, and 12q13, and losses of 4q28, 6q26-qter, 9p21-p22, 9p23-pter, chromosome 10, 11q23-qter, and 15q13-14. Changes present in some cases of desmoplastic melanoma but not MPNST included gains of 1p12-p34, 9q21-31, and 12p13, and losses of 5p14-pter, 11p, 13q14-21, 15q22-qter, and 17q11-12. Selective validation with fluorescence in-situ hybridization (FISH) was performed. We conclude that desmoplastic melanoma and MPNST share both morphologic and cytogenetic similarities. Genetic loci that may be involved in the tumorigenesis of these tumors will be presented.

## 1295/W/Poster Board #953

**A novel unknown translocation partner on chromosome 2 or chromosome 5 for the EWSR1 gene in Ewing Sarcoma.** *S. Shetty[1], N. Uddin[1,2], K.B. Geiersbach[1], S.T. South[1,2]*. 1) University of Utah, School of Medicine, Department of Pathology, Salt lake City, Utah and Cytogenetics/Molecular Cytogenetics, ARUP Laboratories, Salt Lake City, UT; 2) University of Utah, School of Medicine, Department of Pediatrics, Salt lake City, Utah and Cytogenetics/Molecular Cytogenetics, ARUP Laboratories, Salt Lake City, UT.

Ewing sarcoma is a common malignant round cell tumor of childhood and adolescence. Cytogenetic analysis to identify a common t(11;22)(q23;q12) or less frequently a t(21;22)(q22;q12) or t(7;22)(p22;q12) plays an important role in confirmation of the clinical diagnosis. The genetic mechanism, in majority of the cases, involves fusion of EWSR1 gene on 22q12 with a member of the ETS family of transcription factors. We report a case of a 20-year-old male who had a soft tissue tumor on the left scapula. Conventional cytogenetic analysis on unstimulated suspension culture revealed a complex karyotype with numerical and structural abnormalities. No chromosomal rearrangement involving the EWSR1 region was recognized and all cells from the monolayer culture were karyotypically normal. However, the biopsy results were consistent with the diagnosis of Ewing Sarcoma. Immunohistochemistry was positive for CD99 and vimentin and negative for AE1/3, CAM 5.2, CD45 and Fli1. To identify a cryptic rearrangement, fluorescence in situ hybridization (FISH) on interphase cells was performed utilizing the LSI EWSR1, dual-color break-apart rearrangement probe. FISH findings revealed two normal fusion signals and an extra signal for the 5' EWSR1 region suggestive of a rearrangement and deletion of the 3' EWSR1. FISH on G banded metaphases from unstimulated suspension culture identified that the extra 5' EWSR1 signal was on chromosome 2. Based on G-banding and FISH findings, the derivative chromosome 2 was shown to consist of a translocation between 2q and 5q with an insertion of the long arm of the 22q12 (approximately between bands 2q12~13 and 5q13. These findings suggest that an insertion event involving the EWSR1 locus on chromosome 22 is another mechanism that could lead to EWSR1-unknown partner gene fusion. To our knowledge, this is the first case report of an insertion of a segment of the EWSR1 region with a possible novel translocation partner on either chromosome 2q12~13 or 5q13 in Ewing sarcoma. This case also highlights the importance of chromosome studies in addition to FISH to understand the genetic mechanism and aid in identifying new partners for the EWSR1 gene on unstimulated suspension cultures in round cell tumors. Based on FISH findings alone, rearrangement of EWSR1 in this case would have been just a postulate and the rearrangements would have been missed if only monolayer culture was analyzed.

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### 1296/W/Poster Board #954

**Acute myeloid leukemia with inv(16), 3'*CBFB* deletion, variant t(9;22) & del(7)(q) in a pediatric patient, a case report.** *N. Uddin[1], W. Chen[2], A. Arbini[2], L. Klesse[3], N. Karandikar[2], M. Almiski[2], I. VIllalobos[1], CA. Tirado[1].* 1) Clinical Cytogenetics, Department of Pathology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX; 2) Hematopathology, Department of Pathology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX; 3) Hematology/Oncology, Department of Pediatrics, University of Texas Southwestern Medical Center at Dallas / Children's Medical Center, Dallas, TX.

Coexistence of inv(16) and t(9;22) has been described, rarely in chronic myelogenous leukemia (CML) and in *de-novo* acute myeloid leukemia (AML) (~7 case reports, only one pediatric case). To the best of our knowledge, we describe the first pediatric case of AML with inv(16) and variant t(9;22) along with 3'*CBFB* deletion and del(7)(q). The patient was a -13-year old male who presented with a 3 month history of night sweats, weight loss and a 2 weeks history of swollen lymph nodes, fever, fatigue, and petechiae. Peripheral blood showed circulating blasts. Bone marrow biopsy revealed a predominant population of large blasts with variably irregular nuclei, dispersed chromatin, variably prominent nucleoli and scant to moderate amounts of cytoplasm. Some of the immature cells had features of promonocytes. Eosinophilia was largely absent. Flow cytometeric analysis showed immunophenotypically aberrant myeloblasts with a small population of mildly aberrant monocytes. Conventional and molecular cytogenetic analysis on blood showed three related abnormal clones, with a stem line showing inv(16), and described as 46,XY,inv(16)(p13.1q22)[2]/46,idem,-del(7)(q22q32)[16]/46,idem,t(9;22;19)(q34;q11.2;p13.1). Interphase FISH with break-apart *CBFB* probe (Vysis, Inc.) showed 3'*CBFB* deletion. FISH analysis using chromosome 16 Telomere probe (Vysis, Inc.) together with *CBFB* probe on abnormal metaphases confirmed the presence of 5'*CBFB* on the short arm of inv(16) while 3'*CBFB* was deleted on the long arm. Deletion 7q was also confirmed by FISH. Metaphase FISH with dual fusion probe for *BCR/ABL* rearrangement [t(9;22)] (Vysis, Inc.) confirmed the presence of Philadelphia chromosome and involvement of chromosome 19 in the variant rearrangement. The patient was initially leukoreduced secondary to respiratory distress and then treated with standard AML chemotherapy. He also received Gemtuzumab as part of a clinical trial and is currently in remission. Limited data suggests that the prognosis in *de-novo* AML cases with both inv(16) and t(9;22) seems favorable. Found rarely, the significance of the 3'*CBFB* deletion with inv(16) is unclear. Few cases of inv(16) with this deletion have shown an aggressive behavior. Deletion 7q occurs uncommonly with inv(16) and there is no apparent prognostic difference. Due to rarity and complex chromosomal aberrations, the exact clinical behavior in this case is difficult to predict. Long-term follow-up would provide further prognostic information.

### 1297/W/Poster Board #955

**Further Evidence of Poor Prognosis For Patients With Variant BCR/ABL1 Fusion Patterns.** *A. Zaslav[1], T. Mercado[1], T. Pardee[2], S. Richard[2], S. Spitzer[3], E. Knorr[1], D. Tully[1], K. Zamkoff[4].* 1) Dept Pathology, Cytogenetics Laboratory, Stony Brook Univ Hosp, Stony Brook, NY; 2) Blood and Marrow Stem Cell Transplantatiion Program, Stony Brook University Medical Center, Stony Brook, NY; 3) Dept Pathology, Molecular Diagnostics Laboratory, Stony Brook Univ Hosp, Stony Brook, NY; 4) Hematology and Oncology, Wake Forest University Medical Center, Winston Salem, NC.

Patients with chronic myelogenous leukemia (CML) typically have the Philadelphia (Ph) chromosome. Ph results from a reciprocal translocation of chromosomes 9 and 22 [i.e., t(9;22)(q34;q11.2)]. Approximately 10% to 15% of patients with CML have an apparently normal karyotype but are Ph+ using fluorescence in situ hybridization (FISH) and or reverse transcriptase polymerase chain reaction (RT-PCR). FISH identified variant abnormal signal patterns in these patients, which have been associated with a more rapid progression to blast crisis and a shorter overall survival. Initial studies on these patients were based on protocols using allopurinol, hydroxyurea, and interferon. With the introduction of imatinib mesylate in 2001 more recent studies demonstrated that this may also be true for these patients on imatinib therapy. We previously reported on three Ph+ CML patients with variant abnormal signal patterns. Here, we present the follow-up data on these patients. The patients were evaluated multiple times during treatment. Two patients failed treatment and one patient demonstrated complete remission. Even though our patient sample was small the data corroborates that CML patients with variant abnormal signal patterns have a worse prognosis than those with typical abnormal signal patterns. Here, we discuss the clinical, cytogenetic, FISH and molecular data on three patients, the possible mechanisms involved in the formation of these variant abnormal signal patterns and the clinical significance of these findings.

### 1298/W/Poster Board #956

**High rate of genetic abnormalities of neoplastic cells in pseudofollicles of CLL.** *Z. Balogh[1], L. Reiniger[1], H. Rajnai[1], J. Csomor[1], A. Szepesi[1], A. Balogh[2], L. Deak[1], E. Gagyi[1], C. Bodor[1], A. Matolcsy[1].* 1) Ist Department of Pathology and Experimental Cancer Research, Faculty of Medicine, Semmelweis University, Budapest, Hungary; 2) TARKI Social Research Institute/ Department of Statistics, Faculty of Social Sciences, Eotvos Lorand University, Budapest, Hungary.

Several lines of evidences suggest that lymph node microenvironment is critical for survival of chronic lymphocytic leukaemia (CLL) cells. In lymph nodes neoplastic cells (prolymphocytes and paraimmunoblasts) form pseudofollicles (PFs) which are also known as proliferation centers. To reveal whether PFs play a role in generation of genetic alterations in CLL we compared deletion at p53, ATM and RB-1 loci and trisomy 12 by fluorescent in situ hybridization (FISH) technique in PFs versus surrounding small lymphocytes in 12 formalin-fixed paraffin-embedded (FFPE) lymph nodes. The FFPE sections were stained with methylene blue and PFs were marked by laser. Subsequent FISH analysis was performed relocalizing the previously defined regions. Loss of 11q was detected in 5 cases, loss of 13q in 2 cases, loss of 17p in 2 cases and trisomy 12 was found in 1 case. In 7 cases PFs contained significantly higher ratio of cells with genetic alterations than the surrounding areas. Our results revealed higher rate of genetic abnormalities in PFs suggesting that these sites play a role in the generation or accumulation of cells with genetic abnormalities. The accumulation of genetic lesions in PFs denotes that genetic progression and transformation of CLL may begin in these proliferation centers.

### 1299/W/Poster Board #957

**FISH ANALYSIS OF BCL-2 AND BCL-6 REARRANGEMENTS IN GERMINAL CENTER AND NON-GERMINAL CENTER TYPES OF DIFFUSE LARGE B-CELL LYMPHOMA IN CHINA.** *C. Simien, M. Littlejohn, P. Hu, Ph.D., A. Lennon, Ph.D., W. Im, R. Pepper, P. Lin, M.D., L. Jeffrey Medeiros, M.D., C. Cameron Yin, M.D., Ph.D.* Program in Molecular Genetic Technology, School of Health Professions and Department of Hematopathology, University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030.

Fluorescence *in situ* Hybridization (FISH) can be used to visualize chromosomal rearrangements in the context of tissue biopsy specimens. Diffuse Large B-Cell Lymphoma (DLBCL) is a heterogeneous disease. In the United States and European patient populations, *BCL-2* and *BCL-6* gene rearrangements have been shown in approximately 20% and 45% of DLBCL cases, respectively. Furthermore, gene expression profiling studies have shown two major prognostic groups: germinal center B-cell-like (GCB) and non-GCB. We investigated the frequency of *BCL-2* and *BCL-6* rearrangements in 63 DLBCL cases from China using FISH break-apart probes, including 21 (33.3%) cases of GCB type and 42 (66.7%) cases of non-GCB type. The GCB and non-GCB types were defined by immunohistochemical analysis using antibodies specific for CD10, BCL-6 and MUM-1 suggested by Hans et al (Blood 2004; 103:275-282). We found that 11 of 50 (22.0%) DLBCL were positive for *BCL-2* rearrangements (range of signals, 8-56%; median 11%). Additionally, 11 of 32 (34.4.0%) DLBCL were positive for BCL-6 rearrangements (range of signals, 12-55%; median, 46%). *BCL-2* rearrangements were found in 5 of 19 (26.3%) of GCB cases and 6 of 31 (19.4%) of non-GCB cases. *BCL-6* rearrangements were found in 5 of 14 (35.7%) of GCB cases and 6 of 18 (33.3%) non-GCB cases. It is noteworthy that three cases (1 GCB, 2 non-GCB) were positive for both *BCL-2* and *BCL-6* rearrangements. The frequency of *BCL-2* rearrangements in our study is similar to what has been reported in the western world. However, *BCL-6* rearrangement appears to be less frequent in the Chinese population. There is no clear relationship between DLBCL subtypes and *BCL-2* or *BCL-6* rearrangement. To our knowledge, this is one of the largest studies to assess the prevalence of *BCL-2* and *BCL-6* rearrangements in different types of DLBCL from China.

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## 1300/W/Poster Board #958

**Mono- and bi-allelic deletions of 13q14.3 in B-cell chronic lymphocytic leukemia (B-CLL): FISH vs. miRNA RT-PCR detection.** *A. Block[1], M.T. Smonskey[2], G. Deeb[3,5], A.A. Chanan-Khan[4], Z.P. Bernstein[4], K.C. Miller[4], P.K. Wallace[5], P. Starostik[2].* 1) Clinical Cytogenetics Lab, Roswell Park Cancer Inst, Buffalo, NY; 2) Molecular Diagnostics Lab, Roswell Park Cancer Inst, Buffalo, NY; 3) Department of Pathology, Roswell Park Cancer Inst, Buffalo, NY; 4) Department of Medicine, Roswell Park Cancer Inst, Buffalo, NY; 5) Laboratory of Flow Cytometry, Roswell Park Cancer Inst, Buffalo, NY.

To develop risk-adapted strategies, prognostic factors are needed to allow for the prediction of individual patient clinical course. Deletion of 13q14.3 (del13q) is the most common molecular cytogenetic abnormality in B-cell chronic lymphocytic leukemia (B-CLL) and is associated with favorable prognosis. This deletion is frequently observed as the sole abnormality in B-CLL, suggesting a pathogenic role for genes in this region. MicroRNAs (miRNA), a new class of gene regulators, have recently been implicated in B-CLL pathogenesis, with frequent deletions and down-regulation of miR-15 and miR-16 at 13q14.3. In this study, we compared fluorescence in situ hybridization (FISH) and real-time miR-15a and miR-16-1 RT-PCR as methods to detect del13q. We analyzed 26 B-CLL patients (pts) showing either mono-allelic (19 pts) or bi-allelic deletion (7 pts) of the locus specific probe D13S25 mapped at 13q14.3 and detected as a sole abnormality by FISH. Both mono-allelic (del13qx1) and bi-allelic (del13qx2) pts were remarkably homogeneous in clinical presenting features (low Rai stage, male predominance, negative ZAP-70 status), with the del13qx2 group older (median age 65 yrs) when compared with the del13qx1 group (median age 58 yrs). miR-15a and miR-16-1 expression was measured as compared to the U6 standard and quantified as a percentage of the U6 using the delta $C_t$ method. Differences in miR-15a and miR-16-1 were examined using a group of trisomy 12 pts as a control. As expected, del13q samples showed lower expression of miR-15a than trisomy 12 samples (mean of 30% vs. 44.8%, respectively); within the del13q samples, the three lowest levels of miR-15a were seen in del13qx2. In contrast, miR-16-1 expression varied widely among these three groups, with the highest levels detected in del13qx1. Due to the high variability of miR-15a levels in the 13q14.3 cases, we were not able to detect a specific cut-off in regards to miR-15a and miR-16-1 expression levels for cases with del13q. As a result, we conclude that discrimination between cases with and without the deletion is better accomplished by FISH.

## 1301/W/Poster Board #959

**IgHV Deletion Detected by Florescence in-situ Hybridization Appears to be a Marker of Poor Prognosis in Plasma Cell Myeloma.** *K. Jabbar, LJ. Medeiros, M. Wang, Lu. Gary.* Department of Hematopathology, and Department of Lymphoma and Myeloma, UT MD Anderson Cancer Center, Houston, Texas, USA.

Plasma cell myeloma (PCM) is a bone-marrow-based, multifocal neoplasm of plasma cells, usually associated with a variety of clinical laboratory findings including an M protein in serum and/or urine. It is genetically heterogeneous. Multiple genomic abnormalities can be observed in up to 90% of cases by Florescence in-situ Hybridization (FISH) during the course of disease development. About 75% of the cases demonstrate rearrangements involving IgH locus, including the unfavorable FGFR3/IgH and the fair prognostic CCND1 XT/IgH. In this study, we report a new genomic marker, IgHV deletion detected by FISH, in 7 PCM cases selected from the MD Anderson Cancer Center database dated from May 2008 to May 2009. All the 7 cases were accessed with complete ancillary studies including routine Cytogenetics, FISH, bone marrow morphologic examination, and flow cytometry. There were 5 men and 2 women, with ages ranging from 52 to 72 years (mean, 62.3). Conventional cytogenetics results were available at time of initial presentation in 5 cases, showing a diploid karyotype in 4, and multiple complex abnormalities in 1. In subsequent follow up samples, FISH showed IgHV deletion in all cases, present in 8.5-49.5% cells (mean, 24.2%), and also demonstrated none of the unfavorable prognostic markers, del(13q) and del(17p), in three cases, two of the three were positive for CCND1 XT/IgH rearrangement. Concurrent routine cytogenetics revealed clonal chromosomal abnormalities in 5 cases. Examination on bone marrow aspirate smears demonstrated increased plasma cells, ranging 29-86%, with greater than 50% replacement of biopsy specimens in 5 of 7 cases; atypical morphology was observed in 4 cases. CD56 expression, an unfavorable immunophenotypic marker, was absent in 5 cases assessed, and CD20 was expressed in 1 case. All 7 patients had increased serum $\beta$2 microglobin levels, ranging from 4.2 to 25.1 with a mean of 10.4 MG/L. Retrospective FISH studies on slides available from samples collected at patient's initial presentation were performed in 3 cases and revealed negative for the IgHV deletion. All 6 patients with adequate follow-up presented with an aggressive clinical course after detection of IgHV deletion; 3 were refractory and 3 showed partial response to high-dose chemotherapy. In conclusion, our results suggest that IgHV deletion in PCM is a marker of unfavorable prognosis and poor response to treatment.

## 1302/W/Poster Board #960

**Hyperdiploid and nonhyperdiploid multiple myeloma subtypes detected by array-based comparative genomic hybridization.** *S.R. Gunn[1,2], M.E. Gorre[1], M.S. Mohammed[1], M.C. Kinney[2], R.S. Robetorye[2].* 1) Combimatrix Molecular Diagnostics, Irvine, CA; 2) Department of Pathology, University of Texas Health Science Center, San Antonio, TX.

Multiple myeloma (MM) is a clinically heterogeneous hematological malignancy recently categorized into prognostically important major aneuploidy groups based on the total chromosomal DNA content of the tumor genome. Classification of MM as hyperdiploid or nonhyperdiploid and identification of associated genomic alterations is an important part of risk stratification and treatment planning in newly diagnosed patients. However, the potential clinical impact of MM risk stratification has been hampered by the limited ability of commonly available clinical diagnostic techniques to consistently determine ploidy status and identify genetic alterations. These limitations include the low percentage of cytogenetic abnormalities detected by conventional G-banded karyotyping, the loss of global genome perspective with targeted FISH, and lack of locus specificity when DNA is quantified by flow cytometry. Here, we describe the application of a customized comparative genomic hybridization microarray (array CGH) for classification of newly diagnosed cases of MM into hyperdiploid and nonhyperdiploid subtypes. Genomic DNA was extracted from bone marrow aspirates of three patients with a clinical diagnosis of MM but whose routine cytogenetic results were uninformative. Tumor genomic profiles were determined with customized targeted bacterial artificial chromosome (BAC) arrays designed and validated for clinical identification of global chromosomal features as well as structural abnormalities associated with specific congenital syndromes. Array CGH results successfully showed genomic imbalances consistent with major MM aneuploidy groups in all patients. Based on global tumor genome presentations, two cases were categorized as hyperdiploid and one case was nonhyperdiploid. These same whole genome profiles also enabled determinations of overall genomic instability associated with hyperdiploid MM. In addition, results revealed structural abnormalities associated with shortened survival such as losses of 1p, 17p, and rearrangements involving the IgH locus. By facilitating incorporation of information about tumor genomic aberrations into clinical decision making, array CGH has great potential to positively impact patient management and care in multiple myeloma and other hematological malignancies.

## 1303/W/Poster Board #961

**Identifying DNA copy number aberrations to predict response to neoadjuvant chemotherapy in breast cancer patients.** *J.H. Woo[1], W. Han[2,3], S.J. Yang[1], K.S. Yang[1], D.Y. Noh[2,3].* 1) Macrogen, Inc., Seoul, South Korea; 2) Department of Surgery, Seoul National University College of Medicine, Seoul, South Korea; 3) Cancer Research Institute, Seoul National University College of Medicine, Seoul, South Korea.

It has been reported that only 40%~60% percent of breast cancer patients respond to neo-adjuvant (pre-surgical) chemotherapy. Without robust criteria for predicting this response, a significant number of patients will incur unnecessary financial burden, and adverse side effects. In this study, we applied array comparative genomic hybridization (array CGH), containing over 4,000 BAC probes, evenly distributed across the human genome at 1 Mb intervals, to identify putative genomic markers associated with chemotherapy response in tumor cells from 63 breast cancer patients that have received Docetaxel and Doxorubicin before surgical treatment. Genomic imbalances were detected at eight chromosomal loci (encompassing 24 clones), and these were correlated with change of tumor size ($p<0.05$ by t-test), as measured by Magnetic Resonance Imaging (MRI) before and after neoadjuvant therapy. Excluding regions that were previously known to be frequently altered in breast cancer cells, and prioritizing regions containing candidate genes, we finally selected two regions, 17p12 and 17q21.32-33, as putative genomic markers for predicting this drug response in breast cancer. The genes located in these regions, included ABCC3, and the HOXB families, members of subfamilies involved in multi-drug resistance and associated with prognosis of breast cancer patients. Twenty-one breast cancer samples were subsequently assembled for an independent validation study to further evaluate the predictive performance of these two genomic regions. In this case, we assessed copy numbers of the two regions using multicolor FISH (fluorescence in situ hybridization) assays as a technical validation. In independent validation data sets, classification models developed through the most commonly used machine learning techniques (artificial neural network, logistic regression, and decision Tree) all showed 80.95% prediction accuracy. We have identified two genomic regions that have >80% prediction accuracy for identifying breast cancer patients that will respond to pre-surgical chemotherapy. Further studies will included identifying the minimal critical regions as well as determining the mechanisms leading to a differential response in this patient population.

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### 1304/W/Poster Board #962

**Genome-wide Association Analysis of Copy-Number Variation (CNV) in Glioblastoma.** *H. Siu[1,2], H. Dong[1,2,3], G. Peng[1], Y. Wang[1,2], R. Chen[4], M. Xiong[1,3].* 1) Laboratory of Theoretical Systems Biology, School of Life Science, Fudan University, Shanghai, 200433, China; 2) State Key Laboratory of Genetic Engineering and MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai, 200433, China; 3) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77225; 4) Department of Molecular and Human Genetics Baylor College of Medicine Houston, TX, 77030.

Copy-number variation (CNV) constitutes a large proportion of total genomic variation and is increasingly recognized to be an extremely important risk factor for cancer. To examine the role of CNVs in glioblastoma, a genome-wide association studies of CNVs in glioblastoma was conducted by assaying 221 tumor tissues and 28 normal tissues samples from primary glioblastoma multiform patients in TCGA project. CNVs were measured by the Affymetrix Genome-Wide Human SNP Array 6.0 with 906,600 SNPs and more than 946,000 probes for the detection of copy number variation. CNVs were called by the modified hidden Markov Models (HMM) and 163024 CNV loci were detected. A total of 197 CNV loci with P-value<2.25E-7 showed significant association with glioblastoma. We also did group association tests of CNV with glioblastoma by gene and pathway. We identified 169 genes with P-values <4.77E-6, including oncogene BCAS1, tumor repress genes CAMTA1, APC and CSMD1, transcription factor ELF2, and transcription activator genes ETV1, CREB5 and ZHX3, which were significantly associated with glioblastoma. Copy number changes in these genes significantly affect the expression of 19 microRNAs and 410 genes. We also identified 19 significantly associated pathways with glioblastoma with FDR <0.05. These significant pathways include Metabolism of xenobiotics by cytochrome P450, Regulation of actin cytoskeleton, Double Stranded RNA Induced Gene Expression pathway, Regulation of eIF2 pathway, Tight junction, Skeletal muscle hypertrophy is regulated via AKT/mTOR pathway, Keratinocyte Differentiation pathway, Drug metabolism - cytochrome P450, Cell cycle, Glioma pathway. Our results provide important clues for investigation of the mechanisms and drug targets of glioblastoma.

### 1305/W/Poster Board #963

**"A cryptic t(1;21;8)(p36.1;q22;q22): A variant involving a single *RUNX1/RUNXT1* fusion in a patient with acute myeloid leukemia (AML-M2)".** *F.J. Valdez[1], W. Chen[1], R. Collins[3], S. Henderson[1], R. Garcia[1], S. Holdridge[1], C. Chastain[1], R. Smart[1], M. Auchus[2], J. Doolitle[3], P. Horna[1], C.A. Tirado[1].* 1) Pathology, UTSouthwestern Medical Center, Dallas, TX; 2) Internal Medicine-Endocrinology, UTSouthwestern Medical Center, Dallas, TX; 3) Internal Medicine-Hematology-Oncology, UTSouthwestern Medical Center, Dallas, TX.

The t(8;21) accounts for 5-12% of AML patients, often occurring in the younger population. This translocation fuses the *RUNX1* gene (formerly *AML1*) on chromosome 21q22 to the *RUNX1T1* (formerly *ETO*) on 8q22 resulting in a *RUNX1-RUNX1T1* hybrid transcript. Under the World Health Organization (WHO) classification, the t(8;21) is distinctly characterized under AML with recurrent genetic abnormalities associated with a favorable prognosis. Variant t(8;21;var) display similar clinical manifestations compared to the classical translocation; however, they are less defined and their clinical significance remains debatable. Complex t(8;21) variants account for approximately 3-4% of all *RUNX1-RUNX1T1* fusion transcripts with approximately 100 variants described in the literature. Here, we report a 45 year-old male patient whose bone marrow revealed an abnormal myeloid blast population with morphologic and immunophentotypic features suggestive of AML with t(8;21)(q22;q22). There were no markers suggestive of a more complex cytogenetic abnormality. However, conventional cytogenetic analysis revealed an apparently balanced translocation between the short arm of chromosome 1 at 1p36 and the long arm of chromosome 8 at 8q22. Fluorescence in situ hybridization (FISH) analyses using the AML1/ETO probe showed a cryptic variant (1;21;8) translocation with a single fusion *RUNX1/RUNX1T1* on the derivative chromosome 8, a small *RUNX1T1* signal on chromosome 1, a *RUNX1T1* signal on the normal copy of chromosome 8, a *RUNX1* signal on the normal copy of chromosome 21 and a small *RUNX1* signal on the derivative chromosome 21. Additional FISH studies utilizing the 1p36 (LSI p58) probe showed that a portion of chromosome 1 at 1p36 was translocated to 21q22. This report demonstrated the existence of a three-way translocation which appeared cryptic by classical cytogenetics as well as the importance of performing FISH studies in conjunction with immunophenotypic, morphologic and clinical information to characterize a specific variant rearrangement for good patient management.

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## 1306/W/Poster Board #964

**Xq Duplication in three member of a family.** *V. Catala[1], C. Garrido[1], E. Gean[2], J. Amstrong[2], E. Cuatrecasas[1], A. Seres-Santamaria[1].* 1) Prenatal Genetics SL, Barcelona, Catalonia, SPAIN; 2) Unitat de Genètica. Hospital Sant Joan de Déu, Barcelona, Catalonia, SPAIN.

Duplication of any region of the X chromosome in 46,XY males, with the exception of pseudoautosomic regions, leads to a disomy of functional genes within the duplicated region. In women, this anomaly may go unperceived because the phenotype is usually normal. The duplication Xq prevalence is unknown. We report a 21 years old patient. Clinical features: severe mental retardation, epilepsy, absence of language and loss of ambulation. His older brother died few years earlier without diagnosis. There was no chromosomal abnormality in the cytogenetic study of the patient. The study of subtelomeric regions using FISH techniques showed the existence of a Xqter duplication and a Xpter deletion (an extra Xqter signal was observed in the Xpter region, indeed an X chromosome with 2 Xqter signals)). The abnormal X chromosome has been inherited from the mother. The mother has a normal X chromosome, and the second X chromosome has a Xqter duplication and Xpter deletion (one Xqter signal in each chromosome end) (demonstrated by FISH ). The patient study using MLPA technique (SALSA MLPA KIT P015-D2 MECP2 and CDKL5 KIT P189) shows overlap in the region Xq28 and Xp22 deletion in the patient. The DNA study of the dead brother and the mother could be made both checking the duplication Xq28 and Xp22 deletion in both cases. In our patient, the application of MLPA and FISH techniques has been decisive to the diagnosis. This has led to genetic counseling and to give the family the possibility of a reliable prenatal or preimplantation diagnosis, in the case of a new pregnancy.

## 1307/W/Poster Board #965

**Prenatal diagnosis of a de novo mosaic isochromosome 18p : karyotype discordance between amniocytes and fetal/neonatal blood.** *M. Kim[1], C. Park[1], S. Park[2], M. Kim[1], B. Lee[2], M. Lee[2], D. Kim[2], H. Ryu[1].* 1) Department of Obstetrics and Gynecology, Cheil General Hospital & Women's Healthcare Center, Kwandong University, College of Medicine, Seoul, Korea; 2) Laboratoy of Medical Genetics,Cheil General Hospital & Women's Healthcare Center, Kwandong University, College of Medicine, Seoul, Korea.

Isochromosome 18p is a rare chromosome abnormality and results in tetrasomy 18p. The tetrasomy 18p syndrome most often expresses itself with moderate to severe mental impairment, very delayed speech, poor ability to self feed, much delayed ability to walk, microcephaly, congenital heart diseases. We report a case of a newborn infant prenatally diagnosed de novo mosaic isochromosome 18p. A 38 year old G4P2 pregnant woman presented at 20 weeks and 4 days due to advanced maternal age. The fetal ultrasound showed an absent cavum septum pellucidum without associated anomaly. The quantitative fluorescent PCR on uncultured amniocytes showed unusual skewed allele ratio at a single locus for short arm of chromosome 18. The conventional cytogenetic study of cultured amniocytes showed a male karyotype with an additional metacentric chromosome in 29/83 colonies . The additional metacentric chromosome was confirmed as isochromosome 18p by FISH probes specific for centromere and short arm of chromosome 18. The parental karyotypes were normal. Karyotpe of lymphocytes of fetal blood at 23 weeks and 6 days was normal 46,XY. After genetic counseling, the couple opted to continue the pregnancy. A living male baby was delivered by Cesarean section at 39 weeks, with a birth weight 2,670 gm. There was no demonstrable external and internal abnormality. The echocardiogram and abdominal ultrasonogram of newborn were normal findings. The newborn had normal reflexes and no feeding disability. The neonatal brain ultrasound confirmed the absent cavum septum but there was no any other abnormality in brain. Conventional cytogenetic study of cord blood at delivery revealed a normal karyotype. The newborn was healthy until 4 weeks after birth. However, long-term evaluation of growth and development is required.

## 1308/W/Poster Board #966

**Recurrent Aneuploidies associated with maternal and paternal non-disjunction in the same sibship.** *L. Martelli[1], C.S. Pereira[1], S.A. Santos[1], F.B. Machado[1,2], L.M. Batista[1], L.A.F. Laureano[1], J. Huber[1], L.C. Veiga-Castelli[1].* 1) Dept Genetics-FMRP, Univ Sao Paulo, Ribeirao Preto, SP, Brazil; 2) UENF, RJ, Brazil.

Chromosome aneuploidy is the major cause of pregnancy wastage. Chromosome segregation errors may arise by non-disjunction during premeiotic mitotic division in the germline of either parent, a first or second meiotic division in either parent, premature separation of sister chromatids or an early embryonic mitotic division. Increasing maternal age remains the only clear risk factor associated with aneuploidy, and has been used predictively to assess risk of meiotic non-disjunction. Genetic variations in centromeric factors, proteins involved in chromatin structure, reduced recombination in meiosis, have also been suggested to affect the chromosome segregation. Down and Turner syndromes are the most common aneuploidies among liveborns and both can be a result of maternal and paternal non-disjunction. We described a young healthy unrelated couple with three children, all of them with chromosome aneuploidies. There was no family history of congenital anomalies, mental retardation or miscarriages. Maternal and paternal ages were respectively 17 and 18 years old at the first gestation. The first child was a 12 year-old boy presenting clinical features compatible with Down syndrome and karyotype 47,XY,+21. The other two children were 7 and 8 year-old girls respectively, both diagnosed as Turner Syndrome with 45,X karyotype. GTG banding analysis revealed normal karyotypes for both parents and somatic mosaicism was excluded. The parental origin of non-disjunctional error was determinated using specific DNA polymorphisms of chromosomes 21 and X. The loci studied were the microsatellites at loci D21S226, IFNAR and D21S1411. The meiotic stage of 21 non-disjunction was assigned on the basis of the pericentromeric marker D21S11. The markers DXS996, DXS1283E, DXS981, DXS1187, HPRT and P39 were used for identification of the parental origin of the X chromosome. Interestingly, the trisomy 21 was found to have arisen by maternal non-disjunction in meiosis II, while the missing X chromosome in the two females with Turner syndrome were found to be paternal. In this family, the observed recurrent paternal origin is not in keeping with the idea that spermatogenesis is more effective in selecting against unbalanced gametes than oogenesis. Identification of parental origin in families with multiple cases of non-disjunction is highly informative for accurate genetic counseling, and provides insight into the causes of recurrent aneuploidy in humans.

## 1309/W/Poster Board #967

**A new case of Double Aneuploidy Mosaicism: 47,XX,+8 / 45,X.** *K. Enomoto[1,2], K. Iju[1], K. Kurosawa[2], M. Ohta[1].* 1) Department of Pediatrics, Toride Kyodo General Hospital, Toride, Japan; 2) Division of Medical Genetics, Kanagawa Children's Medical Center, Yokohama, Japan.

Double aneuploidy mosaicism is a rare chromosomal disorder, characterized by the presence of two different aneuploidy cell lines. The mosaicism of 45,X in combination with trisomy of chromosomes 7, 8, 10, 13, 18, or 21 has been previously reported. We report on an additional case of a Japanese girl with double aneuploidy mosaicism, consisting of a cell line with 45,X and a cell line with 47,XX,+8 without a normal karyotype line. She is a 4-year-old girl seen for the first time at Toride Kyodo General Hospital at age 3 months. She was born at term by normal spontaneous vaginal delivery to a 26-year-old mother who denied consanguinity, use of alcohol, tobacco, or drugs. Birth weight was 2526g, head circumference (OFC) was 32.0 cm, and length was 48.0 cm. She developed epilepsy, which was well controled with treatment by Phenobarbital. Cardiac defect with mitral stenosis was detected by echocardiography. Her development was mildly delayed. Frontal bossing, deep-set eyes, everted lower lip, prominent ears with thick helices, long and slender trunk, and deep creases on soles were suggestive of trisomy 8 mosaicism. She also had a short neck with slight webbing and cubitus valgus, consistent with the Turner phenotype of 45,X. She had been affected with Salmonella sepsis / enterocolitis and recurrent otitis media (acute / with effusion). Cytogenic analysis on her peripheral blood showed 47,XX,+8 [24] / 45,X [6]. Confirmatory FISH studies of lymphocytes revealed 351/500 (70.2%) disomy X with trisomy 8 cells and 149/500 (29.8%) monosomy X with disomy 8 cells. Subsequent analysis on cultured fibroblasts by GTG showed 47,XX,+8 [16] / 45,X [34], and FISH studies on buccal cells showed 26/100 disomy X with trisomy 8 cells and 74/100 monosomy X with disomy 8 cells, which is contrary to the ratio in the peripheral lymphocytes. The parental origins of the alleles in chromosome X and 8 are not yet determined. Several mechanisms have been proposed to explain the double aneuploidy mosaicism involving monosomy X and trisomy 8. Most of these reported cases have biparental alleles for the chromosome X, representing the loss of a chromosome X during an early mitotic division. Two separately arising nondisjunction are assumed to occur in prezygotic and/or postzygotic division. Further analysis of parental origin of aneuploidy cells provides insight to consideration of this rare mosaicism.

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### 1310/W/Poster Board #968

**Analysis of chromosome instability in parents of fetus with aneuploidies using cytokinesis block micronucleus test.** *J. Garcia-Sagredo, A. de Leon, M. Arias, K. Yanowsky, C. Villalon, M. Talavera, E. Garcia-Galloway, M.T. Ferro.* Dept Medical Genetics, University Hospital Ramon y Cajal, Madrid, Spain.

Objective: To analyze if parents with aneuploidic fetus diagnosed in the prenatal diagnosis clinic, have an increment in the rate of chromosomal instability. Methodology: Cytokinesis block micronucleus test (CBMN) to score micronuclei (MN) rate in 1,000 binucleated cells (BNC) and distribution of MN among FISH positive, containing whole chromosome, and FISH negative, containing chromosome fragments, using a pancentromeric probe. Material: Couples recruited in the prenatal diagnosis clinic after a diagnosis of aneuploidy in their fetus. Control couples recruited in the prenatal diagnosis clinic having a fetus with normal karyotype. Taking that a high amount of first trimester abortion are due to trisomies, we also analyzed normal karyotype couples with two or more abortions. Results: Our results did not show any significant differences among parameters analyzed with CBMN test in the three groups analyzed: MN rate $6.89 \pm 2.5$ in parents of aneuploidy, $7.85 \pm 2.6$ in couples with abortions, and $6.44 \pm 2.6$ in controls. FISH distribution using a pancentromeric probe shows slight but non-significant increase of MN FISH+ in couples with an aneuploidic fetus: 53.9% MN FISH+ in parents of aneuploidy; 53.3% MN FISH+ in abortion couples, and 47% MN FISH+ in controls. There are also no differences in buds and chromatin bridges rate in BNC: 0.53 buds and 0.35 bridges per 1,000 BNC in parents of aneuploidy; 0.56 buds and 0.32 bridges per 1,000 BNC in abortion couples, and 0.37 buds and 0.44 bridges per 1,000 BNC in controls.

### 1311/W/Poster Board #969

**LOW LEVEL CHROMOSOME MOSAICISM DETECTED BY SNP/COPY NUMBER MICROARRAY.** *I.K. GADI[1], V.L. JASWANEY[1], S. SCHWARTZ[1], R. BURNSIDE[1], H. RISHEG[1], K. CASAS[2], B. WILLIFORD[1], R. PASION[1], S. GRIFFIN[1], J.H. TEPPERBERG[1].* 1) CYTOGENETICS, LABCORP, RESEARCH TRIANGLE PARK, NC; 2) MEDICAL AND BIOCHEMICAL GENETICS, TRINITY MOTHER FRANCES HOSPITALS AND CLINICS, TYLER, TX.

Structural and numerical chromosome anomalies are considered to be the most frequent cause of unexplained non-syndromic developmental delay and mental retardation. The phenotype of low level mosaicism for chromosomes other than chromosome 13, 18, and the sex chromosomes is well documented, however involvement of other chromosomes has not been clearly established. Classical G-band analysis is limited in detecting low level mosaicism in general and although fluorescence in situ hybridization (FISH) has the sensitivity to detect and resolve low level mosaicism, a specific target chromosome is needed. Recent advances in whole genome microarrays have improved the detection of mosaicism. The Affymetrix 1.8 million SNP/copy number array has shown tremendous power in detecting low level mosaicism of various chromosomes due to copy number change and also in revealing uniparental disomy or consanguinity due to loss of heterozygosity (LOH). We report two patients with low level chromosome mosaicism detected through this SNP array analysis. Patient 1 was referred for SNP microarray at 1.4 year of age because of dysmorphia, growth delay, hypospadia, small kidney, malformed ears and language delay. Previous history included IUGR, gestational diabetes, normal karyotype and array CGH results. The SNP array revealed a low level (10-15%) increased SNP/CN dosage across the whole chromosome 22. A chromosome 22 specific FISH probe (TUPLE1) confirmed 4% mosaicism in lymphocytes. Patient 2 was sent for array analysis due to short stature, limb length asymmetry, and congenital anomalies of abdominal wall, speech and language disorder and swirling hyper pigmentation of legs and arms. The SNP array revealed a low level (~10-20%) increased SNP/CN dosage across the whole chromosome 14. FISH using specific probe for 14q32 (IgH gene) on PHA stimulated cultures from this patient showed 9.5% of cells with three signals for chromosome 14. No other gain or loss of DNA was apparent in the genome wide SNP analysis of these patients. UPD for chromosome 14 and 22 was ruled out since there was no apparent loss of heterozygosity in the SNP analysis for these chromosomes. UPD is of great clinical significance when there is a rescue from trisomy to disomy involving an imprinted chromosome. These reports demonstrate that low levels of mosaicism below levels seen using routine cytogenetic analysis can be detected by SNP microarrays.

### 1312/W/Poster Board #970

**Prenatal diagnosis of a de novo trisomy 12p by array-CGH in a fetus with normal karyotype.** *CH. Lin[1], SY. Lin[2], M. Gorre[3], J. Kim[3], YN. Su[2].* 1) Department of Obstetrics and , National Taiwan University Hospital, Taipei, Taiwan; 2) Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan; 3) CMDX, Irvine, USA.

Objective: Trisomy 12p syndrome is a rare chromosome anomaly with an estimated incidence of 1/50,000. Most cases are inheritance from parental balance translocation. Here we present the prenatal sonographic findings, postnatal features, and molecular cytogenetic analysis of a new de novo case. Case report: The 29 y/o G1P0 healthy woman was pregnant at 30th gestational age. Prenatal sonography showed polyhydramnios, fetal facial dysmorphism: hypertelorism, marked prenasal thickness, broad and flat nasal bridge, cleft palate, large philtrum with thickened everted upper lip, and micrognathia, short lone bones and abnormal spine curvature. She received tocolysis for frequent uterine contraction. Serial amnioreduction with 1500ml, 750ml, and 880 ml were done every 3 days. Cord blood sampling was also performed for karyotyping and BAC array-CGH study. The G-banding chromosome report was 46, XY with no obvious anomaly. aCGH result showed arr cgh 12p13.33p11.1(RP11-28313→RP11-313F23)X3. She terminated this pregnancy at 32nd gestational week for IUFD. Postmortem pictures were similar with ultrasound findings and compatible with previous trisomy 12p syndrome report. Conclusion: Trisomy 12p was characterized by profound psychomotor and mental retardation, generalized hypotonia, seizure, and facial dysmorphism. Traditional karyotyping may be suggested when facial dysmorphism was noted by detail prenatal 2D and 3D ultrasound screening. If there is no abnormal finding of chromosome, array-CGH is an additional sensitive and effective diagnosed choice for the rare syndrome.

### 1313/W/Poster Board #971

**CHROMOSOME 10P DUPLICATION ASSOCIATED WITH CENTRAL NERVOUS SYSTEM DEFECTS AND CRANIAL ANOMALIES. A CASE REPORT AT THE HOSPITAL PARA EL NIÑO POBLANO, MÉXICO.** *J. Aparicio[1,6], H.M.L. Hurtado[2], J.B. Vazquez[3], P.S. Rodriguez[4], P.M. Barrientos[5], O.H. Chavez[6], J.G. Vega[6], M.S. Chatelain[7].* 1) Dept Genetica; 2) Cytogenetics; 3) Neurology; 4) Neurosurgery; 5) Endocrinology, Hosp para el Nino Poblano, Puebla,; 6) Estomatology, Benemérita Universidad Autónoma de Puebla; 7) Biotecnology, Universidad Autónoma Metropolitana, México.

Introduction. Distal 10q duplication is a rare chromosomal disorder that causes slow postnatal growth and severe mental retardation. Humans, like all sexually reproducing species, have somatic cells that are in diploid [2N] state, there are 23 chromosomes that are arranged according to their size, function and genes they carry, but sometimes due to mutations or malfunctions during cell division, mistakes are made that cause serious health problems. One such error is the cause of duplication that causes a trisomy 10q disorder. Among the clinical manifestations in patients with chromosome 10p duplication síndromes are defects including central nervous system disorders (SNC) as hypotonia, poor feeding, and mental retardation, cranial anomalies (CA) with small head , long head , flat back of skull, microcephaly, dolichocephaly, retrognathia, wide sutures, frontal bossing and nose malformations, skeletal deformities like clinodactyly, camptodactyly, and clubfoot, urogenital anomalies, and cardiac defects. Case report. A seven months aged patient was studied by genetics, neurology and neurosurgery. Magnetic resonance imaging (MRI), three dimensional (3D) images of scalp and skull and cytogenetic studies were peformed. Conclusions. Distal 10q duplication disorder was diagnosed by citogenetics, where distal portion of the q (long) arm of the chromosome number 10 appears to be present three times, rather than two times as it is supposed to be. Chromosome 10, distal trisomy 10q is an extremely rare chromosomal disorder in which part of the end (distal) portion of the long arm (q) of one chromosome 10 is duplicated. The severity can vary from case to case. However clinical manifestations of SNC and CA were observed in this study. Often the source of this chromosomal error is a translocation in one of the parents, in over 90 percent of reported cases. Sometimes it occurs spontaneously, in which case it is termed de novo .

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## 1314/W/Poster Board #972

**Comprehensive genetic analyses of PLP1 in patients with Pelizaeus-Merzbacher disease applied by array-CGH and fiber-FISH analyses identified new mutations and variable sizes of duplications.** *K. Shimojima[1], T. Inoue[2], A. Hoshino[3,4], S. Kakiuchi[5], Y. Watanabe[6], M. Sasaki[7], A. Nishimura[7], A. Takeshita-Yanagisawa[8], G. Tajima[9], H. Ozawa[10], M. Kubota[11], J. Tohyama[12], M. Sasaki[13], A. Oka[14,15], K. Saito[16].* 1) International Research and Edu, Tokyo Women's Medical University, Shinjuku-ward, Tokyo, Japan; 2) Division of Child Neurology, Institute of Neurological Sciences, Faculty of Medicine, Tottori University, Yonago, Japan; 3) Department of Pediatrics, Tokyo Metropolitan Hachioji Children's Hospital, Hachioji, Japan; 4) Departments of Neuropediatrics, Tokyo Metropolitan Neurological Hospital, Fuchu, Japan; 5) Department of Neonatology, Tokyo Metropolitan Bokutoh Hospital, Tokyo, Japan; 6) Department of Child Neurology, Okayama University Graduate School of Medicine, Okayama, Japan; 7) Department of Pediatrics, Kyoto Prefectural University of Medicine, Kyoto, Japan; 8) Department of Pediatrics, Faculty of Medicine, Tokyo Women's medical University, Tokyo, Japan; 9) Department of Pediatrics, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan; 10) Shimada Ryoiku Center, Tama, Japan; 11) Division of Child Neurology, National Center of Child Health and Development, Tokyo, Japan; 12) Epilepsy Center, National Nishi-Niigata Chuo National Hospital, Niigata, Japan; 13) Department of Child Neurology, National Center Hospital for Mental, Nervous and Muscular Disorders, National Center of Neurology and Psychiatry, Kodaira, Japan; 14) Department of Pediatrics, Faculty of Medicine, The University of Tokyo, Tokyo, Japan; 15) Department of Pediatrics, Faculty of Medicine, Kyorin University, Tokyo, Japan; 16) Institute of Medical Genetics, Tokyo Women's medical University, Tokyo, Japan.

Pelizaeus-Merzbacher disease (PMD; MIM#312080) is a rare X-linked recessive neurodegenerative disorder. The main cause of PMD is alterations in the proteolipid protein 1 gene (PLP1) on chromosome Xq22.2. Duplications and point mutations of PLP1 have been found in 70 % and 10-25 % of all patients with PMD, respectively, with a wide clinical spectrum. Since the underlining genomic abnormalities are heterogeneous in patients with PMD, clarification of the genotype-phenotype correlation is the object of this study. Comprehensive genetic analyses using microarray-based comparative genomic hybridization (aCGH) analysis and genomic sequencing were applied to fifteen unrelated male patients with a clinical diagnosis of PMD. Duplicated regions were further analyzed by fiber-fluorescence in situ hybridization (FISH) analysis. Four novel and one known nucleotide alterations were identified in five patients. Five microduplications including PLP1 were identified by aCGH analysis with the sizes ranging from 374-kb to 951-kb. The directions of five PLP1 duplications were further investigated by fiber-FISH analysis, and all showed tandem duplications. The common manifestations of the disease in patients with PLP1 mutations or duplications in this study were nystagmus in early infancy, dysmyelination revealed by magnetic resonance imaging (MRI), and auditory brain response abnormalities. Although the grades of dysmyelination estimated by MRI findings were well correlated to the clinical phenotypes of the patients, there is no correlation between the size of the duplications and the phenotypic severity.

## 1315/W/Poster Board #973

**Characterization of the complex 7q21.3 rearrangement in a patient with bilateral split-foot malformation and hearing loss.** *H. Saitsu[1], K. Kurosawa[2], H. Kawara[3], M. Eguchi[3], T. Mizuguchi[1], N. Harada[3], T. Kaname[4], H. Kano[5], N. Miyake[1], T. Toda[5], N. Matsumoto[1].* 1) Dept Human Gen, Grad Sch Med, Yokohama City Univ, Yokohama, Japan; 2) Div of Med Genet, Kanagawa Children's Medical Center, Yokohama, Japan; 3) Dep Molecular Cytogenetics, Kyushu Medical Science, Inc., Nagasaki, Japan; 4) Faculty of Medicine, Dep Med Genet, Univ of the Ryukyu, Okinawa, Japan; 5) Div of Clin Genet, Dep of Med Genet, Osaka Univ Grad Sch of Med, Suita, Japan.

We report on complex rearrangements of the 7q21.3 region in a female patient with bilateral split-foot malformation and hearing loss. G-banding karyotype was 46,XX,t(7;15)(q21;q15),t(9;14)(q21;q11.2)dn. By fluorescence, in situ hybridization (FISH), Southern hybridization, and inverse PCR, the 7q21.3 translocation breakpoint was determined at the nucleotide level. The breakpoint did not disrupt any genes, but was mapped to 38-kb telomeric to the *DSS1* gene, and 258- and 272-kb centromeric to the *DLX6* and *DLX5* genes, respectively. It remains possible that the translocation would disrupt the interaction between these genes and their regulatory elements. Interestingly, microarray analysis also revealed an interstitial deletion close to (but not continuous to) the 7q21.3 breakpoint, indicating complex rearrangements within the split-hand/foot malformation 1 (*SHFM1*) locus in this patient. Furthermore, a 4.6-Mb deletion at 15q21.1-q21.2 adjacent to the 15q15 breakpoint was also identified. Cloning of the deletion junction at 7q21.3 revealed that the 0.8-Mb deletion was located 750-kb telomeric to the translocation breakpoint, encompassing *TAC1*, *ASNS*, *OCM*, and a part of *LMTK2*. Because *TAC1*, *ASNS*, and *OCM* genes were located on the reported copy number variation regions, it was less likely that the three genes were related to the split-foot malformation. *LMTK2* appeared to be a potential candidate gene for SHFM1, but no *LMTK2* mutations were found in 29 individuals with SHFM. Further *LMTK2* analysis of SHFM patients together with hearing loss is warranted.

## 1316/W/Poster Board #974

**Case Report: A new born with plagiocephaly, supernumerary rib, cardiopathy and a 5q11.1q12 duplication.** *I. Briceno[1,2], G. Gordillo[2], I. Zarante[2].* 1) Bioscience, U Niversidad de La Sabana, Chia, Colombia; 2) Instituto Genética Humana, Pontificia Universidad Javeriana, Bogota, Colombia.

The 5q duplications have been well described. The phenotype of trisomy 5q31& 5qter it's well know: severe psychomotor retardation, low birth weight, microchepaly, high forehead, hypertelorism, downward slanting palpebral fissure, epicanthal folds, strabismus, large outstanding pinnae, prominent nasal bridge, long philtrum, large upper lip and micrognathia; there's also brachydactyly, preaxial polydactyly and hernias. Other well know phenotype is the one that belongs to the trisomy 5q13 q22 in with high forehead, bulbuous nose, short philtrum, large, protruding pinnae and micrognathia. Also, Trisomy 5 is found in both acute lymphoblastic leukaemia and acute myeloid leukaemia, but very rarely as the sole karyotypic abnormality. We report the case of a 2-year-old boy product of a second controlled pregnancy, without perinatal history of importance; Coarctation of aorta and persistent Ductus Arteriosus are diagnosed at 4 days of life; Estenosis of the Mitral Valve is diagnosed at 3 months of age. Later Supernumerary Rib is found in thorax X-ray and plagiocephaly in TAC of cranium. The family comes to the service for genetic evaluation because of multiple malformations. To the physical examination the weight and the height were normal for the age, but the cephalic perimeter, the external intercantical distance and the interpupilar distance were higher than the percentile 97. As positive findings he had right-posterior plagiocephaly, low nasal bridge, narinas antervertidas, flat facial profile, long filtrum, rotated ears with folded helix; right prominent thorax and systolic murmur II/VI. Genitalia with scrotal hypoplasia with right inguinal testicle and left cryptorchidism. There wasn't deficit of the nervous central system. Inside the arranged laboratories the band G karyotype reports 46, XY, dup (5) (q11.1-q12) in all studied cells. The trisomy of chromosome 5q also called duplication of 5q, does not present typical genotype - phenotype correlation, but every patient described up to the date presents a variety of personal clinical signs. The Craniosynostosis has described related to the gene MSX2, located in the locus 5q53, which is not involved in the rearrangement of our patient; this opens new questions about the genes located in this region and possibly epigenetic factors related to their expression. This one is the first case of duplication q11.1-q12 described up to the date as we checked in the literature.

## 1317/W/Poster Board #975

**Molecular Characterization of a Complex Chromosome 9p Rearrangement in an Infant with Multiple Congenital Malformations with SNP Oligoneuclotide Microarray Analysis (SOMA).** *K. Anyane-Yeboa[1,2], B. Levy[1], V. Jobanputra[1], C. Paley[2], A. Revah[2], A. Woods[1,2].* 1) Dept Pediatrics, Columbia University Medical Center,NY; 2) Depts of Pediatrics and Obstetrics, ST. Luke's-Roosevelt hospital, NY.

Microarray analysis is now the preferred method for clarification of chromosome deletions and rearrangements in situations where routine cytogenetic studies are inconclusive. We report on and infant with multiple congenital malformations and a complex chromosome 9p translocation that was resolved using SOMA. He was the 2090g SGA product of a 39-week gestation to a 32-year-old mother. He was noted at birth to have multiple anomalies including relatively large head, bulbous nose, mild micrognathia, severe peno-scrotal hypospadias, cryptorchidism, short 5th fingers, clinodactyly, and bilateral simian creases. His muscle tone was mildly increased.Follow up examination at 18 months revealed failure to thrive, relatively large but normocephalic head, broad forehead, mild hypertelorism, large floppy ears, micrognathia, and hypotonia. His hypospadias and cryptorchidism had been surgically repaired.His motor and speech development were severely delayed. He was just beginning to crawl, and his only vocabulary was"-mama". Chromosome studies revealed a dicentric chromosome 9 with a complex unbalanced translocation of 9p and an acrocentric chromosome. Additional chromosome material translocated to 9p was NOR positive, and C-band positive, but no additional details of 9p could be gleaned from cytogenetic studies. SOMA revealed a 5.67 Mb deletion of 9p24.3-p24.1 and a 31.43Mb duplication of 9p24.1-p13.2. The contribution of the acrocentric chromosome is therefore confined to the centromere and short arm while the additional euchromatic material on 9p derived from an interstitial duplication of distal 9p. There are 6 OMIM disease genes in the deleted segment and 17 in the duplicated segment. We believe that haploinsufficiency for two genes in the deleted segment: DMRT1 and DOCK8, are responsible for the phenotypic features in this infant. High DMRT1 expression is necessary for testicular diffrentiation and low levels to ovarian differentiation. Haploinsufficiency may cause sex reversal or incomplete male genital differentiation as in this infant. Haploinsufficiency or dominant mutations in DOCK8 have recently been shown to cause severe mental retardation. Other genes in this region are recessive genes that would not contribute to his phenotype. There are a large number of genes in the duplicated region, 17 of them are OMIM disease genes that are expressed as dominant or recessive traits. Effects of over expression of these genes are not known.

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## 1318/W/Poster Board #976

**MECP2 duplications in five patients with complex sex chromosome rearrangements.** *A.M. Breman[1], M. Ramocki[2], S.H. Kang[1], M. Williams[3], D. Freedenberg[4], A. Patel[1], P.I. Bader[5], S.W. Cheung[1].* 1) Medical Genetics Laboratories, Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Section of Pediatric Neurology and Developmental Neuroscience, Baylor College of Medicine and Texas Children's Hospital, Houston TX; 3) Vanderbilt University School of Medicine, Division of Medical Genetics, Nashville, TN; 4) Department of State Health Services, Austin, TX; 5) Northeast Indiana Genetic Counseling Center, Fort Wayne, IN.

Duplications of the Xq28 chromosome region resulting in functional disomy are associated with a distinct clinical phenotype including dysmorphic facial features, severe developmental delay and hypotonia. Increased expression of the dosage-sensitive MECP2 gene, located within this region of the X chromosome, is considered responsible for the severe neurological impairment in affected individuals. While cytogenetically visible duplications of Xq28 have been well documented in the published literature, recent advances using aCGH analysis have revealed an increasing number of cases of microduplication involving MECP2. In rare cases, the duplication results from an intrachromosomal rearrangement or translocation between the X and Y chromosomes. We report 5 cases with complex X-X or X-Y rearrangements involving duplication of MECP2. Cases 1-3 are unbalanced rearrangements involving MECP2 on chromosome Xq28 and chromosome Y. In case 1, aCGH detected a gain in copy number of at least 0.38 Mb at Xq28. FISH analysis revealed an insertional translocation of Xq28 material into the terminal region of chromosome Yp. In case 2, a loss in copy number was detected in the pseudoautosomal region of chromosomes Xp/Yp, spanning at least 0.6 Mb, as well as a gain in copy number of at least 1.5 Mb at Xq28. FISH analysis revealed a de novo unbalanced translocation between the distal segments of chromosomes Xq and Yp. In case 3, aCGH indicated a duplication of ~ 2.1 Mb on Xq28. FISH analysis showed the additional Xq28 translocated to Ypter, but in this case, with no detectable loss of Ypter material. Interestingly, the FLNA gene is also duplicated in cases 2 and 3, but not case 1. Duplications of FLNA have been associated with a more severe phenotype, consistent with the clinical features of these patients. Finally, two cases (4 and 5) were identified by aCGH to have a loss in copy number at Xp22.32, spanning at least 5 Mb, as well as a gain in copy number at Xq28 spanning at least 10 Mb involving the MECP2 and FLNA genes. In both cases, FISH analysis revealed a recombinant X chromosome deleted at Xp22.32 and containing the duplicated material from Xq28 on Xp, resulting from a maternal pericentric inversion. The cases described here add to a growing number of submicroscopic Xq duplications involving MECP2 that have been detected by aCGH. These data demonstrate the utility of aCGH analysis for the accurate delineation of breakpoints in patients with complex rearrangements.

## 1319/W/Poster Board #977

**Chromosome abnormalities in spontaneous abortuses.** *J. Choi[1], M. Woo[1], N. Song[1], H. Ko[1], S. Oh[1,2], J. Park[2], J. Jun[2], Y. Choi[1,2], S. Moon[1,2].* 1) Institute of Reproductive Medicine and Population; 2) Department of Obstetrics and Gynecology, College of Medicine, Seoul National University, Seoul, Korea.

Chromosomal aberrations usually lead to spontaneous abortions which occur in 10-15% of pregnancies. The majority of clinically recognized spontaneous abortions occur during the first trimester, and abnormal chromosome is identified in 50-80% of these early miscarriages. This study reviewed chromosomal analysis of 47 abortues referred at the Institute of Reproductive Medicine and Population, Seoul National University. The spontaneous aborted specimens were performed using conventional tissue culturing and karyotype analysis. Among 47 specimens, chromosome abnormality was detected in 13 cases (27.7%). Five cases (14.9%) were consistent with numerical abnormalities for autosome, including two cases of trisomy 21 and one case of trisomy 18, trisomy 5, and triploidy (69,XXY), respectively. Two cases (4.3%) showed X monosomy. Four cases (8.5%) were mosaicsim. Unbalanced structural rearrangements were detected in two cases (4.3%) which derived due to the unbalanced chromosome segregation from female carriers with balanced reciprocal translocations. These data showed that cytogenetic analyses of reproductive failure are necessary for an approach to determining the causes of spontaneous abortion, and should be enable to provide valuable information for genetic counseling and prenatal diagnosis.

## 1320/W/Poster Board #978

**10, 11 a novel translocation in an Indian azoospermic man.** *R. Dada[1], S. Venkatesh[1], R. Goswami[2], AC. Ammini[2], N. Tandon[2].* 1) Dept Anatomy, All India Inst Medical Sci, New Delhi, India; 2) Department of Endocrinology, AIIMS, New Delhi.

Aim: Reporting a case of novel 10, 11 translocation in an infertile man with azoospermia. Methods: Semen analysis was performed according to WHO criteria (1999). GTG banding karyotyping was done in 72 hours blood culture. Hormone estimation was done by radio-immuno assay. Results: Repeated semen analysis showed azoospermia even after centrifugation. Semen volume was found to be 1 ml and the liquefaction was incomplete even after 60 min. The pedigree analysis showed that his elder brother was also found to be infertile and unavailable for the study. Patient had late virilzation and appeared to be partial. Hormone analysis showed FSH-17.47 mIU/ml, LH-22.28 mIU/ml and testosterone -1305.86 ng/dl. Peripheral karyotype analysis revealed 46, XY t (10; 11) (q11.2; p15) chromosome complements Conclusions: To the best of our knowledge this translocation has not been previously reported in an infertile man. However, such translocation involving chromosomes 10 and 11 have been frequently observed in acute myeloid leukemia (AML) (46,XY,t (10;11)(p12;q231[9]/46,XY[31, 46, XY, t(10;11)(p13;q14), 46,XY, t(10;11)(p13;q15). However, this patient showed no symptoms of such disorder as the translocation break points are different from those reported in AML. Nil sperms in the semen and elevated testosterone levels showed completely blocked germ cell division might have resulted from impaired quadrivalent synaptic complex due to novel translocation. Also, genes that were disturbed during this translocation may also responsible for impaired fertility, which cannot be ruled out.

## 1321/W/Poster Board #979

**Array based genomic analysis reveals hidden complexity of chromosome aberrations.** *J. Gerfen[1], L.K. Conlin[1], B. Kamath[1], B. Thiel[2], L. Ernst[1], H. Hakonarson[3], N.B. Spinner[1,2].* 1) Dept of Pediatrics, The Children's Hosp Philadelphia, Philadelphia, PA; 2) Dept of Pathology, The Children's Hosp Philadelphia, Philadelphia, PA; 3) Center for Applied Genomics, The Children's Hosp Philadelphia, Philadelphia, PA.

In the course of analysis of over 1,000 patients by genome-wide SNP array, we have identified several examples of complex rearrangements that were unexpected. Here we present data on 3 patients with mosaic deletions and duplications occurring on the same chromosome, that we believe are associated with an inversion. Patient 1 was studied at 1 month of age, because of fetal death, associated with a diagnosis of a hypoplastic left heart. Array analysis revealed a 518 kb mosaic deletion in 25-30% of cells, at chromosome 14q24.2 and a 3.5 Mb mosaic duplication (also at 25-30% of cells) at chromosome 14q32. The fact that the deletion and duplication occur with similar frequency, lead us to hypothesize that they are associated with a paracentric inversion, with the breakpoints marked by the deletion and duplications. To confirm this, we carried out FISH using probes from across the putative inversion, and confirmed both the copy number alterations, and the suspected inversion. Patient 2, was known to have a 900 kb mosaic deletion of 20p12.2, including the JAG1 gene. She had been identified subsequent to the birth of her daughter, who had a non-mosaic deletion of JAG1, and clinical characteristics of Alagille syndrome. Array analysis revealed that both the proband and her mother in fact had both the deletion and a 2.4 Mb duplication within 20p11, with the mother's deletion and duplication being mosaic, again at similar levels (30-35%). Patient 3 also had a 4.8 Mb mosaic deletion of 20p12 (JAG1 deleted, with clinical features of Alagille syndrome) and a 1.8 Mb mosaic triplication of 20q13.2 in 30-35% of cells. In each of these cases, the similar mosaicism levels for the deletion and duplication provided a clue to the structural relationship. Given the large distances between the deletion and duplication in each case (27, 13 and 38 Mb respectively), we hypothesize that there is an inversion between these events. And further suggest that the inversion event has resulted in the copy number changes we observe. In each of the cases we report, the event occurred mitotically, since these events were mosaic. Array analysis allows finer detail in characterization of chromosome alterations and will result in further understanding of the mechanisms of chromosome abnormalities.

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## 1322/W/Poster Board #980

**A recurrent translocation between chromosomes 4 and 18 is mediated by homologous recombination.** *K. Hermetz[1], U. Surti[2], J.D. Cody[3], K. Rudd[1]*. 1) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA; 3) Department of Pediatrics, University of Texas Health Science Center at San Antonio, TX.

Chromosome rearrangements are a significant cause of intellectual disability as ~16% of affected individuals referred for diagnostic array comparative genome hybridization (CGH) carry a pathogenic copy number abnormality. Despite the frequency of structural rearrangements in the human genome, the mechanisms of chromosome breakage and repair are usually unknown. Recurrent chromosome abnormalities provide an opportunity to unravel mechanistic factors, as their frequency is suggestive of rearrangement-prone genomic architecture. Such is the case for the segmental duplications that flank recurrent microdeletion and microduplication loci and the palindromic AT-rich repeats that underlie the breakpoints of the common translocation between chromosomes 11 and 22. Here we report the breakpoint junction and mechanism of a recurrent translocation between chromosome arms 4q and 18q. At least 3 unrelated individuals with the apparently same unbalanced translocation have been described in the literature; FISH and array CGH studies have defined the derivative chromosome as der(18)t(4;18)(q35;q23). Subsequently, we have combined high-resolution array CGH and breakpoint cloning to resolve the breakpoints and determine the mechanism of rearrangement in two unrelated individuals carrying the derivative chromosome 18. We designed a custom oligonucleotide array targeting the 5 megabases (Mb) flanking the original 4q and 18q breakpoints with a mean probe spacing of one oligonucleotide per 100 basepairs. Array CGH revealed the same breakpoints in both individuals with a net loss of 6.96 Mb on 18q and a net gain of 7.30 Mb of 4q. Based on the array CGH data, we designed PCR primers to amplify the breakpoint junction of the derivative chromosome 18. Cloning and sequencing the junction revealed that both of the breakpoints occurred in a cluster of long terminal repeats (LTRs). The LTR clusters on 4q and 18q are ~5 kilobases in size and 95% identical. Such genomic architecture is a signature of rearrangements mediated by homologous recombination. Given the mechanism of translocation, we anticipate that other individuals carry the same imbalance, and future phenotypic studies will better inform patient outcome. Interestingly, the reciprocal unbalanced translocation [der(4)t(4;18)(q35;q23)] has not been reported in the literature, possibly do to lethality of this unbalanced product.

## 1323/W/Poster Board #981

**An SRY-deleted XXY female resulting from a paternally inherited t(Y;22).** *J. Kim[1], S. Kim[2], H. Kim[3], K. Lee[1]*. 1) Laboratory medicine, Kangnam severance hospital, Seoul, Kangnam, Korea; 2) Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; 3) Department of Pediatrics, Kangnam severance hospital, Seoul, Kangnam, Korea.

The Individuals found with 47,XXY appear as males, but are rarely seen in females. Most of these females were reported to show androgen insensitivity due to mutations in androgen receptor genes. To my knowledge, there was only one case report of an SRY negative 47,XXY female resulted from an aberrant X-Y interchange leading to transfer of Yp material to one of the X chromosome. We report a case of 46,XXYp- female with unbalanced Y-22 translocation inherited from the father. A 7 year and 9 months-old girl was referred to the endocrinology department of our hospital because of fast progressing signs of precocious puberty. The girl with a birth weight of 3800 g was delivered by ceasarian section due to delayed time of delivery. The height of the father and mother were 175 cm and 153 cm. At 7 years and 9 months the height of the girl was 127.3 cm (80 percentile) and her weight was 36 kg (>97 percentile). Her body mass index was 22.21 (>95 percentile). The skeletal age was around 94 to 106 months. The ultrasonography of the abdomen and pelvis showed uterus (3.7 x 0.6 cm) and ovaries (right: 1.4 cm, left 1.5 cm) slightly small in size. There were no other abnormalities on further physical examination. The endocrinology lab results were all within the reference interval. In G-banding analysis, an enlarged short arm of one chromosome 22 consisting of heterochromatic component was detected. The additional material attached to chromosome 22p was characterized by metaphase fluorescence in situ hybridization with whole chromosome painting probes as being derived from Y chromosome. The SRY gene was not amplified in the DNA analysis showing the deletion of SRY gene. Thus, the karyotype of the patient was 46,XX,der(Y;22)(q10;q10). Her father showed 46,XY,der(Y;22)(q10;q10), which revealed that the derivative chromosome has arisen from the segregation of the paternal rearrangement. Her mother showed a normal karyotype. Although it is likely that she will be fertile since she has both X chromosome intact, close follow up of the patient is necessary for relatively small sized reproductive organs. Enlargement in acrocentric short arms should be carefully examined to clarify whether it is a normal polymorphism or whether it hides a cryptic rearrangement.

## 1324/W/Poster Board #982

**Prenatal diagnosis of a maternally derived unbalanced der(4)t(4;11) leading to an overlapping phenotype of Wolf-Hirschhorn and Russell-Silver syndromes in two siblings: novel recurrent translocation, aCGH and molecular cytogenetic analyses.** *E. Kolomietz[1], A. Smith[1], K. Chong[2].* 1) Pathology/Lab Med, Mount Sinai Hosp/Univ Toronto, Toronto, ON, Canada; 2) Prenatal Diagnosis Unit, Mount Sinai Hosp/Univ Toronto, Toronto, ON, Canada.

Constitutional reciprocal translocations were thought to arise randomly with the only exception of recurrent rearrangements being the t(11;22). Recent evidence indicates that recurrent breakpoints may be more common than previously thought and mediated by non-B DNA structures, such as cruciforms, caused by palindromic AT-rich repeats. The clinical significance of these rearrangements may be underestimated because certain rearrangements, most notably subtelomeric translocations, can be difficult to detect by conventional G-banding. We report on two siblings of same sex and apparently normal karyotypes both presented prenatally with severe intrauterine growth restriction (IUGR). The mother had an apparently balanced translocation, t(4;11)(p16.3;p15.5), and both sibs had an adjacent -1 segregation variant with loss of terminal 4p and gain of terminal 11p regions which was detected by aCGH and FISH using subtelomeric probes. Each of these rearrangements is associated with well known clinical phenotypes. Paternal duplication of 11p15 is associated with the Beckwith-Wiedemann syndrome while maternal duplication is associated with Russell-Silver syndrome. As expected both fetuses presented with severe IUGR consistent with both Wolf-Hirschhorn and Russel-Silver phenotypes. This familial case of what appears to be a novel recurrent translocation demonstrates the value of aCGH in the diagnosis of submicroscopic chromosome abnormalities in those patients for whom routine chromosome analysis does not adequately explain clinical findings. Breakpoint sequence analysis in our cases is compared to previously published cases and an analysis of the 4p and 11p regions for palindromic repeat sequences may help to elucidate mediators of this recurrent chromosomal aberration.

## 1325/W/Poster Board #983

**Identification of 3q26.3 deletion in a family with blepharophimosis/ptosis/epicanthus inversus syndrome (BPES).** *M. Kumar[1], M. Tanwar[1], B. Chawla[2], R. Dada[1]*. 1) Department of Anatomy; 2) Dr R.P. Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, India.

Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) is an autosomal dominant disease of the eyelids that may severely impair visual function. The frequency of BPES has been estimated to be 1 in 50,000. Patients with BPES have a combination of congenital anomalies as small palpebral fissures, epicanthus inversus, low nasal bridge and congenital ptosis. Other features like microphthalmos, anophthalamos, microcornea, hypermetropia, divergent strabismus, high arched palate, cup shaped ears, mental retardation and infertility in females has been reported. Based on the presence and absence of premature ovarian failure BPES has been categorized into two types: type I with infertility in females and type II involves eye malformation in both males and females. It has been shown that penetrance is 100% in type I when the transmission is by males only and 96.5% in type II in which transmission occurs through both sexes. From reviews it has been concluded that a locus for eyelid development is situated at the interface of long arm of chromosome 3. Since blepharophimosis, ptosis and microphthalmia are consistent features in Patients with BPES an interstitial deletion of band 3q2, the location of BPES gene at this position seems highly likely. Various reports linked the deletion in 3q21, 3q22, 3q23, 3q24, 3q25 and translocations t(3:7)(q26-qter:q+), t(X:3)(p22:q21), t(3:8)(q23:p22.1) to the BPES. Thus cytogenetically different deletions and translocations of chromosome 3 have been described in patients with BPES. We report a familial case of BPES. Pedigree shows autosomal dominant pattern of inheritance. We have done the Cytogenetic analysis of the family. Standard GTG banding showed deletion of band 3q26.3 in two members. This finding may represent a severe manifestation of the disease. BPES is a heterogeneous entity, and evaluation and counseling of affected individuals should be undertaken with caution.

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## 1326/W/Poster Board #984

**Complex chromosomal rearrangements in a girl with Pelizaeus-Merzbacher disease.** *K. Kurosawa[1], M. Tanaka[2], H. Osaka[1], H. Ohashi[2], S. Hamano[2], K. Enomoto[1], A. Ishikawa[1], N. Furuya[1].* 1) Kanagawa Children's Med Ctr, Yokohama, Japan; 2) Saitama Children's medical Ctr, Saitama, Japan.

Pelizaeus-Merzbacher disease (PMD) is a rare congenital dysmyelinating disorder, characterized by nystagmus, psychomotor developmental delay, spasticity, and ataxia. PMD is caused by predominantly submicroscopic duplications of Xq22.2 including the entire PLP1 gene. The duplications vary in size between the families, and are detected as a double signal using PLP1 probe in interphase FISH. Several cases have been reported in which the duplication is found in a cytogenetically distinguishable band on the X chromosome or autosomes. We report here a female patient with PMD in which complex rearrangements involving the seemingly balanced translocation t(X;6) is observed. She was born after uneventful pregnancy at term by normal delivery with a birth weight of 2660 g. At age of 7 months, she visited the neurological clinic of Saitama Children's Medical Center because of horizontal nystagmoid movements present from birth, and delayed motor development. Brain MRI showed diffuse dysmyelination, and ABR study revealed no consistent waveforms after wave II. These results were consistent with those of typical PMD, but inconsistent with the trait of inheritance of PMD. FISH analysis with a BAC clone with PLP1 detected three independent signals in interphase nuclei, of those third signal was observed in the autosome in the metaphase. Cytogenetic analysis by standard GTG revealed balanced translocation t(X;6)(q22.2;p21.2). To determine the ranges of duplication and breakpoints of both chromosomes, we used Affymetrix 250K Mapping array and detected 820 kb duplication of the Xq22.1-q22.1 including the PLP1. In addition, array analysis unexpectedly detected submicroscopic 400 kb deletion in the breakpoint of chromosome 6. Both parents had normal karyotype. A small set of PMD cases is caused by gene duplication arising by a sub-microscopic transposon, jumping gene, inversion, or translocation. Diversity of genomic rearrangements found in these PMD patients suggests the unique architecture of PLP1 region in Xq22.2.

## 1327/W/Poster Board #985

**Characterization of a Large Pericentric Inversion of Chromosome One in a Multi-Generational Pedigree: Risk for Pregnancy Loss, Recombinant Offspring and the Possibility of Meiotic Drive.** *E. McCready[1,2,3], c. Honeywell[1], S. Douglas[1], J. Allanson[1,2,4], J. McGowan-Jordan[1,2,4].* 1) Dept of Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada; 2) Children's Hospital of Eastern Ontario Research Institute, Ottawa, ON, Canada; 3) Department of Pathology and Laboratory Medicine, University of Ottawa, Ottawa, ON, Canada; 4) Department of Pediatrics, University of Ottawa, Ottawa, ON, Canada.

Although generally not associated with phenotypes in carriers, pericentric chromosome inversions can be associated with increased risks for miscarriage, infertility and in some cases, the birth of children with mental and/or physical differences. We have identified a large, multi-generational family with an inversion of chromosome 1 between bands 1p36.21 and 1q42.13. To our knowledge this is the largest familial inversion ever described, comprising approximately 88% of the total chromosome 1 length. The family was ascertained through the birth of a child with multiple congenital anomalies including significant cardiac and brain abnormalities. Cytogenetic studies performed during the prenatal period showed that the proband had a duplication of chromosomal material distal to band 1q42 and loss of material distal to band 1p36.2, consistent with a recombinant chromosome from a maternal pericentric inversion. Subsequent family studies have led to the identification of fourteen inversion carriers across five generations. Miscarriages were reported or observed in 35% of pregnancies to known inversion carriers (13/37 pregnancies), indicating a substantial risk for pregnancy loss associated with this inversion. There have been no additional children, or conceptuses identified with a recombinant chromosomes, although the risk of having a child with significant health issues due to chromosomal imbalances remains a possibility for family members carrying the inversion. Surprisingly, of twenty healthy family members for whom chromosome studies have been performed, 14 (70%) had inherited the inv(1) chromosome, while only 6 (30%) had inherited the normal chromosome 1. This suggests a possible meiotic drive that favors transmission of the inverted rather than the normal chromosome 1. Specific chromosomal breakpoints have been mapped to within approximately 52 kb at 1p36.12 and 532 kb at 1q42.12. A FISH protocol has also been developed to investigate the meiotic segregation of the inversion in sperm of inversion carriers. In addition to providing valuable information about risks for adverse pregnancy outcomes in this family and other families with similar rearrangements, further characterization of the above inversion and its meiotic segregation may provide valuable information about genes that influence fertility and gametic fitness.

## 1328/W/Poster Board #986

**Ring chromosome 20 syndrome: a new case with duplications of two contiguous BAC clones in 20q11.22 without deletions of the telomeric regions, studied by Genome Array-CGH.** *A. Nucaro[1], R. Rossino[3], S. Zorco[2], N. Santini[2], F. Boscarelli[2], A. Milia[5], M. Loi[4], C. Montaldo[2].* 1) CNR, Inst Neurogen Neurofarmacolog, Monserrato, Italy; 2) Dip. Scienze Chir. Odontostomatologiche, Università, Cagliari, Italy; 3) Dip.Scienze Ped. Med Clinica, Azienda Osp-Università, Cagliari, Italy; 4) Div. Neuropsichiatria Infantile, AOB, Cagliari, Italy; 5) Dip. Biol. Sperimentale, Università, Cagliari, Italy.

Ring chromosome 20 syndrome is a rare disease characterized by refractory epilepsy, moderate mental retardation and particular electroencephalographic disorder with non-convulsive status epilepticus. Herein, we report on a new case of r(20) syndrome in a 15 year old female that presented minimal dysmorphism, generalised tonic e clonic and absence seizures refractory to medical therapy and behavioural disorders. The female was the first child of an unrelated couple, and she was born after uneventful pregnancy and delivery. Among 50 cytogenetically analyzed cells, 30 showed a 46, XX, karyotype (60%) and 20 exhibited a 46,XX,r(20)(p13q13.3)(40%). FISH using whole specific probe of chromosome 20 confirmed the presence of the ring chromosome 20. Telomeric-FISH using BAC clones RP5-1103C7, RP5-1059L7, RP5- 1005F21, RP11-476I15 revealed no deletions of telomeric regions. Genome Array- CGH using Cytochips Blue-gnome (Technogenetics - Bouty) detects the absence of any deletion in the ring chromosome 20 and the presence of an amplification of about 350 kb in 20q11.22. We think that the lack of detection of any deletion could be attributed to the mosaicism,and we hypothesize that clinical symptoms of r(20)syndrome could be attributed to telomeric partial monosomy generated by ring chromosome and causing an haplo-insufficiency of two epilepsy genes CHRNA4 and KCNQ2. To the best of our knowledge, our patient was the first case presenting the typical epilepsy disorder but no detectable deletion in the ring chromosome 20 associated with amplification of about 350 kb in 20q11.22. Further studies are in progress to better definition and characterization of the duplicated region.

## 1329/W/Poster Board #987

**Insertional translocation detected by array-based comparative genomic hybridization (aCGH).** *Z. Ou, SH. Kang, C. Shaw, PA. Eng, ML. Cooper, AN. Pursley, T. Sahoo, CA. Bacinos, AC. Chinault, P. Stankiewicz, A. Patel, JR. Lupski, SW. Cheung.* Dept Molec & Human Gen, Baylor Col Med, Houston, TX.Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA.

Insertional translocations (IT) are rare events that require at least 3 breaks in the chromosomes involved and thus qualify as complex chromosomal rearrangements (CCR). In the current study, we identified 41 IT cases from approximately 18,000 clinical cases (1:500) using array comparative genomic hybridization (aCGH) in conjunction with fluorescence in situ hybridization (FISH) confirmation of the aCGH findings, and parental follow-up studies. Both submicroscopic and microscopically visible IT events were detected. There were 3 major categories; 1) simple intrachromosomal and interchromosomal IT resulting in pure segmental trisomy, 2) complex IT involving more than one abnormality, 3) deletion inherited from a parent with a balanced IT resulting in pure segmental monosomy. Interestingly, of the cases where follow-up parental studies were available, over half were inherited from a parent carrying the same unbalanced rearrangement detected in the proband, thus, decreasing the likelihood that these IT events are clinically relevant. Nevertheless, we identified six cases where small submicroscopic events were detected that involved known disease-associated genes/genomic segments and are likely to be pathogenic. We recommend that copy number gains detected by clinical aCGH analysis should be confirmed using FISH analysis whenever possible in order to determine the location of the duplicated segment. We hypothesize that the increased use of aCGH in the clinic will demonstrate that IT occur more frequently than previously predicted, but can identify many genomic rearrangements with unclear clinical significance.

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## 1330/W/Poster Board #988

**Cytogenetic and SNP Microarray Analysis of a Ring Chromosome 22 with a Duplicated Segment.** *V.R. Potluri[1], J.L. Smith[1], T. Solomon[1], D.L. Rodriguez[2], S. Schwartz[3], P. Papenhausen[3].* 1) Cytogenetics, Dynagene/ LabCorp, Houston, TX; 2) Pediatric Neurology, Texas Childrens Hospital, Houston, TX; 3) Cytogenetics, LabCorp, Research Triangle Park, NC.

The utilization of SNP array technology has provided a better understanding and insight into the delineation of chromosomal abnormalities. In this study, we report a two and a half year old female referred for chromosome studies with the clinical features of developmental delay, microcephaly, pulmonary stenosis, ADHA-poor behavior and stereotypical habits. The pregnancy history was uneventful and full term; birth weight was normal. Chromosome analysis on peripheral blood revealed a female chromosome constitution with a ring chromosome 22 in every cell; breakpoints were determined to be at p12 and q13.3. Initially it was believed that there could be a small deletion in 22q13.3. Subsequent studies with a whole genome chromosome SNP microarray copy number analysis revealed an interstitial 8.04Mb duplication at 22q13.2 - q13.33. No deletions at the breakpoints, or elsewhere, on chromosome 22 were detected by microarray analysis. FISH analysis of this ring with BAC RP11-938G7 confirmed that the ring chromosome contained the duplicated segment. Further FISH studies using ARSA and chromosome 22 subtelomere probes both exhibited single copy signals showing that this duplication is interstitial. These findings are very intriguing for several reasons. To our knowledge, this is the first non-mosaic, non-supernumerary ring chromosome 22 reported with a duplicated segment rather than loss of chromosomal material. This indicates that it cannot be presumed that a ring formation results in a deletion which leads to the phenotype. More importantly, it elegantly shows the need for both cytogenetic and array analysis to clearly delineate chromosome abnormalities.

## 1331/W/Poster Board #989

**CYTOGENETIC STUDY AND CLINICAL CO-RELATION IN PREMATURE OVARIAN FAILURE.** *A. Sharma[1], A.C. Ammini[2], A. Kriplani[3], R. Dada[1].* 1) Department of Anatomy; 2) Department of Endocrinology & Metabolism; 3) Department of Obstetrics & Gynaecology, All India Institute of Medical Sciences, New Delhi-110029.

Premature Ovarian Failure (POF) is defined as the occurrence of menopause before the age of 40 years, biochemically low levels of gonadal hormones (estrogen & inhibin) and high levels of gonadotrophins (FSH & LH). POF is a heterogeneous disorder affecting approximately 1% of women <40 years, 1:10,000 women by age 20 and 1:1000 women by age of 30. Several causes of POF have been identified, including X chromosome aberrations. This study finds out the proportion of genetic causes of POF and analyze different chromosomal pattern and clinically co-related. Fifty patients of POF were referred for cytogenetic analysis. Family history, age, occupation, disease information and all other medical records were reviewed carefully. Blood was collected and lymphocyte cultures were set up. Twenty GTG banded metaphases were analysed for chromosome complement. Mean age and height was found to be 20.2 years and 150cm respectively. FSH level was found to be >40mIU/ml in all patients. 82% of cases had a normal karyotype. In 6% cases karyotype was 45,XO whereas 16% of the cases showed mosaicism with normal cell lines. Two patients had a chromosomal constitution 46,X,i(X) whereas other two had the karyotype 46,XX,del Xq(13.3-21.1) and 46,XX,delXq(22-24). Various reports suggested that X chromosome deletions associated with POF are more common than translocations. In this study X chromosome aberration are involved in all cases as reported in the earlier studies. This mosaicism could explain an accelerated loss of gametes in the ovary. Cytogenetic analysis in association with molecular techniques could allow finding the molecular pathogenesis of the ovarian failure. These POF patients most commonly clinically presented with irregular menstrual cycle with night sweats , irritability, poor concentration, vaginal drying , infertility.

## 1332/W/Poster Board #990

**Novel 1q31-32 deletion in a patient with ocular coloboma.** *H. Singh[1], M. Kumar[1], M. Tanwar[1], P. Venkatesh[2], R. Dada[1].* 1) Department of Anatomy,; 2) Dr R.P.Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, India.

Coloboma (from the Greek koloboma, meaning ''mutilated'' or ''curtailed'') may be defined as a congenital defect in uveal tissue in a site consistent with abnormal closure of the embryonic fissure during embryogenesis. Ocular features commonly associated with coloboma are microphthalmos, microcornea, cataract, and retinal detachment. Typically, coloboma are clefts caused by absent tissue in the inferonasal quadrant of the eye, but subtype, severity, and visual prognosis vary, depending on location and associated eye defects. Iris coloboma involves the pigment epithelium and stroma giving rise to the so-called ''keyhole'' pupil, which can be unilateral or bilateral. Coloboma can be caused by genetic, environmental (teratogens) or unknown factors. Autosomal dominant (AD) inheritance is the most common mechanism reported in the literature. The genetic causes can be chromosomal or monogenic. Coloboma associated with chromosomal abnormalities is usually associated with systemic abnormalities. An extraordinary number of conditions are associated with coloboma either as Mendelian inherited traits or as chromosomal aberrations. To date, 27 genetic loci have been mapped to specific chromosomal regions and 21 of the genes have been identified. Eleven chromosomal aberrations have been documented and three of these overlap with known coloboma-associated genes (SHH, CHX10, MAF). The coloboma phenotype is therefore genetically heterogeneous. We report a case with sporadic case of iridofundal coloboma. We have done the Cytogenetic analysis of the patient. Standard GTG banding showed deletion of band 1q31-32. To the best of our knowledge this is the first report. This finding may represent Novel a severe manifestation of the disease. Karyotyping and examination by clinical geneticist should be performed to identify associated syndromes. Parents should be examined for minor forms of coloboma.

## 1333/W/Poster Board #991

**Molecular cytogenetic evaluation of a proband with 12p13.32 terminal deletion and her parent with inv(12)(p13.33p12.1).** *K. Wakui[1,2], T. Kosho[2,1], A. Nishimura[3], M. Uhara[4], K. Matsuda[4], E. Hidaka[4], R. Kawamura[1], N. Matsumoto[3], Y. Fukushima[1,2].* 1) Department of Medical Genetics, Shinshu University School of Medicine, Matsumoto, Nagano, Japan; 2) Division of Clinical and Molecular Genetics, Shinshu University Hospital, Matsumoto, Nagano, Japan; 3) Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Kanagawa, Japan; 4) Department of Laboratory Medicine, Shinshu University Hospital, Matsumoto, Nagano, Japan.

The proband, a two-year-8-month-old girl, is the first child of healthy nonconsanguineous parents. The pregnancy was uneventful. At birth at 39 weeks of gestation, the child weighed 2205g (-2.1SD), measured 44.3cm (-2.6SD) and head circumference was 29.3cm (-2.8SD). Developmental delay was pointed out at her 10 months of age. G-banded chromosomal analysis was performed, and the terminal deletion of 12p13.3 was suspected, and confirmed by 12p/12q subtelomere FISH analysis. Her parents were visited to our division to get detailed information of the chromosome abnormality of their child. At age of 2 9/12 years, the proband had obvious growth failure (weight: 8.8kg (-3.3SD), height: 77.7cm (-4SD) and head circumference: 42.2cm (-4SD)), and mild developmental delay. Microarray analysis confirmed the deletion of an approximately 4 Mb fragment from the 12p telomere region without any other abnormalities. Thus, the chromosome abnormality of the proband was the terminal deletion, and the deletion breakpoint was 12p13.32. On other hand, one of the parents was suspected as paracentric inversion of 12p, according to G-banded chromosome and 12p/12q subtelomere FISH analyses. Additional metaphase FISH analyses revealed inv(12)(p13.33p12.1). These results indicated that breakpoint identified in the proband and that of the parent were somehow different. The relationship between the 12p terminal deletion in the proband and the paracentric inversion 12p in her parent will be discussed.

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## 1334/W/Poster Board #992

**Microdeletion and triplication around 17p13 including PAFAH1B/LIS1 in three patients with MR and epilepsy.**
*T. Yamamoto[1], K. Shimojima[1], C. Sugiura[2], H. Takahashi[3], Y. Kubota[3], Y. Takahashi[3], K. Saito[4].* 1) International Research and Edu, Tokyo Women's Medical University, Shinjuku-ward, Tokyo, Japan; 2) Division of Child Neurology, Institute of Neurological Sciences, Tottori University Schoold of Medicine, Yonago, Japan; 3) Department of Pediatrics, National Epilepsy Center Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan; 4) Institute of Medical Genetics, Tokyo Women's Medical University, Shinjuku-ward, Tokyo, JAPAN.

Array-based comparative genomic hybridization (CGH) analyses enabled to identify cryptic microdeletions and/or duplications in patients with idiopathic mental retardation and epilepsies. Subtelomeric region of the short arm of chromosome 17 is well-known as the responsible region for Miller-Dieker syndrome which manifests lissencephaly and characteristic facial features. We report on three patients with epilepsy and psychomotor developmental delay in whom we identified cryptic microdeletions and/or duplications of 17p13.3. Patient 1 is 25 years old female, who started intractable seizures when she was 6 years and severely delayed with bedridden. Brain MRI is compatible with lissencephaly but there was no deletion of PAFAH1B/LIS1 by FISH analysis. Patient 2 is 11-month old girl manifesting severe psychomotor delay and West syndrome. Brain MRI showed atypical hypoplasia of the brain. Patient 3 is 22-year old male. He suffered seizures when he was 7 years old. Samples derived from the three patients were analyzed by Oligoarray CGH (Agilent Technologies). Patient 1 showed partial deletion of PAFAH1B. Patient 2 showed chromosomal triplication including PAFAH1B. Patient 3 showed small subtelomeric deletion including YWHAE but not PAFAH1B. Intra genic neucleotide mutations or deletions cause isolated lissencephaly, and the neighboring gene YWHAE is believed to be necessary for dysmorphic features. In our study, microdeletions identified in patient 1 and 3 included YWHAE and PAFAH1B, independently, and nobody showed a facial feature characteristic with Miller-Dieker syndrome. Heterozygous triplication of PAFAH1B identified in patient 2 was similar to that recently reported by Bi et al. (Nature Genet 2009).

## 1335/W/Poster Board #993

**A fetus with multiple gastro-intestinal atresias, limb defects and a de novo translocation 46,XY,t(4;9)(q27-p23).**
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We report on a fetus with multiple malformations and a de novo translocation 46,XY,t(4;9)(q27-p23). The family history was unremarkable: there was no consanguinity and no maternal diabetes. During the pregnancy there was no infection or drug exposure. The most striking features were the multiple gastro-intestinal atresias: the esophagus and stomach were blind ending, even so the cecum. There was no proximal, transversal and distal colon. The rectum was also blind ending with anal atresia. The right upper limb was severely dysplastic with one 'humero-radial bone', one metacarpal and one digit. At the left hand there was an oligodactyly. Further malformations were cardiac abnormalities and absence of one lung. On the microarray a microdeletion was found of about 8 Mb (4q26-4q28.1). We comment the deleted genes and the possible involvement of the FGF2 gene (and the adjacent genes NUDT6 and SPRY1) in the defective morphogenesis. The deletion of the FGF2 region does not explain all the malformations, so we suppose that (an)other missing gene(s) may play an important role during the morphogenetic period (NDST3 gene ?).

## 1336/W/Poster Board #994

**Recurrent deletions mediated by complex low-copy repeats on chromosome 10q11.21q11.23 in eight patients with variable phenotypes.**
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Using clinical oligonucleotide array CGH analysis, we have identified recurrent deletions in a low-copy repeat (LCR)-rich chromosome region 10q11.21q11.23 in eight patients with variable phenotypes, including mild to severe developmental delay/mental retardation, autistic spectrum disorder, dysmorphic features, ataxia and multiple congenital anomalies. Custom designed high-resolution array CGH analysis allowed us to narrow the breakpoint regions. The deletions range in size from ~ 1.5 to 6 Mb. No CNV has been reported in the smallest unique sequence region of deletion overlap (between ~ 49.2 and 50.6 Mb). FISH analysis revealed that the deletion was inherited from an apparently normal parent in six cases, three from the father and three from the mother. One of the maternally inherited deletions was accompanied by a de novo ~ 12 Mb deletion in 3q13.12q13.32 and the other by a maternally inherited ~ 2 Mb duplication in 13q22.3. In two cases, no parental samples were available. More than half of the analyzed 6.5 Mb region in 10q11.21q11.23 is occupied by 13 large LCR clusters of complex structure; there are also seven genomic gaps. Four same-sized ~ 6 Mb deletions are flanked by LCRs with directly homologous subunits ~ 100 kb in size with 98.2% DNA sequence identity and the fifth similar deletion is flanked by ~ 112 kb subunits with 99% DNA sequence identity, indicating a non allelic homologous recombination (NAHR) as the mechanism of formation. The other two deletions were likely stimulated but not mediated by LCRs. In addition, we describe a 21-year-old patient with developmental delay, very limited vocabulary, and poor motor conditioning, in whom we identified an ~ 9 Mb deletion in 10q11.2q21.1 present also in his healthy father and brother. We propose that the phenotypic diversity and lack of distinct syndromic features associated with the deletion CNVs in 10q11.21q11.23 warrant further careful examination of this genomic region. Variable expression and incomplete penetrance should be considered.

## 1337/W/Poster Board #995

**Exploiting 15q11.2-q13 segmental duplication structures to distinguish different classes of chromosomal rearrangements *in situ*.**
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Genomic regions flanked by segmental duplications in Prader-Willi and Angelman syndromes (PWS/AS) show a great proclivity for chromosomal structural changes. The most common rearrangements in PWS/AS involve large (Class I) and small (Class II) deletions sharing a common telomeric breakpoint. Based on genomic array studies, the deletion breakpoint hotspots coincide with locations of segmental duplications. Genomic arrays have determined the approximate locations of the breakpoints of these rearrangements. Greater precision in determining breakpoints can be gained by using pre-defined, short repeat-free sequences from the reference human genome in a FISH-based assay. In the present study, we devised a strategy for breakpoint analysis by mapping approximate locations of Class I and Class II breaks to overlapping adjacent single copy or duplicated intervals. Sequences of defined copy number and context of 1500-5000 base pairs were designed and overlapped across boundaries of previously reported breaks. Of a total of 47 sequences, 8 occurred at the interfaces of greatest density of breakage activity were selected for probe development. Two of the probes, embedded within segmental duplicons, which intersect the proximal end of Class I deletions, contain paralogous sequences at the telomeric breakpoint. Two different probes embedded within duplicons that intersect proximal end of Class II deletions are not found at the telomeric breakpoint. Of the remaining 4 probes, 2 are adjacent to segmental duplicons at the proximal end of Class I deletions and two are adjacent to duplicons at the telomeric breakpoint. A positive control probe is also designed for the imprinted domain in PWS/AS specific to the *MAGEL2* locus. FISH studies using probes for the proximal ends of Class I and Class II deletions have demonstrated high specificity. Departure from the expected hybridization patterns for normal chromosomes dictates the probe combinations to be used in the subsequent hybridizations. A two-step hybridization algorithm is capable of differentiating multiple classes of breakpoints by co-hybridizing probes sequences labeled with different fluorophores to discriminate deleted from non-deleted chromosome 15 homologs. This approach will accurately determine breakpoints and the genomic structure of PWS/AS deletions. These strategies should be useful for delineation of chromosome boundaries for other disorders associated with segmental duplication.

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## 1338/W/Poster Board #996

**Mosaic deletion of TCF4 in a child with Pitt-Hopkins syndrome.** *D.J. Stavropoulos[1], D.L. MacGregor[2], G. Yoon[2,3].* 1) Dept Cytogenetics; 2) Division of Neurology; 3) Division of Clinical and Metabolic Genetics, Hosp Sick Children, Toronto, ON, Canada.

We present the clinical and cytogenetic findings in an 8 year old girl with mental retardation, absent speech, acquired microcephaly, delayed motor skills and stereotypical hand movements. Craniofacial exam revealed coarse facial features, up-slanting palpebral fissures, a broad nasal bridge and tip with prominent lips held open in a Cupid's bow pattern. She experienced tonic clonic seizures since the age of 14 months, and an MRI at 17 months revealed delayed myelination of the frontal lobes as well as prominence of the ventricles. Molecular testing for Rett and Angelman syndromes was negative. Array comparative genomic hybridization identified a de novo deletion of approximately 6 Mb in chromosome region 18q21.1q21.31, resulting in the loss of one copy of the TCF4 gene as well as 21 other RefSeq genes. Fluorescence in situ hybridization (FISH) analysis of peripheral blood confirmed the deletion in approximately 50% of cells, consistent with a mosaic deletion. The deletion likely occurred early in development as this child has clinical symptoms affecting multiple organ systems, consistent with those observed in Pitt-Hopkins syndrome (PHS; OMIM 610954). PHS is a rare neurodevelopmental disorder caused by haploinsufficiency of the TCF4 gene on chromosome region 18q21.1. Individuals with a deletion or de novo heterozygous mutation of TCF4, have been described with severe mental retardation with absent or limited speech, characteristic facial gestalt, unsteady gait and hypotonia. Other features include episodes of hyperventilation followed by apnea, congenital or acquired microcephaly, seizures, stereotypic movements of the hands, and constipation. The fact that our patient does not manifest all the typical features of PHS may be due to her being haploinsufficient for TCF4 in mosaic form only. This case report represents the second known example of a mosaic deletion resulting in clinical symptoms consistent with Pitt-Hopkins syndrome.

## 1339/W/Poster Board #997

**Extra structurally abnormal chromosomes: experience of a prenatal diagnostic laboratory during 11 years.** *F. Tihy, L. Oprea, E. Lemyre.* Dept Genetics, CHU Ste-Justine, Montreal, PQ, Canada.

Extra structurally abnormal chromosomes (ESACs) are one of the challenges in prenatal diagnosis. Their identification was not evident by classical cytogenetics but has become easier with the development of fluorescent in situ hybridization and recently, of CGH micro-arrays. Nonetheless, literature is still missing precise genotype-phenotype correlation for counselling once the marker chromosome has been characterised. We report here the finding of eleven years of prenatal diagnosis, with 32015 amniocenteses in which 58 had an ESAC, 51 of them characterised. 42 ESACs were de novo, 12 were inherited and 4 were extra derivative chromosomes from a parental translocation. The incidence of ESACs in our laboratory was 1/552. Of those, twenty six were from acrocentric chromosomes, eight were isochromosomes of the short arm of chromosomes 9, 12 and 18, three were complete short arms of chromosome 4, 5 and 20, eight were ring chromosomes from chromosome 1, 2, 3, 19 and X and two were centromeres of chromosomes 2 and 12. The 7 non-characterised ESACs were mostly found in the years 1998 and 1999. 4 ESACs seemed to contain euchromatin and were associated with ultrasound abnormalities and 3 were very small without evidence of euchromatin (2 inherited). Of the 58 ESACs, 32 contained some euchromatin, all of them de novo or extra derivative from a parental translocation. Most of the pregnancies were then terminated but a few continued: two pregnancies with a derivative 22 who developed signs of cat eye syndrome, one with a derivative 15 containing the PWS region, two with a derivative 14, one with a ring chromosome 3 containing mostly part of the long arm, one with a small ring 2. These children are evaluated regularly at our clinic. For a good genotype-phenotype correlation, a CGH array analysis from a blood sample of those children can assess more precisely the euchromatin present but only when the ESACs are not at a low level of mosaicism.

## 1340/W/Poster Board #998

**A healthy birth after preimplantation genetic diagnosis in two couples of reciprocal translocations.** *M. Woo[1], J. Choi[1], H. Seol[1], S. Kim[2], M. Kang[2], H. Kim[2], S. Oh[1,2], S. Ku[2], S. Kim[2], Y. Choi[1,2], S. Moon[1,2].* 1) Institute of Reproductive Medicine and Population; 2) Department of Obstetrics and Gynecology, College of Medicine, Seoul National University, Seoul, Korea.

Balanced reciprocal translocations which lead to errors in gametogenesis are known to be an increased risk of infertility, spontaneous abortions, and abnormal offspring. The application of preimplantation genetic diagnosis (PGD) has been mostly attempted by fluorescent in situ hybridization (FISH) using specific DNA probes to reduce the risk transferring embryos with aneuploidy. The FISH analysis has been used to select a chromosomally normal or balanced segregation from blastomere of preimplantation embryos. We describe our experience on the PGD analyses using FISH for couples repeated pregnancy loss. Case A, a 32-year-old female, was a carrier of balanced reciprocal translocation, 46,XX,t(9;14)(p24;q32). Her husband had normal karyotype. The couple experienced two spontaneous abortions at the first trimester. The case A was used by FISH with two telomeric probes, 9pter and 14qter probes, and centromeric probe. We identified six embryos with a normal or balanced combination of probe signals from case A, and achieved in one pregnancy. The amniocentesis was confirmed at 16 weeks gestation and a girl with a normal karyotype was born. Case B, 36-year-old female, was a carrier of balanced translocation with karyotype 46,XX,t(18;21)(q11.2;q22). The karyotype of male partner was normal. The case B was referred due to the two pregnancy losses. The FISH analysis was studied with two telomeric probes, 18qter and 21qter probes. The case B showed two embryos derived from 3:1 segregations, one from adjacent-1segregation, and one from mosaic. No embryo transfer could be carried out because all the embryos of case B were unbalanced. In conclusion, PGD-FISH for infertile couples of reciprocal translocations should be considered as a treatment method for transfer of normal embryos after in vitro fertilization, and these results will be useful information in reproductive counseling.

## 1341/W/Poster Board #999

**Evolution of centromeric sequences in Primates: new insights from the White-cheeked Gibbon and Marmoset.** *C.R. Catacchio[1], A. Cellamare[1], C. Alkan[2], G. Giannuzzi[1], F. Antonacci[2], M.F. Cardone[1], G. Della Valle[3], M. Malig[2], M. Rocchi[1], E.E. Eichler[2], M. Ventura[1].* 1) Department of Genetics and Microbiology, University of Bari, Bari, Italy; 2) Department of Genome Sciences, Howard Hughes Medical Institute, University of Washington School of Medicine, Seattle, Washington, USA; 3) Department of Biology, University of Bologna, via Selmi 3, Bologna, Italy.

Alpha-satellite DNA is the major component of centromeric DNA and forms the centromere central domain of both human and all studied primate chromosomes, ranging in size up to 5Mb. It is the most studied repeated DNA family, is made up of a basic 171bp unit tandemly organized and was first described in the african green monkey as a 340bp repeated unit consisting of two 171 bp different monomers. Human alphoid DNA is organised into higher order structures and suprachromosomal subfamilies that hybridize to a distinct subset of chromosomes. Besides higher order repeats $\alpha$-satellite in HSA can organize into monomeric repeats which are likely to be emerged first during karyotype evolution. $\alpha$-satellite pancentromeric localization clearly suggests its functional involvement in an active centromere. These long homogenous stretches of repetitive DNA could work as a spacer to protect the centromeric locus from transposable sequences that would disrupt centromeric activity rather than having a sequence-mediated specific role. The evolutionary history of $\alpha$-satellite DNA is hardly defined because of the difficulty in its sequence assembly and its rapid evolution. By using several approaches we have cloned, sequenced and characterized $\alpha$-satellite sequences from two species of Primates: the white-cheeked gibbon and marmoset. Sequence analyses demonstrate that white-cheeked gibbon and marmoset $\alpha$-satellite sequences are formed by units of ~171 bp and ~342 bp, respectively, and they both lack high-order structure found in humans and great apes. Further, FISH characterization shows a broad dispersal of alpha-satellite in the white-cheeked gibbon genome including centromeric, telomeric and chromosomal interstitial localizations. On the other hand centromeres in marmoset appear organized in highly divergent dimers roughly of 342bp that show a similarity between monomers much lower than previously reported dimers thus representing an ancient dimeric structure. Our results shed light on the evolution of the centromeric sequences in Primates. We suggest radical differences in the organization and evolution of $\alpha$-satellite DNA among different primate species, showing that centromeric function is not linked to higher-order structure and all the centromeric sequence in Primates evolved by genomic amplification, unequal crossover and sequence homogenization using a 171bp monomer as basic seeding unit.

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### 1342/W/Poster Board #1000

**Mapping of functional domains at the human chromosome 5 centromere.** *B. Grimes[1], C. Steiner[1], J. Bhavsar[1], T. Schwarz[2], S. Christan[2], M. Yoder[3], D. Schindelhauer[2], R. Slee[1].* 1) Medical & Molecular Gen, Indiana Univ Sch Medicine, Indianapolis, IN; 2) Department of Medical Genetics, Ludwig Maximilians Univ, Munich, Germany; 3) Wells Center for Pediatric Research, Indiana Univ Sch Medicine, Indianapolis, IN.

This study examines the relationship between sequence and chromatin domains at the human chromosome 5 centromere to improve our understanding of centromere specification. Alpha satellite DNA is present at all centromeres and forms chromosome specific higher order repeats (HORs). Structurally, the centromere comprises two chromatin domains: the kinetochore (mitotic spindle attachment region) characterized by deposition of the CENP-A (a histone H3 variant) and flanking pericentromeric heterochromatin important for sister chromatid cohesion. Here, the contribution of the two adjacent HOR families, D5Z1 and D5Z2 to the chromosome 5 centromere has been investigated. FISH analysis revealed deletion of all detectable D5Z1 sequence and retention of D5Z2 on a mitotically stable patient derived ring 5 chromosome (with breakpoints at 5p10-p13). Consistent with D5Z2 assembling the kinetochore, combined immunofluorescence (IF) with a CENP-A antibody and Fluorescence In Situ Hybridization on chromosome spreads from a cancer cell line (HT1080) showed overlap between CENP-A and D5Z2 but not D5Z1. To refine this analysis, chromatin immunoprecipitation (ChIP) and semi-quantitative real-time PCR (qPCR) was performed on HT1080 cells and primary human umbilical vein endothelial cells (HUVECs) with antibodies to CENP-A and H3K9me3. In all cases CENP-A was restricted to D5Z2 while D5Z1 was comparable to negative controls confirming that D5Z2 assembles the kinetochore. In contrast, H3K9me3 (a heterochromatin marker) was detected on both D5Z2 and D5Z1. In HT1080, H3K9me3 was present at a lower level on D5Z2 relative to D5Z1 while in HUVECs, similar amounts of H3K9me3 were detected on each sequence. Furthermore, amounts of H3K9me3 at the centromere in HT1080 were reduced more than five-fold relative to HUVECs indicating loss of pericentromeric heterochromatin in HT1080 cells. Support for this conclusion was obtained using IF with H3K9me3 and CENP-A antibodies on chromosome spreads. H3K9me3 was rarely detected in the region between the CENP-A double dot kinetochore signals in HT1080 cells but was detected frequently between the CENP-A signals in HUVECs. These data provide an integrated DNA and chromatin map of centromere 5 and raise the possibility that loss of pericentromeric heterochromatin in HT1080 cancer cells may contribute to aneuploidy and cancer progression.

### 1343/W/Poster Board #1001

**Phenotypic effects of a pericentric inversion of chromosome 9.** *C. Albu[1], D.F. Albu[1], A. Toma[2], E. Severin[1].* 1) Dept of Human Genetics, Carol Davila Univ Med Pharm, Bucharest, Romania; 2) Panait Sirbu Hospital, Bucharest, Romania.

Aim: Inversion of chromosome 9 is a cytogenetic abnormality quite common in the general population. Phenotypic effects often do not exist. We present a case of a premature neonate with this abnormality and a congenital malformation of the midline. Material and methods: A case of a premature neonate is presented. For examination there were used: clinical and neurological examinations, ultrasound examinations, cytogenetic tests. Results: The patient is a male newborn, delivered at 33 weeks gestational age for suspicion of hydrocephalus. The postnatal ultrasound showed a hypoechogenic formation of 1.81/1.97 cm in the posterior fossa, with no vascular supply.This image was consitent with an arachnoidal cyst. The heart and abdominal ultrasound exams showed no added malformations. There were noted dysmorphic features with wide facies and low nasal bridge. The karyotype showed a pericentric inversion of chromosome 9 [46 xy, inv (9)(p12q13)]. On follow up visits the infant showed a normal neurologic development and no modification in the dimensions of the cyst. The patient is now 6 months old. Conclusion: Posterior fossa cysts can occur in the case of pericentric inversion of chromosome 9, with some added dysmorphic features.

### 1344/W/Poster Board #1002

**Micronuclei in human lymphocytes exposed to sodium pertechnetate, in vitro.** *I. Aranha.* Inst Biol, PHLC S-525-6, Univ Estado Rio de Janeiro, Rio de Janeiro, Brazil.

Technetium-99m ($^{99m}$Tc)has become the most widely used radioisotope in the detection of inflamatory sites as well as in the diagnosis of transplanted tissues. The goal of the present work was to study the effect of sodium pertechnetate on human lymphocytes in vitro, using the micronucleus assay. Micronuclei appear during cell division as a result of acentric chromosome fragments or whole chromosomes outside the nucleus. Peripheral whole blood cells collected from healthy donors, 18 to 30 years old, were incubated at 37ºC for 48 hours in the presence of $^{99m}$Tc (3.7MBq/100µL). Cells not exposed to the radionuclide served as control for the experiment. Cytochalasin B (3µg/mL) was added to the cultures 20 hours postinitiation. After fixation, cells were stained with Giemsa Gurr (2%) and were observed under optical microscope. In the test group, 12077 binucleated cells were studied and 212 of them showed alterations (18 micronuclei and 194 nucleoplasmic bridges). Nucleoplasmic bridges are biomarkers of genomic instability and are a result of chromosome rearrangements involving more than one centromere (dicentric chromosomes). In the control group 12056 cells were obsreved and 6 micronuclei were seen. The chi-square test with Yates correction indicated that the results were extremely significant ($p<0.0001$) suggesting that sodium pertechnetate was responsible for the chromosome alterations observed.

### 1345/W/Poster Board #1003

**Effect of 99m-technetium combined to its reducing agent stannous chloride on chromosomes from human lymphocytes in vitro: Micronucleus assay.** *E.C.M. Passos, I.P. Aranha.* IBRAG, Univ. do Estado do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil.

Technetium-99m ($^{99m}$Tc) has been used in the detection of inflamatory sites as well as in the diagnosis of transplanted tissues. Its efficiency is increased by a reducing agent, stannous chloride ($SnCl_2.2H_2O$). Previous work inn our laboratory has shown that the use of the reducing agent (12µg/mL) in the culture of whole blood lymphocytes neither decreased the mitotic rate nor caused any numerical or structural chromosome abnormality. The goal of the present work was to observe if sodium pertechnetate combined to its reducing agent might cause micronuclei in human lymphocytes, in vitro. Peripheral whole blood cells, collected from healthy donors, 18 to 30 years old, were incubated at 37ºC for 48h. One sample was exposed to stannous chloride (12µg/mL), another was exposed to the radionucleide combined to its reducing agent and a third one contained cells that were not exposed to any of the agents and served as control for the experiment. Cytochalasin B (3µ/mL) was added to the cultures 20h postinitiation. After fixation, cells were stained with Giemsa Gurr (2%) and were observed under optical microscope. In the group exposed to stannous chloride 13003 cells were observed and none showed micronuclei. In the group exposed to sodium pertechnetate combined to its reducing agent 12413 cells were studied and 222 had micronuclei. In the control group 13907 cells were observed and 2 had micronuclei. The chi-square test for independence indicated that the results were extremely significant ($p<0.0001$) suggesting that sodium pertechnetate was responsible for the micronuclei observed.

### 1346/W/Poster Board #1004

**Translocation 20p to 21 q in mother and son.** *A. Toma[1], C. Albu[2], E. Severin[2], D. Albu[2].* 1) “Panait Sarbu” Obstetrics and Gynecology Hospital , Bucharest, Romania; 2) “Carol Davila” Univ Med Pharm.

Aim: The paper reports a 20p;21q translocation present in the mother and in her newborn son, affecting the neonate but not the mother. Material and method: For the description of the case there were used the clinical examination of the mother and infant, the ultrasound examinations of the infant, blood samples and the karyotype test. Results: This is the case of a term male newborn, delivered by cesarean section at 38 weeks gestational age. The clinical examination at delivery noted axial hypotonia. The baby presented at 12 hours of age with digestive bleeding . The hemorrhagic disease of the newborn was excluded and head, heart and abdomen ultrasound were performed in order to exclude other hemorrhages. The head ultrasound showed the presence of a cyst in the posterior fossa. The karyotype sampled from the baby showed the following result: 47, xy, t(20p; 21q1). The same translocation was noticed in the mother, who was asymptomatic, phenotipically normal. The evolution of the infant was unfavorable with hypotonicity, apneic spells, repeated respiratory infections. There is present a severe mental retardation. Conclusion: The particularity of the case consists in the absence of symptoms in the mother and the presence of malformations and severe mental retardation in the infant.

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## 1347/W/Poster Board #1005

**Cytogenetic Alterations in Peripheral Blood Lymphocyte Cultures of Occupationally Exposed Steel Welders.** *A.R. Patel, S.S. Chettiar, M.V. Rao.* Department of Zoology, Gujarat university, Navarangpura,Ahmedabad, Gujart, India.

Purpose: More than three million workers worldwide involve in welding profession. The stainless steel welding fumes contains higher content of reactive oxides of the chromium compounds. Epidemiological studies have reported a significant elevated risk for lung cancer among the welders. The overall objective of this study is to investigate the underlying relationship between airborne exposure to welding fumes and their genotoxic effects on occupationally exposed workers. Materials and Methods The selection criteria for the welders were based on a questionnaire. The questionnaire was intended to elicit information on the subject's age, smoking habits, alcohol consumption and duration of exposure and medicine usage. Individuals in control group belonged to the same age group and socio-economic status as the welders. We ensured that the welders and the controls did not markedly differ from each other except for occupational exposure. The heparinized blood of welders was collected in sterile vials and brought to laboratory in coolant box and further procedure was done within 4 hours of collection. The blood was cultured in duplicates with equal number of age matched controls. After 69 hours of culture metaphase plates were prepared as mentioned above and subjected to analyses of several genotoxic endpoints such as Single cell gel electrophoresis and Chromosomal aberration. Blood profile of these welders was also analysed using automated cell counter (sysmax). The chromium levels were also measured in the blood and water by Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP AES). Results and Discussion: The welders are divided in the three major groups depending upon the exposure years (0-5 years, 6-10 years and exposed more than 10 years). The result revealed that there is a significant increase in the tail length and frequency of the chromatid and chromosomal breaks and gaps suggesting increased DNA damage. The blood chromium levels were significantly higher than that of the control individuals. The increase in DNA damage is correlated to the chromium levels in blood of welders and drinking water at work place. The result of this study indicates a probable human genetic risk at these work places.

## 1348/W/Poster Board #1006

**Microduplication 22q11.2 in a boy with dysmorphic features and speech delay.** *L. Telvi[1], M. Minz[1], O. Brichet[2], P. Bougneres[2].* 1) Cytogenetics Laboratory, Hosp St Vincent de Paul, Paris, France; 2) Department of Pediatric Endocrinology, Hosp St Vincent de Paul, Paris, France.

Recently, the microduplication of 22q11.2 region has been reported as a new chromosomal syndrome (Portnoi, 2009). The phenotype of these patients is extremely variable ranging from normal to behavorial abnormalities to multiple defects. We report a five-years-old boy with dysmorphic features with no intellectual disability after Denver scale testing who carrying a duplication of 22q11.2. The dysmorphic features consisted with macrocephaly, unique palmar crease, pterygium colli, pectus excavatum, small upper lip with absence of philtrum. He showed also speeching difficulties, sleeping troubles, but have not development delay, no autistic trait and no seizures. The karyotype with RHG banding of the patient was found normal, the FISH analysis showed the duplication of the region 22q11.2 after using Tuple1 probe (Cytocell). A PCRq analysis and a CGH-array confirmed the duplication. The analysis of parental karyotypes are undergoing. Compared to microdeletions, few microduplications of 22q11.2 have been identified to date. Chromosome 22, particularly the 22q11.2 region predisposed to rearrangements due to misalignements of low-copy repeats (LCRs). The described phenotypes of these patients with microduplications is extremely diverse. This diversity will make ascertainment difficult and will necessitate a rapid-screening method. A correlation between the size of the duplication and the severity of the phenotypic presentation is not well defined to date. This suggests that in addition to gene dosage other mechanisms such as genetic, environmental interactions and possibly imprinting may be important in determining the phenotypic outcome of patients with 22q11.2 microduplication.

## 1349/W/Poster Board #1007

**Complex chromosomal 3:1 segregation after a chromosome breakage and reunion within the quadrivalent formation in a balanced carrier.** *C. Yu[1], C. Thompson[1], H. Huang[1], S. Gagneaux[1], A. Bhatt[2].* 1) Dept Pathology and; 2) Dept Pediatrics, University of Mississippi Medical Center, Jackson, MS.

Balanced translocation carriers could produce unbalanced gametes when there is an adjacent or 3 to 1 segregation of a quadrivalent occurring during meiosis. We report a newborn who had a complex unbalanced chromosome constitution possibly resulting from a chromosome breakage and reunion in a quadrivalent and followed by a 3 to 1 segregation in his parents during the meiosis. A 34 5/7-week infant was born to a 32 year G4P2Ab2 mother. The birth weight was 2.55kg, length was 43cm and circumference was 32.5cm. Infant was referred for chromosome study due to multiple congenital anomalies. The Mother had polyhydramnios and ultrasound fetal anomaly during pregnancy and had underwent amniotic fluid reduction at 32 weeks of gestation. On physical examination, the newborn had small and low set ears, small eyes, prominent nose, mild micrognathia, low hair line, webbed neck, wide spaced nipples, fused 4th and 5th toes on both sides. The new born expired at 57 days with renal and respiration failure. Blood chromosome study by GTG banding method indicated that this infant had 47 chromosomes with a translocation between chromosomes 3 and 9 and an extra marker chromosome. G banding indicates that this marker chromosome is dicentric and contains segments of 9pter to 9q21.2, 9q12 to 9p22 and 3q26.2 to 3qter. FISH study confirms the presence of locus 305J7-T7 and locus D3S4560 on the 9pter and 3qter end of the marker chromosome, respectively. Parental chromosome studies are not available. Thus this infant could have inherited all the abnormal chromosomes from one of his parents who is a carrier of a balanced 3/9 translocation and has a 3:1 segregation in meiosis after a possible chromosome breakage and reunion had occurred between the normal 9 and the abnormal chromosome 9 of the quadrivalent formation. The karyotype of this infant could be described as 47,XY,t(3;9)(q26.2;p22),+der(9;9)(q21.2;q12)t(3;9)(q26.2;p22). The infant had tetrasomy of 9p22 to 9q12 and trisomy of 9pter to 9p22, 9q12 to 9q22, and 3q26.2 to 3qter.

## 1350/W/Poster Board #1008

**Performance characterization of the Affymetrix Cytogenetics Research 2.7M microarray system.** *D. Abdueva, J. Veitch, V. Huynh, P.J. Collins.* Affymetrix, Emeryville, CA.

The resolution of CNV regions that can be reliably detected in a microarray platform is formulated in terms of signal to noise ratio at any given resolution. The recently released Affymetrix Cytogenetics Research 2.7M array interrogates Copy Number state at about 2.7 million locations evenly spaced along the Human. Here we present the performance characterization using Chromosome X aneuploidy cell lines with Copy Numbers ranging from 1 through 5. We use the Signal to Noise (S/N) ratio to evaluate performance. Using the CNV calling algorithm that comes with the Affymetrix supplied software, at a 50 KB resolution for gains and losses the Affymetrix 2.7M array has a False Positive Rates substantially less than 1% at a true positive rate better than 99.9%.

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### 1351/W/Poster Board #1009

**Long-range expression consequences of copy number variation suggest that chromosome gene assortment is not random.** *G. Ricard[1], N. Gheldof[1], J. Chrast[1], J. Molina[2], S. Pradervand[1], F. Schutz[1], J. Lupski[3], K. Walz[2], A. Reymond[1].* 1) Ctr Integrative Genomic, Lausanne, Switzerland; 2) Centro de Estudios Cientificos (CECS), Valdivia, Chile; 3) Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA.

To examine the effect of genome structural changes on gene expression, we implemented genome-wide expression arrays in genomic disorder mouse models. Both a microdeletion and its reciprocal microduplication mapping to mouse chromosome 11 (MMU11), which model the rearrangements present in patients with Smith-Magenis (SMS) and Potocki-Lupski (PTLS) syndromes, respectively, were studied. We profiled the transcriptome of five different tissues that are affected in human patients in mice with 1n (Deletion/+), 2n (+/+), 3n (Duplication/+) and balanced 2n compound heterozygous (Deletion/Duplication) copies of the same region in an otherwise identical genetic background. The most differentially expressed transcripts between the four studied genotypes were ranked. A highly significant propensity of transcriptional changes are mapping to the engineered SMS/PTLS interval in the different tissues. A statistically significant overrepresentation of the genes mapping to the flanks of the engineered interval was also found in the top-ranked differentially expressed genes; an observation robust across multiple cell lineages and that extends along the entire length of the chromosome, tens of megabases from the breakpoints. These long-range effects are uncoupled from the number of copies of the CNV genes, i.e. both the deletion and the duplication, influence expression of these flanking genes in a similar manner. Thus, our data indicate that a structural change at a given position of the human genome may cause the same perturbation in particular pathways regardless of gene dosage. Consistently, conditions affecting the central nervous system were shown to be associated with both the deletion and the duplication of the same DNA segment. The uncoupling between the number of copies of the CNV genes and the phenotype, here the effect on expression of genes outside of the rearrangement, is further illustrated by the fact that we detect the same changes in expression for these genes in the compound heterozygote in which gene copy number was restored. Consistently, not all the phenotypic manifestations observed in the SMS and PTLS mice models were rescued in these animals. Finally, our results suggest that the assortment of genes mapping to a chromosome is not random.

### 1352/W/Poster Board #1010

**Case Report: A boy with a 46XY,del(14)(p11.2) Karyotype and phenotype correlation.** *C. Duran[1], A. Lopez[1], G. Gordillo[1], O. Gutierrez[2], R.A. Lopez[1], J.C. Prieto[1,2].* 1) Instituto de Genética Humana, Universidad Javeriana, Bogotá, Colombia; 2) Hospital La Victoria, Secretaría de Salud, Bogotá, Colombia.

Deletions are chromosomal disorders characterized by loss of genetic information. Terminal deletion and interstitial deletion may be instigated by agents that cause chromosome breaks and by unequal crossover during meiosis. The phenotype and clinical characteristics of the patient show a direct relationship between the involved chromosome and the amount of genetic material lost. This abnormality can also be present without having a clinical effect. However, deletion of the short arm of the chromosome 14 is characterized by hepatocellular carcinoma, squamus cell carcinoma, and prostate cancer. We report on a 6 month old boy, a product of a first pregnancy (37 weeks), born of non consanguine teenage parents. The patient required hospitalization in the neonatal unit due to low weight, jaundice and suspected neonatal sepsis. On physical examination, thepositive findings were microtia Grade I/ II bilateral with external ear canal and right helix indent. In addition there was upslanting palpebral fissures, bilateral single palmar fold and brachydactyly. During hospitalization the patient received a Renal US, chest X-rays, neonatal TSH (all reported as normal) and the band R karyotype report: 46XY,del(14)(p11.2). Until now, there have been reports of patients with this chromosomal inbalance associated with squamus cell carcinomas and prostate cancer. Deletion of information in the short arm of those acrocentric chromosomes is usually complete and is associated with Robertsonian traslocation between 13, 14 in 75%, 14, 21 in 8% and 14, 15 in 2.5%. By contrast, deletions of the long arm of chromosome 14 have been associated with variable phenotype.

### 1353/W/Poster Board #1011

**A first case of mosaic double aneuploidy (46,X,+14/46,XX) in a patient with short stature, pigmentary abnormalities, mental retardation, dysmorphic features and hypogonadism.** *T.-J. Chen, Y. Wang, G. Steen, A. Lee, C. Tuck-Muller, H. Hobart, J.E. Martinez.* Dept Medical Genetics, Univ South Alabama, Mobile, AL.

We report the first case of a patient with an unusual mosaic double aneuploidy with phenotypic features of Turner syndrome and Trisomy 14 mosaicism syndrome. The patient is a 15 year-old Asian female. She was born prematurely, at 7 months gestation and spent about three weeks in the NICU. She was later noted to be short with failure to thrive, areas of skin depigmentation, and developmentally delayed. Physical examination revealed a short and dysmorphic female with head circumference: 53 cm (25$^{tho}$%tile); Height: 128 cm (<2$^{ndo}$%tile) and Weight: 38 kg (<2$^{ndo}$%tile). Craniofacial dysmorphism includes hypertelorism, prominent forehead, micrognathia, short philtrum, low set and small dysplastic ears with outstanding pinnae and folded helix and a short neck with low posterior hair line. Striae of skin hyperpigmentation in trunk and lower back were noted and a Tanner I stage of sexual development suggested hypogenitalism that requires further investigation. The diagnoses of Hypomelanosis of ITO and CHARGE syndrome were considered and a chromosome analysis revealed a 46, XX karyotype. Array CGH analysis was performed on a blood specimen revealing an unusual double mosaicism of both monosomy X and trisomy 14. Array CGH result suggested mosaicism of both monosomy X and trisomy 14 in roughly equal percentages (10-15%). Chromosome analysis of 100 metaphases found 4 cells that were 46,X,+14. Interphase FISH analysis on 200 nuclei yielded 6.5% of nuclei with coexistent trisomy 14 and monosomy X, confirming the patient's karyotype 46,X,+14[4]/46,XX[96].nuc ish (DXZ1x1)(IGHx3)[13/202]. To our knowledge, this chromosome abnormality has not been previously reported, though Figuera et. al. (2008) noted a patient with a similar phenotype, a combination of Turner syndrome and trisomy 14 mosaicism, but whose karyotype was 45,X[45]/47,XX,+14[5]. Genotyping using chromosome 14 STR markers demonstrated only two alleles at each locus. Thus, trisomy 14 is unlikely due to a parental meiosis I non-disjunction event. Our patient's phenotype also suggests mosaicism in tissues other than blood. The primary event resulting in this double mosaicism should occur at early post-zygotic stages. However, without additional evidence it is difficult to envision a mechanism for the two numerical, coexisting chromosome abnormalities in a cell line. Further study is needed to clarify the etiology of this unusual abnormality.

### 1354/W/Poster Board #1012

**Utility of SNP arrays in detecting, quantifying, and determining meiotic origin of tetrasomy 12p in blood from Pallister-Killian patients.** *L.K. Conlin[1], M. Kaur[1], D. Clark[1], E. Zackai[1], M.A. Deardorff[1], H. Hakonarson[1,2], N.B. Spinner[1,3], I.D. Krantz[1].* 1) Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) The Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA; 3) Department of Pathology, The Children's Hospital of Philadelphia, Philadelphia, PA.

Diagnosis of the isochromosome 12p (i(12p)) associated with Pallister-Killian syndrome is complicated by the low frequency of this supernumerary chromosome in PHA stimulated peripheral blood lymphocytes, and frequently requires cytogenetic analysis of fibroblast cells for diagnosis. Recently, it has been shown that array CGH techniques are able to detect tetrasomy 12p in peripheral blood, even when not identified by cytogenetics. We studied 15 patients with a clinical diagnosis of Pallister-Killian syndrome using a genome-wide SNP array to investigate the ability to identify i(12p) in blood and tissue. Tetrasomy 12p was previously identified using traditional cytogenetic techniques in 12 patients, with the average age at diagnosis less than 1 year old. For this study, patient age at the time of tissue collection was between 2 months to 6.75 years. Array analysis verified tetrasomy 12p in all 13 samples from fibroblasts. Percent mosaicism was calculated using both probe intensity and allele frequency. The percent mosaicism ranged from 35% to 100%, and patterning of allele frequencies suggested Meiosis II origin in 12 patients. Tetrasomy 12p was detected in 6 of the 13 samples from unstimulated blood, with the percent mosaicism ranging from 0% to 75%. Previous cytogenetic testing results in PHA stimulated peripheral blood did not correlate with the array results; three of four patients with previous findings in blood were verified by array (5% to 75%), while two of six patients with previous negative findings in blood were found to have detectable tetrasomy 12p by array (10% and 20%). Of the eleven patients with both blood and tissue samples, two had similar percent mosaicism in both tissues, 3 patients had evidence for tetrasomy 12p in both tissues with much lower percent in blood, and 6 patients had no evidence for the tetrasomy 12p in blood. Analysis of the percentage of abnormal cells with patient age at time of study demonstrated that the frequency of the i(12p) decreased with age in blood, but not in fibroblasts. Two patients had unusual structural findings not detectable by array, with one patient with hexasomy 12p and one patient with a derivative 12p, containing tandem copies of 12p. Taken together, these findings stress the importance of traditional cytogenetic techniques, in addition to array technology, in the diagnosis of Pallister-Killian syndrome.

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## 1355/W/Poster Board #1013

**Microdeletion of the Down syndrome critical region at 21q22.** *R. Kosaki[1,2], H. Fujita[1,2], C. Tori[2], J. Kudoh[3], T. Takahashi[2], K. Kosaki[2].* 1) Department of Clinical Genetics and Molecular Medicine, National Center for Child Health and Development, Tokyo, Japan; 2) Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan; 3) Department of Molecular Biology and Center for Bioinformatics, Keio University School of Medicine, Tokyo, Japan.

The concept of Down syndrome critical region implies that there exist several dosage-sensitive genes of which duplication leads to abnormal phenotype. Considering that an altered amount of expression from crucial target genes, as a result of regulatory imbalance, tends to produce phenotypic effects in both monosomics and trisomics, haploinsufficiency for the Down syndrome critical region is expected to be associated with abnormal phenotype. We report a patient with severe microcephaly, developmental delay accompanied with hypospadias and corneal opacity who had microdeletion spanning Down syndrome critical region including DYRK1A and SIM2. A Japanese boy was born by a cesarean section at 39 weeks gestation due to intrauterine growth retardation. At birth, hypospadias and corneal clouding were noted. At age 14 months, he was referred to us for evaluation of multiple congenital anomalies with developmental delay. He had arched eyebrows, upslanting and narrow palpebral fissures, corneal clouding, left preauricular pit, bifid uvula, prominent nasal root, short columella, prominent cental incisors, pegged shaped teeth, retrognatia, hypoplastic nipples, thick toenails, and hypospadias. At age 3 years, surgical correction for hypospadias was performed. He never developed petechia and his thrombocyte count was normal. He could stand without support at age 3 years and walked alone at age 5 years and his gait continued to be ataxic. At age 8 years, he had no meaningful words but understood simple commands. His G-banded karyotype was normal. Array comparative genomic hybridization using the Agilent 105K Whole Human Genome CGH array revealed a de novo deletion of 3.97Mb, extending from 34984142 to 38959902 on 21q22 (hg18; NCBI Build 36.1). Mouse gene targeting experiments of the mouse homologs of genes within the deleted interval suggest that some of the phenotype of the proband might be attributed to haploinsufficiency of these two genes: Extreme degree of microcephaly in the present case may be ascribed to haploinsufficiency of DYRK1A because brain size is severely reduced in heterozygotes for Dyrk1a null mutation. Cleft uvula, a microform of cleft palate, may be due to haploinsufficiency of SIM2 because cleft palate is observed in heterozygotes for Sim2 null mutation. Third, ataxia may be associated with haploinsufficiency of KCNJ6, an inwardly rectifying K channel because the “weaver” mice with Kcnj6 hypomorphic mutation present with ataxia.

## 1356/W/Poster Board #1014

**Small Supernumerary Marker Chromosomes characterization elucidated by array techniques.** *L. Kulikowski[1,3], F.M.S. Jehee[2], R. Pelegrino[4], D.C.B Rosolen[3], L. Antonangelo[3], M.N. Buratini[3], M.A.C. Smith[1], M.I. Melaragno[1].* 1) Morphology & Gen, Univ Federal de Sao Pa, São Paulo, Brazil; 2) Instituto de Biociências Univers de São Paulo, Brazil; 3) Divisão de Laboratório Central - HC LIM 03 - FMUSP Univ de São Paulo, Brazil; 4) Molecular Core AFIP, Brazil.

Supernumerary marker chromosomes (SMCs) are a frequent chromosome alteration, although their molecular unbalanced content and their mechanism of origin are often not precisely characterized. We reevaluated four phenotypically abnormal patients, with autosomal SMCs identified previously by G-banding. We used arrayCGH or oligo-array techniques for the chromosome characterization in order to allow a better genotype-phenotype correlation.In two patients, who presented developmental delay and SMCs derived from chromosome 15, molecular investigation revealed a duplication of about 13.8 Mb, region that contain genes associated to psychiatric diseases. One of the patients presents infantile psychosis. In another patient, the SMC was inherited from her mother who presents the extra chromosome in mosaic form. Array studies revealed the marker was originated from chromosome 19 with about 44.9 Mb duplication. This unbalance has been associated with ophthalmologic disease, as found in this family. One of the patients presents a complex SMC derived from duplication of short arms of chromosome 18 and 19 with about 10,6 Mb e 26,8, respectively. These regions are associated with neurologic disease, in accordance with psychomotor retardation present in the patient.Thus, high-resolution genome-wide analysis has greatly facilitated the characterization of SMCs and has proven to be advantageous in the detection of complex rearrangements. The increasing use of array techniques in clinical cytogenetic laboratories will allow for more accurate SMC/phenotype correlation improving the clinical diagnosis genetic counseling and risk evaluation. (Financial support: FAPESP, CAPES).

## 1357/W/Poster Board #1015

**Case Report: A girl with a 46,XX/46.XX,inv(1)(p21;q44) karyotype and a characteristic phenotype.** *J. Prieto[1,2], A. Lopez[1], G. Gordillo[1], R. Romero[2].* 1) Instituto de Genética Humana, Universidad Javeriana, Bogotá, Colombia; 2) Hospital La Victoria, Secretaría de Salud, Bogotá, Colombia.

Pericentric inversions are structural intrachromosomal rearrangements resulting from two breaks on both sides of the centromere followed by the 180° rotation of the chromatin segment between these breaks. This is not an infrequent structural chromosomal anomaly in humans, with a frequency from 0.089 to 0.34% in the general population or even from 1 to 2% depending on the type of inversion reported. Although the evidence is still too limited to allow a confident conclusion, it seems that pericentric inversions are more frequent in certain chromosomes and at certain breakpoints. It is commonly noted in chromosome 3 with breakpoints at 3p25, 3q21 or 3q25; also common is inv (18)(p11q21) and inversions of chromosomes 10-12 with breakpoint close to the centromeres. Until now, there have been reports of patients with this kind of chromosomal rearrangements associated with male infertility, multiple miscarriages, papillary carcinoma of the thyroid and Bardet-Biedl syndrome. The female patient was born of non consanguineous healthy parents, a product of a third controlled pregnancy with no pathological neonatal events referred. She has a history of left eye strabismus surgically corrected and nephritis at 4 years old. Her psychomotor development was normal. At physical examination she had normal antropometrical parameters but some characteristic findings as ocular hypertelorism, epicanthal folds, palpebral ptosis of left predominance, flat nasal bridge, flat facial profile, prominent ears with folded helix; she also presents with palmar keratoderma. The band R karyotype report: 46,XX/46.XX,inv(1)(p21;q44) in 1 metaphase of 80. In this case we report a mosaic inversion of the 1 chromosome accompanied by specific phenotype characteristics. This has been the first case described to date. We continue to check literature.

## 1358/W/Poster Board #1016

**Demonstration of recombination resulting in paternal transmission of an Xp deletion originating on the Y chromosome to multiple offspring.** *N. Hoppman-Chaney[1], W. Smith[2], D. Oglesbee[1], E. Thorland[1], S. Jalal[1], J. Keefe[1], K. Olson[1], J. Yuhas[1], G. Velagaleti[1].* 1) Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; 2) Division of Genetics, Maine Medical Center, Portland, ME.

Subtelomeric deletions of the X and Y chromosomes are generally thought to be benign variants unless they include adjacent genes. We report a case of an 8 year-old-female with a history of developmental delay, severe autism, and short stature. G-banded chromosome analysis revealed a normal karyotype; however, subtelomere FISH studies identified a cryptic deletion of Xp. Subtelomere FISH on the parents showed mother with normal results, while father was positive for an Yp deletion with a normal X chromosome. Two of her three younger siblings were found to have a similar Xp deletion, while one of a set of fraternal twins did not. Additional follow-up using arrayCGH and BAC-FISH mapping indicated that this deletion was approximately 700-940kb in size and includes the SHOX gene, consistent with short stature observed in the individuals with this deletion. Also interesting is the occurrence of cleft palate in both the father and the twin daughter with the deletion, but not in the other affected daughters. Likewise, the features of autism are variable among the daughters ranging from severe autism requiring admission to a psychiatric facility in the proband, high functioning in another and normal development in the third; the twin without the deletion has concerning behaviors. The fact that the three affected daughters had a demonstrable Xp deletion with normal Xp in both parents and a Yp deletion in the father suggests that a crossover occurred in the pseudoautosomal region during paternal meiosis which led to the transfer of this deletion onto the paternal X chromosome. This hypothesis was supported by microsatellite genotyping of dinucleotide repeats spanning the X chromosome of both parents, 2 daughters with the deletion, and the daughter without the deletion. The observation that one daughter did not inherit this deletion, while her fraternal twin sister did, also argues against the requirement of an obligate crossover in the pseudoautosomal region during male meiosis. The heterogeneity of clinical features among the affected daughters and the father might be explained by variation in the size of deleted segment since each recombination event is independent and crossover might have occurred at different regions in the pseudoautosomal regions. Oligonucleotide arrayCGH studies are in progress to determine the extent of deletion in each of the affected daughters and the father to further explore the phenotypic variation.

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## 1359/W/Poster Board #1017

**del(1)(p21p22) syndrome: a Prader Willi like phenotype.** *J.G. Pappas[1], J. Borsuk[1], P.R. Papenhausen[2].* 1) Dept Pediatrics,Human Gen, New York Univ, Sch Med, New York, NY; 2) Dept Cytogenetics, Labcorp America, Res Triangle Park, NC.

We present a 22 month old boy with poor growth, developmental delay and 46,XY,del(1)(p21.1p22.1) karyotype. There are three cases reported in the medical literature with deletion breakpoints in bands 1p21 and 1p22 (Finelli P et al, 2001; Stockton DW et al, 1997). The subband of the breakpoints is different in each case and our case. These three cases and our case share common features including cardinal features of Prader Willi syndrome (PWS). Our patient was born 2.73 kg at full term via vaginal delivery to a 33 year old gravida 7 para 7 mother. During infancy he was feeding poorly and his weight was below the 5th centile. He was diagnosed with hypotonia and laryngomalacia. He had normal echocardiography and renal ultrasound. At 16 months, he started sitting unsupported and his muscle tone and food intake started improving with physical therapies. He could not stand unsupported, he had no speech, his head circumference was below the 2nd centile, his length below the 5th and his weight between the 10th and 25th centile. He had closed fontanelles, palpable metopic ridge, micrognathia, narrow and high arched palate, bilateral epicanthal folds, green eyes (rest of family had brown eyes), light brown hair (rest of family had dark brown hair) and very light skin complexion. The deletion in our case was ascertained with chromosome microarray and subsequently with routine karyotype. Whole genome chromosome microarray utilizing 1,800,000 region specific SNP/CN targets (Affymetrix, Inc.) revealed a 9.738 MB interstitial deletion 1p21.1->p22.1 with base pair linear position from 93610986 to103348942. The parents had normal karyotypes and whole genome chromosome microarray tests. The deleted segment in our case includes 30 genes annotated in OMIM. There is no known association of haploinsufficiency of any of these genes with phenotypic characteristics of our case. The case presented by Finelli at al was an 11 year old boy with hyperphagia and similar developmental history and facial features to our case. The DNA methylation test for PWS was negative in our case and the previously reported case (Finelli et al, 2001). The del(1)(p21p22) syndrome resembles PWS because of infantile hypotonia and poor feeding that improves in childhood, microcephaly, short stature, hypopigmented hair, eyes and skin as well as hyperphagia, obesity and developmental delays in childhood. Our case provides precise breakpoints and phenotypic data to delineate the del(1)(p21p22) syndrome.

## 1360/W/Poster Board #1018

**A 7Mb terminal deletion of chromosome 3p with normal phenotype: case report and review of the literature.** *G. Tiller, S. Morton, K. Wendt.* Dept Genetics, Kaiser Permanente, Los Angeles, CA.

The advent of comparative genomic hybridization by microarray (array CGH, or aCGH) has led to the characterization of genomic deletions and duplications, some of which appear to have no clinical consequence. We report an adult male with a 7Mb terminal deletion of chromosome 3p with a normal phenotype. His wife presented for prenatal counseling due to advanced maternal age; amniocentesis revealed a 46,XX,del(3)(p26) karyotype in the fetus. The couple elected to terminate the pregnancy despite determination that the paternal karyotype demonstrated the same terminal 3p deletion. The deletion was further characterized by aCGH, and found to span 36,006-7,028,403. Fourteen genes have been identified in the region, including two (CHL1 and CRBN) which are associated with mental retardation. Takagishi et al. (2006) reported a mother and daughter with an 8.8Mb terminal deletion of 3p and normal phenotype, whereas Fernandez et al. (2008) and Dijkhuizen et al. (2006) reported patients with mental retardation and mild dysmorphic features who carried 4Mb deletions within the 7Mb region deleted in our patient. These cases demonstrate the difficulty in predicting the consequences of individual chromosomal aberrations, and underscore the importance of establishing collaborative aCGH clinical databases to assist in effective genetic counseling.

## 1361/W/Poster Board #1019

**Balanced reciprocal translocation 46,XX,t(1;4)(p13.1;q34.3) associated with severe unexplained emphysema.** *N. Jinawath[1], R. Yonescu[2], N. Rush[4], K.S. Schweitzer[4], K. Murphy[2], D.B. Pearse[3], I. Petrache[4], C.A. Griffin[1,2].* 1) Institute of Genetic Medicine, Johns Hopkins Medical Institution, Baltimore, MD; 2) Department of Pathology, Division of Molecular Pathology, Johns Hopkins Medical Institution, Baltimore, MD; 3) Department of Medicine, Johns Hopkins Medical Institution, Baltimore, MD; 4) Department of Medicine, Indiana University, Indianapolis, IN.

Emphysema is a chronic obstructive pulmonary disease leading to significant morbidity and mortality. While most cases are caused by tobacco abuse, genetic defects such as mutations of alpha-1 antitrypsin (AAT) causing decreased AAT levels underlie some cases. We report a case of unusually severe emphysema (FEV1 =0.5 L/s) in a 45 year-old woman. Her clinical history of 15 pack-year cigarette smoking and MZ AAT genotype with normal circulating A1AT levels were considered insufficient to explain the severity of her illness. We hypothesized her constitutional balanced translocation, 46,XX,t(1;4)(p13.1;q34.3) may have resulted in an increased susceptibility to her developing severe and accelerated emphysema. Under an IRB-approved protocol, we mapped the breakpoint using BAC-FISH and DNA fiber-FISH, and identified the breakpoint to be inside BAC clones RP4-686J16 and RP11-763N18 on chromosome 1p13.1 and 4q34.3, respectively. Subsequent mapping using additional BAC clones on chromosome 1p13.1 identified the breakpoint between the genomic location 116,974,711 - 117,009,114 (NCBI Build 36.1), which mapped to intron 2 of *IGSF3*, a member of immunoglobulin superfamily known to be expressed in alveolar basal epithelial cells, but with unclear function. The chromosome 4 counterpart of this breakpoint contained no known gene. Affymetrix SNP6.0 array analysis using DNA from the lymphoblastoid cell line identified no genomic gain or loss in the vicinity of the breakpoint or elsewhere in the genome. Since the coding sequence of both *IGSF3* variants 1 and 2 start from exon 2, we predict that expression of *IGSF3* in this patient should be disrupted and may lead to reduced expression of IGSF3 protein. Immunohistochemistry was performed using IGSF3 antibody (R&D Systems) on human emphysematous lung tissue sections from 4 individuals and 4 control lungs and on lung tissue from mouse models of emphysema (cigarette smoke-exposed DBA/2 and C57BL/6 mice). We found significantly reduced IGSF3 expression in the human lungs with emphysema and in the cigarette smoke-exposed mice compared to normal lung tissues. Further studies on the roles of IGSF3 in lung physiology may elucidate its involvement in the pathobiology of emphysema.

## 1362/W/Poster Board #1020

**Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioral problems, dysmorphism, epilepsy, and abnormal head size.** *M. Shinawi[1], P. Liu[1], S-H. King[1], J. Shen[2], A. Pursley[1], A. Patel[1], P. Stankiewicz[1], A.L. Beaudet[1], S.W. Cheung[1], J.R. Lupski[1], CLINICAL 16p11.2 CONSORTIUM.* 1) Dept Molec & Human Gen, Baylor Col Med, Houston, TX; 2) Children's Hospital Central California, Madera, CA.

Microdeletion and the apparent reciprocal microduplication on 16p11.2 were recently associated with autism and developmental delay. We identified 27 deletions and 18 duplications of 16p11.2 in 0.6% of all samples submitted to the Medical Genetics Laboratories at Baylor College of Medicine (BCM) for clinical array-CGH analysis. Detailed molecular and phenotypic characterizations were performed on 17 subjects with deletion and nine subjects with duplication. One subject was asymptomatic and another had a normal cognitive and behavioral phenotype. The most common clinical manifestations in our cohort were speech delay and cognitive impairment. Other phenotypes associated with deletion included motor delay (50%), seizures (~40%), behavioral problems (~40%), and congenital anomalies (~30%). The autism phenotype was observed in only ~20% of deletion cases. The phenotypes associated with duplication included motor delay (6/9), behavioral problems (especially ADHD) (5/9), congenital anomalies (4/9) and seizures (2/9). Patients with the 16p11.2 microdeletion had statistically significant ($p<0.0017$) large mean head size and 2/3 of them had absolute or relative macrocephaly. Five of the nine patients with the microduplication had microcephaly. The broad forehead, macrocephaly, and flat midface render a distinct facial gestalt to the deletion patients. The chromosomal rearrangement was *de novo* in most of the deletion cases in which parental studies were available but equal inherited and *de novo* cases were observed for the duplication. Our data expand the spectrum of phenotypes associated with the 16p11.2 rearrangements. The incomplete penetrance and variable expressivity in these rearrangements complicate the interpretation of the molecular data and the genetic counseling. *CLINICAL 16p11.2 CONSORTIUM:* ***J. Belmon[1], D. Scott[1], F. Probst[1], W. Craigen[1], B. Graham[1], G. Clark[2], J. Lee[2], M. Proud[2], A. Stocco[2], D. Rodriguez[2], S. Sparagana[3], B. A. Kozel[4], E.R. Roeder[5], S.G. McGrew[6], T.W. Kurczynski[7], L.J. Allison[8], S. Amato[9], S. Savage[9].*** *[1]Baylor College of Medicine, [2]Texas Children's Hospital, [3]Texas Scottish Rite Hospital for Children, [4]Washington University School of Medicine, [5]University of Texas Health Science Center at San Antonio,[6]Monroe Carell, Jr. Children's Hospital at Vanderbilt,[7]Akron Children's Hospital, [8]Monarch Medical Clinic,[9] Eastern Maine Medical Center..*

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## 1363/W/Poster Board #1021

**Frequency of Cytogenetic Abnormalities and Testing in Children with Congenital Defects, Metropolitan Atlanta 1986-2005.** *J. Jackson, S.A. Rasmussen, J. Cragan, K. Crider, R. Olney.* Birth Defects & Dev Disabilities, Ctr Disease Control Prevention, Atlanta, GA.

We assessed changes in the usage of cytogenetic testing of children born with congenital defects from 1986 through 2005 using data from the Metropolitan Atlanta Congenital Defects Program (MACDP). MACDP, a population-based surveillance system covering the five central metropolitan Atlanta counties, uses active methods of ascertainment to identify infants with birth defects from birth and pediatric hospitals, and certain laboratories and physician offices. Data on cytogenetic testing were obtained from hospital records and from a local academic cytogenetic laboratory, with the addition of data from a commercial laboratory for the years 1997-2005. The frequency of cytogenetic testing in children with birth defects more than tripled from 7.6% in 1986 to 23.2% in 2005 ($p<0.001$). However, prenatal cytogenetic testing in mothers aged 35 years and older declined from 33.1% in 1996 to 19.5% in 2005 ($p<0.001$); prenatal testing in mothers less than 35 years old remained fairly constant (6.4% in 1996, 5.6% in 2005; $p=0.4$). The number of infants identified with cytogenetic abnormalities increased significantly through the years: autosomal trisomies increased from 13.0 to 18.3 per 10,000 births ($p<0.001$) and other autosomal abnormalities from 3.0 to 5.4 per 10,000 births ($p<0.001$) from 1986 to 2005, respectively. Finally, we showed the value of adding an additional data source. Inclusion of results from a commercial laboratory for the years 1997-2005 increased the number of MACDP records with recorded cytogenetic test results by approximately 2% (330 cases), of which 6.5% of these were abnormal. Our results indicate an overall increase in the use of cytogenetic studies of infants with birth defects; however, a decrease in prenatal testing in women aged 35 years and older was also observed. The reasons for this decrease are unknown, but could be related to changes in noninvasive prenatal screening technologies that might be used instead of diagnostic testing by amniocentesis or chorionic villus sampling.

## 1364/W/Poster Board #1022

**The diagnostic relevance of genotyping data derived from diagnostic SNP arrays.** *R. Pfundt[1], N. de Leeuw[1], J. Hehir-Kwa[1], L. Moruz[1], C. Ruivenkamp[2], A. Gijsbers[2], H. Yntema[1], B. de Vries[1], B. van Bon[1], N. Leijsten[1], I. Neefs[1], T. Machielsen[1], S. van Gessel[1], M. Wunderink[1], J. Veltman[1], D. Smeets[1].* 1) Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 2) Center for Human and Clinical Genetics, Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands.

Cytogenetic centers worldwide are on the verge of a revolution since, for a number of patient groups, classical chromosome analysis by karyotyping is being replaced by molecular karyotyping using microarrays. In our diagnostic cytogenetic laboratory, array analysis was implemented for patients with mental retardation with/without multiple congenital anomalies (MR/MCA) in 2003, initially using BAC arrays and only after a normal karyotype. In 2007 we switched to the Affymetrix 250k SNP array platform and more than 2000 patients and over 500 parental samples have now been diagnostically screened. This has resulted in the identification of many (novel) relevant copy number variations (CNVs) and a diagnostic yield of more than 13%. Based on these results we decided in January 2009 to make array analysis, instead of karyotyping, the “first pass” diagnostic test for MR/MCA patients. SNP based microarrays do not only provide reliable data for CNV analysis, but also generate a genotyping profile for every individual that is hybridized. Does this additional SNP data have an added value, compared to non-SNP based array platforms, or is it a byproduct without diagnostic relevance? In this presentation we present our experience in using genotype information in the diagnostic workflow of MR/MCA patients. Firstly, SNP data of patient and parent trios are screened for Mendelian inconsistencies. Based on this analysis we can rule out sample mix-up, potential non-paternity and screen for uniparental disomies (UPD). So far we have encountered one case of non-paternity and have identified 4 cases of UPD. Secondly, SNP information can be used determine the parent-of-origin of a CNV (deletions and duplications). In a number of cases this could be used to determine the de novo occurrence of a CNV even when one of the parents was not available for genetic analysis. Thirdly, the SNP data of individual patients is screened for stretches of homozygosity, indicating consanguinity (in case of many large regions) or identity by descent (single regions ranging in size from 2 - 60 megabases). We conclude that the genotyping information from SNP based array platforms is very helpful for the practical diagnostic array workflow and increases the diagnostic yield of molecular karyotyping. In addition, this genotype information from large patient cohorts will be enormously useful in research projects aimed at identifying recessive loci and for SNP-based association studies.

## 1365/W/Poster Board #1023

**Nonrecurrent genomic rearrangements associated with CMT1A or HNPP and their underlying mechanisms.** *F. Zhang[1], M. Khajavi[1], C.F. Towne[4], S.D. Batish[4], E. De Vriendt[5], P. De Jonghe[5], A. Vandenberghe[6], F. Palau[7], L. Van Maldergem[8], V. Timmerman[5], J.R. Lupski[1,2,3].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030; 2) Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030; 3) Texas Children’s Hospital, Houston, TX 77030; 4) Athena Diagnositics, Inc., Worcester, MA 01605; 5) Department of Molecular Genetics, VIB and University of Antwerp, Universiteitsplein 1, 2610 Antwerpen, Belgium; 6) Unité de Neurogénétique Moléculaire, Laboratoire de Biochimie, Hôpital de l’Antiquaille, F-69005 Lyon, France; 7) Institut de Biomedicina, Consejo Superior de Investigaciones Cientificas, Valencia, Spain; 8) Centre de Génétique Humaine, Institut de Pathologie et de Génétique, Loverval, Belgium.

Genomic rearrangements involving the peripheral myelin protein gene (*PMP22*) in the human chromosome 17p12 are associated with neuropathy; duplications cause Charcot-Marie-Tooth disease type 1A (CMT1A; MIM 118220), whereas deletions can lead to hereditary neuropathy with liability to pressure palsies (HNPP; MIM 162500). Our previous studies showed that most of these rearrangements are recurrent and mediated by the mechanism of non-allelic homologous recombination (NAHR), whereas nonrecurrent rearrangements also exist and their underlying mechanisms are not well investigated. In this study, we investigated 17 CMT1A/HNPP-associated nonrecurrent rearrangements by high-resolution oligonucleotide array comparative genomic hybridization (aCGH) analysis and breakpoint sequence analyses. We identified two identical complex duplication-triplication rearrangements (of which *PMP22* is triplicated), four *PMP22* duplications (two of them are identical), two identical *PMP22* deletions from the same family, and nine deletions involving only one or several *PMP22* exons (one of them is a complex exonic deletion). Our observations suggest that various molecular mechanisms, including non-homologous end-joining (NHEJ), replication-based fork stalling and template switching (FoSTeS) and/or microhomology-mediated break-induced replication (MMBIR), are implicated in the generation of nonrecurrent rearrangements of *PMP22*. Interestingly, the identical FoSTeS/MMBIR-mediated complex exonic deletion was also identified in the patient’s affected sibling and found to be mosaic in the unaffected mother, which suggests that this complex deletion is generated mitotically, consistent with a replication-based event.

## 1366/W/Poster Board #1024

**Inherited Duplication of 22q11.2 Concomitant with Mosaic Turner Syndrome.** *S. Beiraghi[1], A. Thurmes[1], S. Holzwart[1], B. Hirsch[2].* 1) Cleft-Palate Craniofacial, Univ of Minnesota, Minneapolis, MN; 2) Lab Medicine & Pathology, Univ of Minnesota, Minneapolis, MN.

Microduplication 22q11.2 syndrome is associated with variable, often subtle phenotypic findings, that typically include cleft palate, speech and neuropsychological issues. Here we present the first report of a female infant with Mosaic Turner Syndrome and a paternally inherited 22q11.2 duplication. This infant was born at full term, to a 31-year-old ($G_1P_1ab_0$); APGAR scores were nine at 1 and 5 minutes respectively. Cleft palate, left pre-auricular pit, patent foramen ovale, hypocalcemia, and congenital hip dysplasia were noted at birth. G-banded chromosome analysis and FISH analysis for 22q11.2 were ordered. G-banding revealed mosaicism for two cell lines: one with 45,X (95% of metaphases), and one with 46,XX (5% of metaphases).. FISH revealed a duplication of the TUPLE1 locus in 50 of 50 interphase cells. A follow-up specimen, analyzed by array comparative genomic hybridization (a-CGH) using a 44K oligonucleotide platform, confirmed a 2.4 Mb duplication within 22q11.2. The boundaries of the duplication were the same as those typically seen in the deletion associated with DiGeorge/VCSF. Parental FISH studies showed paternal 22q11.2 duplication. These findings highlight the ability of a-CGH to detect and characterize genomic imbalances and further define the molecular etiology of clinical phenotype seen in microduplication 22q11.2 syndrome. Furthermore, without the input of the craniofacial team, the duplication of 22q11.2 would not have been diagnosed, as the abnormality was too subtle to be seen in the newborn G-banded chromosomal study.

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### 1367/W/Poster Board #1025

**Constitutional Abnormalities of Chromosome 18: Hacettepe University Experience.** *D. Aktas, G.E. Utine, Y. Alanay, K. Boduroglu, M. Alikasifoglu.* Dept. of Genetics, Hacettepe University Medical School, Ankara, Turkey.

Clinical and cytogenetic characterizations of partial monosomy 18p and partial trisomy 18q have been well documented. Deletions involving the long arm of chromosome 18 have also been reported in many patients. Most of them are localized in distal part of the long arm. However, smaller interstitial deletion 18q12.3 is less common. Furthermore, isochromosome 18q is a rare but distinctive syndrome. The common clinical manifestations associated with i(18q) closely resemble those of trisomy 18 and 18p deletion syndrome. Moreover the paracentric inversion of the long arm of chromosome 18 is also rare and the phenotypic findings are similar to those seen in 18q deletion syndrome. Additionally, due to the formation process of the ring chromosome 18, the phenotype of the r(18) patients is similar to the features of the 18q deletion syndrome. We presented patients carrying constitutional abnormalities of chromosome 18. Eight patients recruited: one with an 18p deletion syndrome; two with an 18q deletion syndrome and one of them has an interstitial deletion; one with trisomy 18q syndrome, two with isochromosome 18q syndrome and one with inversion 18q and one with ring chromosome 18q. The clinical phenotype and cytogenetic/molecular cytogenetic findings of our patients are reviewed and discussed.

### 1368/W/Poster Board #1026

**CHROMOSOME POLYMORPHISMS ASSOCIATED WITH RECURRENT FETAL LOSS.** *C.L. Campanhol[1], S.C.S. Lima[3], E. Couto[2,4], J.K. Heinrich[1], R. Barini[2,4].* 1) Cytogenetics Core at CAISM - Women's Hospital - State University of Campinas - UNICAMP, Campinas,SP, Brazil; 2) Department of Gynecology and Obstetrics - Faculty of Medical Sciences - State University of Campinas - UNICAMP, Campinas, SP, Brazil; 3) BSL Reproduction Immunology Laboratory - Campinas, SP, Brazil; 4) Obstetrics Area at CAISM - Women's Hospital - State University of Campinas - UNICAMP, Campinas, Brazil.

Chromosomal abnormalities play an important role for the etiology of the recurrent fetal loss as well as other immunological, endrocrine, morphological and gene mutational causes. Chromosomal polymorphisms have always been considered as silent alterations with no clinical repercussion. More recently many groups have indicated that recurrent fetal loss is a frequent finding in individuals with heterochromatin length polymorphisms and pericentromeric inversions of the chromosome 9. The objective of this study was to determine the frequency and distribution of specific chromosome abnormalities in individuals with recurrent spontaneous abortion, fetal abnormality and/or fetal death in a Women's University Hospital and a private outpatient service. Samples were 60% retrospectively and 40% prospectively included in the study in an 8-year time frame. Patient data were retrieved and the karyotype was accessed using conventional G and C banding. A total number of 1236 karyotypes were analyzed. Having at least two episodes of fetal loss, fetal abnormality and/or fetal death was assigned as an inclusion criteria. The total number of cases included from the University Hospital was 202 and from the private clinics was 1034. Normal karyotypes were found in 166 (82,17%) and 964 (93,23%) individuals. Twelve (5,94%) and 17 (1,64%) displayed aneuploidy; 7 (3,46%) and 13 (1,25%) displayed sctructural chromosome abnormalities; 18 (8,91%) and 40 (3,86%) showed polymorphic variants. Among women, ages varied from 19 to 42 (average of 29,59) and from 25 to 47 (average of 33,85). Among men, ages varied from 16 to 46 (average of 31,14) and from 26 to 55 (average of 35,51). Our results corroborate with the recent hypothesis which shows a possible contribution of chromosome polymorphisms to the recurrent fetal loss and also emphasize the importance of cytogenetic evaluation for an accurate approach to elucidate the etiology of fetal loss. Genetic counseling should be offered as the individuals should be assisted to decide as to deal with future reproductive risks.

### 1369/W/Poster Board #1027

**Multiple intrachromosomal rearrangements leading to a derivative chromosome 9 detected by conventional cytogenetic analysis and characterized by array CGH.** *N. Christacos, J. Meck, J. Kelly, S. Schonberg, J. Scheerle, N. Kamara, P. Mowrey.* Cytogenetics Dept, Quest Diagnostics Nichols Institute, Chantilly, VA.

We report the cytogenetic findings on a 5-year old female with multiple congenital anomalies including microcephaly, seizures and mental retardation. Conventional cytogenetic analysis identified a structurally abnormal chromosome 9 interpreted as add(9)(q33). Fluorescence in situ hybridization (FISH) studies and array CGH (aCGH) using the ClariSure 1MB BAC array chip (Quest Diagnostics Nichols Institute) revealed a complex intrachromosomal rearrangement. Specifically, the array CGH showed five abnormal regions on the long arm of one chromosome 9, which were all FISH confirmed using the appropriate BACs as FISH probes. Briefly, there was a large region of duplication from bands 9q13 to 9q21.33 (16.65 Mb/70,459,999-87,110,000) as well as a region of deletion at band 9q33.3 (2.62 Mb/ 122,650,001-125,269,996). In between these two regions, there were two separate single clone gains and one two clone gain. The most proximal and largest duplication and the most distally located deletion, both with multiple genes involved, are considered to be causative in the abnormal phenotype and cognitive development seen in this child. The other three smaller abnormalities may be copy number variants. Parental aCGH testing has been requested and will be instrumental in making this determination. Furthermore, additional molecular testing will be performed to help further define this complex intrachromosomal abnormality and elucidate a possible mechanism for its formation. To conclude, partial trisomy of 9q is rare and, to our knowledge, cases with a similar pattern of gains and loss have not been reported.

### 1370/W/Poster Board #1028

**Characterization of Contiguous Gene Syndromes in the NIGMS Human Genetic Cell Repository by the Affymetrix GeneChip SNP 6.0.** *N. Gerry[1], L. Toji[1], C. Beiswanger[1], J. Collins[2], J. Leonard[1].* 1) Coriell Institute for Medical Research, Camden, NJ; 2) Affymetrix Inc., Santa Clara, CA.

The Affymetrix SNP Array 6.0 was used to define the chromosomal segments gained and lost in one hundred and seventy-one micro or sub-micro deletion or duplication abnormalities from twenty-two disorders that were previously characterized by FISH or PCR. Data were analyzed for copy number changes using both Affymetrix Genotyping Console Software and Partek Genomics Suite. In each case the segments detected were of the expected size and included the critical regions for the respective disorder: Deletion 1p36 syndrome, Wolf-Hirschhorn, Cri-du-chat, Williams-Beuren, Greig Cephalopolysyndactyly, Trichorhinophalangeal II, Wilms Tumor-Aniridia-Genitourinary Anomalies and Mental Retardation, Potocki-Shaffer, Angelman, Prader-Willi, Chromosome 15q11-q13 Duplication, Charcot-Marie-Tooth A1, Miller-Dieker, SmithMagenis, Deletion 18p,Deletion 18q, DiGeorge, and Velocardiofacial syndromes, X-linked Ichthyosis, Duchenne Muscular Dystrophy, Pelizaeus-Merzbacher Disease, and Azospermia. The resolution of the Affymetrix SNP Array 6.0 allowed characterization of chromosomal copy number changes in the kilobase range. Within the samples studied, the smallest change detected was a 35 kb deletion of an exon in SNRPN of a Prader-Willi sample. This result, along with many other submicroscopic changes detected on the array agreed with prior molecular mapping experiments. The high resolution of the array also provided the ability to distinguish between chromosome breakpoints in multiple samples of the same disorder that appeared nearly identical by traditional G-band analysis, but in fact were all unique. In addition, the array provided more accurate breakpoints for submicroscopic changes that had previously been identified by FISH using only one or at most a few markers in the syndromes critical region. Finally, previously unrecognized complexities such as cryptic translocations and duplications adjacent to syndrome specific deletions were identified by the array. The copy number segment data and graphic output from Affymetrix Genotyping Console are being used to upgrade the descriptions of these samples on the Coriell Cell Repositories web-site (http:// ccr.coriell.org/) to better inform users in their decisions for experimental design and choice of reference samples. Each of the samples is publicly available as a culture and many are available as DNA.

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### 1371/W/Poster Board #1029

**22q12.1 deletion: a new cause of syndromic mental retardation.** *A.P Jinnah[1], C. Bacino[1], S.W Cheung[1], A. Patel[1], C.W Brown[1,2].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, Baylor College of Medicine, Houston, TX.

We report a six year old girl with short stature, moderate mental retardation, bradycardia, and facial dysmorphic features including hypertelorism, preauricular tags, unilateral simplified ear, broad nasal tip, and high arched palate with bifid uvula. Family history is significant for several members, including her mother, with variable severity of mental retardation, hearing loss, cleft palate, and facial dysmorphism. Although the craniofacial features were reminiscent of velocardiofacial syndrome (VCFS), chromosomal microarray analysis revealed a 2.157 Mb loss in 22q12.1 occurring between (but not including) the VCFS (22q11.2) and McDermid region (22q13) regions. The microdeletion encompasses three genes, MN1 (meningioma 1), PITNB (phosphotidylinositol transfer protein beta) and TTC 28 (tetraticopeptide repeat protein 28). Haploinsufficiency of these genes has previously been reported in at least one other unrelated patient with similar clinical features. MN1 is a transcription factor that functions in part as a nuclear receptor coactivator protein. It is expressed in fetal brain, cardiac myocytes, osteoblasts, skeletal, and smooth muscle. Expression of PITNB and TTC 28 have been identified in many tissues, including brain. Targeted homozygous deletion of MN1 mice is post-natal lethal due to severe cleft palate. In addition abnormalities of intramembranous ossification processes of craniofacial bones, lethargy and poor sucking behavior have also been described in this animal model. We propose that deletion of 22q12.1 represents a new familial mental retardation syndrome in patients presenting with VCFS-like facial features and other congenital anomalies.

### 1372/W/Poster Board #1030

**Emerging Challenges in Interpreting Microduplications 1-10 Mb in Size in a Clinical Setting.** *S.-H.L. Kang, S. Neill, C.A. Shaw, C.A. Bacino, S.R. Lalani, P. Stankiewicz, A. Patel, S.W. Cheung.* Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

The increased use of array comparative genomic hybridization (aCGH) based assays has revealed extensive structural variation in the human genome. In the clinical setting, interpretation of these variations can often present a diagnostic dilemma. We evaluated a total of 4186 patient samples on a custom designed whole-genome oligonucleotide microarray (Agilent 105K). At least one copy number variation (CNV) was detected in 1356 patients (32.4%) and a total of 1569 CNVs were detected (some patients had more than one CNV). The abnormal detection rate in our cohort of patients was 16.6% (693/4186). Of the 693 pathogenic CNVs, approximately twice as many deletions (N=356) as duplications (N=176) were identified. These CNVs included many known syndromic regions that are commonly observed or have been newly described. However, a major huddle when using microarrays with whole-genome coverage is interpreting the clinical significance of genomic imbalances with no clear disease association. In particular, determining the clinical consequence of large (>1 Mb) microduplications, including reciprocal rearrangements of disease associated regions, frequently pose a challenge. We identified 164 duplication CNVs between 1-10 Mb in size. Parental analyses were completed for 78 patients, and remain pending on 86. Of the cases with follow-up parental studies, 35 were shown to be *de novo* and interpreted as pathogenic, and 43 were inherited from a parent carrying the same genomic imbalance. Two inherited duplications were in known disease associated regions (22q11.2 microduplication and CMT1A duplication on 17p12), and also interpreted as pathogenic. Nine involved duplication in a region associated with a known microdeletion syndrome and 32 were found in regions with no clear disease association. Given the size of the CNV, one presumes that the genomic imbalance would have clinical relevance. However, this hypothesis is confounded by inheritance from a seemingly phenotypically "normal" parent. This category of aCGH results blurs the boundaries between incomplete penetrance (and therefore interpreted as abnormal) and clinically benign. Understanding the clinical consequence of these observations will require compilation of genotype-phenotype data in larger cohorts of patients and controls.

### 1373/W/Poster Board #1031

**Molecular Characterization of a Chromosome 12q Telomeric Terminal Deletion in a Patient with Dysmorphia.** *N. Kaya[1], J. Al-Zahrani[1], N. Al-Dosari[1], D. Colak[1], T. Al-Sheddi[2], O. Al-Habit[1], B. Meyer[1], P. Ozand[1], N. Sakati[1].* 1) Genetics, KFSH & RC, Riyadh, Saudi Arabia; 2) College of Science, King Saud University.

Chromosome 12q deletions are rare cases in general and only a few cases have been reported in the literature without any mosaicism. In this study we describe the first Saudi patient with a 12q telomeric terminal deletion with mosaicism. The patient is 8-years-old boy affected with varying degrees of dysmorphia, developmental delay, abnormal genitalia, growth retardation and had normal standard chromosomal karyotype. We performed high resolution aCGH assay on the patient's DNA using Agilent's 244K Human CGH Assay. The aCGH results indicated presence of a hemizygous 12q telomeric terminal deletion (46,XY,del(12)(q24.31q24.33)). Besides this large deletion one novel and seven previously reported copy number variations throughout the whole genome were also identified. Confirmatory Real Time PCR and MLPA experiments were performed and validated the aCGH results in the region. Genome-wide gene expression profiling experiments were also performed to understand the pathways involved. To further validate and physically show the deletion a FISH assay was also performed on the interphase nuclei and metaphase spreads. These results indicated presence of mosaicism in the labelled nuclei and metaphase spreads. Altogether to the best of our knowledge, this is a first study showing a mosaicism among Chr12q terminal deletions and the deletion is the largest in size (around 9.3 Mb) among the previously reported cases.

### 1374/W/Poster Board #1032

**The Low-Lands-Consortium, a collaborative approach for the identification and interpretation submicroscopic chromosomal aberrations in patients with MR/MCA.** *K. Kok[1], E. van Binsbergen[2], J. Saris[3], A.C. Knegt[4], E.A. Sistermans[5], B. Ylstra[6], G.B. van der Vries[1], M. Poot[2], H.B. Beverloo[3], A.M. Polstra[4], A.W.M. Nieuwint[5], T. Dijkhuizen[1], J.K. Ploos van Amstel[2].* 1) Genetics, UMCG, Groningen, The Netherlands; 2) Medical Genetics, university Medical Center Utrecht, The Netherlands; 3) Erasmus Medical Centre, Rotterdam, The Netherlands; 4) Clinical Genetics, Academic Medical Centre, Amsterdam, The Netherlands; 5) Clinical Genetics, VUMC, Amsterdam, The Netherlands; 6) VUMC Cancer Center, Amsterdam, The Netherlands.

Array-based comparative genomic hybridization has become an indispensable tool in the detection of submicroscopic chromosomal aberrations in patients with multiple congenital anomalies and/or idiopathic mental retardation (MCA/MR). However, the discovery of presumably neutral copy number variants complicates the interpretation of CNVs found in these patients. Thus, there is a need for procedures that efficiently distinguish between benign and pathogenic aberrations. Early 2008, five Dutch clinical genetics groups have joined forces, and formed the "Low-Lands-Consortium". One goal was to implement an oligo-based array platform (2x105K Agilent custom array, designed by L. Connell , Oxford), for the postnatal genome-screening of patients with MR/MCA for cryptic microdeletions and duplications. Consensus on the hybridization procedures, reference DNA sample and methods of data-analysis (DNA analytics, Agilent, Santa Clara, USA), have resulted in a smooth and efficient data-exchange between the groups. From May 2008 to May 2009 the consortium has analysed ~1500 patients on the 105K array. In the course of this study 500 healthy parents have also been analysed on the same array. The software package NEXUS (Biodiscovery, CA, USA) has been used to generate a database of CNVs for the Dutch population for the data obtained for the healthy parents. All CNVs consist of at least 4 consecutive oligos and are, with few exceptions, larger than 100 Kb in size. The database now contains ~3500 entries, representing ~500 CNV loci. The one-hundred CNV loci with the highest frequency, represented by 2556 entries, all contain more than two hits. Export files from this database can be visualized as custom track in the UCSC genome browser and can be uploaded into several commercial data analysis platforms, including DNA analytics (Agilent). This database has proven to be invaluable for the identification of neutral (benign) CNVs in the patients. Recently within the consortium a custom 4x180K oligo-array has been designed that replaces the 2x105K design. Results on the analysis of ~1500 patients using the 2x105K array, and preliminary data on the 4x180k array will be presented.

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## 1375/W/Poster Board #1033

**Frequency and inheritance of CNVs less than 1 Mb in a clinical cohort. How do we interpret these?** *A. Patel, S-H.L. Kang, S. Neill, M. Strivens, C.A. Shaw, P. Stankiewicz, S.R. Lalani, C.A. Bacino, S.W. Cheung.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Application of Microarray analysis for detection of copy number variation (CNV) has revealed that the human genome is awash with structural variation. The locus specific mutation rate for these structural variations is two to four orders of magnitude greater than single nucleotide changes (NG 39:S43-47,2007). An enormous amount of data has been deposited in various databases for structural variations but the basic question of what role, if any, do they play in human disease remains to be elucidated. These databases have been routinely used by clinical laboratories to guide the interpretation of CNVs less than 1 Mb in size in non-disease regions. We reviewed CNVs of less then 1 Mb in a cohort of 4186 consecutive patients examined using a custom chromosomal microarray (CMA V7 105K OLIGO) that interrogates the whole genome at an average resolution of 30 Kb with increased coverage at disease loci . Included in the CMA are probes for all the known microdeletion/duplication syndromes (>270) while excluding repetitive sequences through a combination of bioinformatics and computation. Excluding clinically significant CNVs (>1 Mb), small CNVs in clinical significant regions, and known polymorphic regions, the frequency of these CNVs was 14% (586 patients). Of these, parental analyses were available in 42% of the cases. Forty six percent of these CNVs were maternally inherited, 43% were paternally inherited and 10% were de novo. Interestingly, when the CNV was present only once (i.e. unique to that patient) in our cohort, the likelihood that the CNV contained a gene was 72%. The detailed collected data will be presented. Compiling these data may lead to a more clinically relevant interpretation of smaller CNVs and identification of new genomic syndromes.

## 1376/W/Poster Board #1034

**CHRNA7 15q13.3 Interstitial ~ 500 kb Deletion/Duplication Associated with Speech Delay, ADHD, Autism and Agressive Behavior.** *J.H. Tepperberg[1], S. Schwartz[1], R. Pasion[1], R.D. Burnside[1], V. Jaswaney[1], I.K. Gadi[1], H. Risheg[2], E. Keitges[2], C. Bullen[1], B. Williford[1], P.R. Papenhausen[1].* 1) Dept Cytogenetics, Lab Corp America, Res Triangle Pk, NC; 2) Dept Cytogenetics, Lab Corp America, Seattle, WA.

The recurrent 15q13.3 microdeletion/microduplication has been reported to cause mental impairment, autism spectrum disorder, facial dysmorphism, seizures and epilepsy. The 1.5 Mb 15q13.3 critical region (chr:15:28,719,136-30,298,264) contains 6 reference sequence genes and three OMIM annotated genes (TRPM1, KLF13 and CHRNA7). This region appears to have an unequal exchange mediated by segmental duplication breakpoints 4 and 5 (BP4 and BP5). We identified 22 individuals using the Affymetrix 6.0 SNP microarray with 15q13.3q13.3 interstitial microdeletions/duplications - 12 individuals with the commonly reported ~1.5 Mb deletion or duplication, and 11 cases with a smaller ~ 500 kb microdeletion or duplication (chr15:29,754,362-30,298,264). The latter only includes the involvement of the nicotinergic acetycholine receptor gene, CHRNA7, a neuronal ion channel gene, which has been implicated as a candidate gene associated with seizures and epilepsy. The smaller 15q deletion/duplication has, to the best of our knowledge, not been previously reported. Many of the classical clinical phenotypic features of the larger 1.5 Mb deletion and duplication were observed in some these patients, including seizures and epilepsy. The smaller 15q13.3 deletion/duplication region appears to be flanked by a 109 kb segmental duplication between BP4, (BP4a - 29,806,912-29,697,285) and the distal segmental duplication BP5 (30,540,143-30,232,699). Eight of the 11 ~500 kb CHRNA7 cases were duplications and three cases were deletions, one of which was inherited from a reportedly unaffected mother. Some of the common clinical features of the BP4a - BP5 ~500 kb deletion were speech delay, developmental delay, ADHD, aggressive behavior, ADHD, and hearing loss. None of the three deletion cases were reported to have either seizures or epilepsy. Phenotypic features of individuals with the ~500 kb duplication include growth delay, midface hypoplasia, speech delay, mild autism and one case exhibiting seizures. Further studies are needed to determine whether CHRNA7 duplications are more likely polymorphic variants with little clinical significance or possibly causative with incomplete penetrance and/or variable expressivity. These results suggest that the deletion/duplication of the CHRNA7 gene by itself may not be sufficient by itself to cause seizures and epilepsy, and may instead be a susceptibility gene related to speech delay, ADHD, autism and aggressive behavior.

## 1377/W/Poster Board #1035

**DNA array-based copy number analysis in chorionic villus samples (CVS) of spontaneous abortions with normal karyotypes.** *T. Yamada[1,3], T. Ohta[3], K. Hosoki[2,3], S. Shimada[1], M. Morikawa[1], T. Yamada[1], K. Yoshiura[4], H. Minakami[1], N. Niikawa[3], H. Yamada[5].* 1) Dept. Obstet. Gynecol., Hokkaido Univ., Sapporo, Japan; 2) Dept. Pediatr., Hokkaido Univ., Sapporo, Japan; 3) Res. Inst. Personalized Health Sci., Health Sci. Univ. Hokkaido, Tobetsu, Japan; 4) Dept. of Hum. Genet., Nagasaki Univ., Nagasaki, Japan; 5) Dept. of Obstet. Gynecol., Kobe Univ., Kobe, Japan.

A half of spontaneous abortions (SA) in the first trimester are caused by chromosome abnormalities. There is a group of patients whose pregnancies end in recurrent spontaneous abortions (RSA). Environmental factors in uterus may also contribute to RSA/SA. To investigate reasons of RSA/SA with normal karyotypes, we analyzed on whether they have a microdeletion/duplication using DNA arrays. We collected 40 chorionic villus samples (CVS) (26 from RSA and 14 from SA) with normal karyotypes. Genomic DNA from 20 CVS was directly applied to DNA arrays, while whole genome amplification (WGA) was performed in the other 20 samples before array analysis as the amount of DNA was not enough. Consequently, we detected copy-number changes at 69 loci on 22 chromosomes. Of the 69 loci, 8 and 61 were those unregistered and registered in UCSC database, respectively. As all the 8 unregistered loci contain structural genes, we focused our attention on them for further analysis, and detected two deletions in one SA sample. As one of them was also found in a parent, it is likely to be a copy-number variant (CNV), whereas the other deletion, i.e., a de novo 11-Mb deletion at 5p14 may directly contribute to SA. Although 32 of the 61 registered loci contain genes, 20 (14 registered and 6 unregistered) loci were those detected after WGA, and thus they remain inconclusive.

## 1378/W/Poster Board #1036

**The effect of large *de novo* chromosomal deletions on gene expression.** *M. Ghahramani Seno[1,2], C. Marshall[1,2], P. Hu[1,2], J. McDonald[1,2], T. Paton[1,2], G. Casallo[1,2], S. Scherer[1,2].* 1) Programme in Genetics and Genome Biology, Hospital for Sick Children, Toronto, Ontario, Canada; 2) The Centre for Applied Genomics, Hospital for Sick Children, Toronto, Ontario, Canada.

In a study of a cohort of 427 individuals with autism we identified that ~7% carried *de novo* CNVs, some of which encompassed megabase-sized genomic regions. In order to study the effect of these large *de novo* CNVs (~3-20 MB) on the expression levels of genes they encompass and also on global genes expression, we carefully selected 8 individuals with large *de novo* deletions. The criteria for selection included: 1) having *de novo* deletions confirmed by qPCR on blood DNA, 2) presence of functionally interesting genes and/or miRNAs at the CNV region, and 3) presence of ultra conserved elements with potential regulatory functions at the CNV region. Included in this study is an individual with ~2.8 Mb *de novo* deletion at chromosome 22q11.21 region. This genomic segment and the genes it harbours have been associated with Schizophrenia in a number of studies. Lymphoblstoid cell lines from the selected individuals and their available parents and siblings were cultured under controlled conditions in three biological replicates. RNA was extracted and run on Illumina gene and miRNA expression arrays. Statistic analysis of the data indicated approximately one-half reduction in gene expression of the majority of the expressed genes directly encompassed by the deletion. Moreover, several genes located distant to the deletions and on different chromosomes also showed significant variation in individuals carrying the deletions compared to their family members. For instance, in the individual with deletion of 22q11.21, genes involved in the TP53 network were found dysregulated as indicated by Ingenuity pathway analysis tool. The data is further being mined to evaluate/identify gene networks affected by these large CNV alterations.

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## 1379/W/Poster Board #1037

**Microdeletion at 15q11.2 between breakpoints 1 and 2 of the PWS/AS critical region: A new genomic disorder?** *X. Hu[1,2], Y.S. Fan[3], P. Li[4], T.J. Chen[5], B. Xiang[3], M. Li[1,2].* 1) 1Hayward Genet Ctr,; 2) Louisiana Cancer Research Consortium, Tulane Univ Sch of Med, New Orleans, LA; 3) Univ of Miami Miller Sch of Med, Miami; 4) Yale Univ Sch of Med, New Haven, CT; 5) Dept Med Genet, Univ of South Alabama, Mobile, AL.

Chromosome 15q11-q13 region contains multiple low-copy repeats (LCRs). These LCRs lead to a high frequency of chromosome rearrangements including deletion, duplication, and inversion. Two typical deletions, one between breakpoint 1 (BP1) and BP3 (type I) and the other between BP2 and BP3 (type II) of the PWS/AS critical region on chromosome 15q11-q13 have been described in 70% of patients with Prader-Willi syndrome (PWS) or Angelman syndrome (AS). Here we report thirteen cases of microdeletion between the two proximal PWS/AS deletion breakpoints, BP1 and BP2, using high resolution olignucleotide microarray-based comparative genomic hybridization. The patients include eleven males and four females aged from one to twenty-one years old. Common clinical features in these patients include short stature, motor and speech developmental delay, cardiac anomalies, and behavioral and psychosocial disturbances such as attention deficit disorder, attention deficit hyperactivity disorder, and autism spectrum disorder. Nine of the thirteen patients carry a deletion of minimum 300 Kb with the proximal and distal breakpoints located at the BP1 and BP2, respectively. The remaining four cases have smaller deletions (minimum 100 to 200 Kb) with only one deletion breakpoint, proximal or distal, located in BP1 or BP2. There are four highly conserved genes, GCP5, CYFIP1, NIPA2, and NIPA1, between BP1 and BP2, which have been postulated to be responsible for the compulsive behavior and lower intellectual ability in PWS/AS patients with the type I deletion. Among the 13 cases of 15q11.2 deletion, 9 deletions include all four genes, 3 contain 3 genes (CYFIP1, NIPA2, and NIPA1), and one involves only one gene (GCP5). Although the phenotypes of these patients vary, they share some common clinical features. The most frequent features include delayed motor and speech development and behavior disturbance. These results support the hypothesis that the 15q11.2 deletion between BP1 and BP2 is a novel microdeletion syndrome. The presence of deletions inherited from apparently normal parents and deletions that are de novo in these patients implies incomplete penetrance. Further study of more patients with similar 15q11.2 deletions and detailed clinical evaluation of apparently normal parents with the same 15q11.2 deletion are essential in defining this potential novel microdeletion syndrome.

## 1380/W/Poster Board #1038

**Identification and characterization of somatic copy number variants (CNVs) in embryonic cell lineages using a novel CNV-targeted array comparative genomic hybridization (aCGH) platform.** *A.A. Ghazani[1,2], M. Blake-Kinnin[1], S. Kantarci[2,3], C. Lee[1,2].* 1) Brigham & Women's Hospital, Department of Pathology, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Beth Israel Deaconess Medical Center, Department of Pathology, Boston, MA.

The identification of widespread copy number variation in the human genome has made a significant contribution to understanding the extent of genomic diversity in the general population. As a result, it has become necessary to categorize CNVs as benign, pathogenic or unknown clinical significance, in a clinical genetic diagnostic setting. In some cases, CNVs can arise somatically and are detected when two or more distinct somatic cell lines from a single zygote / individual contain different CNV profiles. Recent published studies have shown the detection of a limited number of somatic CNVs between monozygotic twins or between different tissues in a given individual. The aim of this study is to more comprehensively investigate the occurrences of somatic CNVs by studying different tissues derived from the different embryonic cell lineages (i.e., ectoderm, endoderm and mesoderm). This investigation has been conducted using a novel custom-designed 105K CNV-targeted array-based comparative genomic hybridization (aCGH) platform. The CNV array is composed of well-annotated probes from Database of Genomic Variant, probes from Phase II studies of the Structural Genomic Variation Consortium (unpublished data), and probes covering segmental duplications - that are commonly associated with CNVs. The test samples include DNA obtained from somatic cell lineages of ectoderm (skin), mesoderm (heart, kidney) and endoderm (liver, lung, stomach and large bowl) from a cytogenetically normal fetus at 27 weeks. The test samples were compared to a reference DNA obtained from the small bowel of the same fetus. Preliminary studies showed that the skin and liver samples exhibited similar CNV profiles compare to the small bowel. The lung exhibited distinct CNV profile regions compared to the remaining tissues, most likely attributed to somatically-derived CNVs. This data supports the notion that somatically-derived CNVs are more common than previously appreciated and can account for detectable genomic imbalances between one tissue and another in the same individual.

## 1381/W/Poster Board #1039

**Characterization of "new" human genome sequences variable among individuals.** *F. Antonacci[1], J.M. Kidd[1], N. Sampas[2], N.A. Yamada[2], P. Tsang[2], A. Tsalenko[2], H.S. Hayden[3], W. Gillett[3], T. Graves[4], C. Alkan[1], I. Hajirasouliha[6], M. Ventura[5], R.K. Wilson[4], L. Bruhn[2], R. Kaul[3], E.E. Eichler[1].* 1) Department of Genome Sciences and Howard Hughes Medical Institute, University of Washington, Seattle, WA; 2) Agilent Technologies, Santa Clara, CA; 3) University of Washington Genome Center, University of Washington, Seattle, WA; 4) Washington University Genome Sequence Center, St. Louis, MO; 5) Department of Genetics and Microbiology, University of Bari, Via Amendola 165A, Bari, Italy; 6) School of Computing Science, Simon Fraser University, Burnaby, BC, Canada.

Since the human genome reference assembly is now viewed as a patchwork of structurally variant sequence, it is expected that sequencing projects of other individuals will reveal novel human euchromatic sequence. We developed an approach using a fosmid clone-based resource to identify and characterize orphan euchromatic sequences that are not represented even once within the human genome reference sequence assembly. Based on our analysis of nine human genomes, we have identified 3,944 sequence contigs (from 10,516 distinct clones) corresponding to new insertions ranging from 1 kbp to 130 kbp in size. We estimate that 497 of these correspond to uncharacterized genomic segments greater than 40 kbp in size with half not associated with known gaps in the reference genome assembly. We developed a customized (Agilent) genotyping platform to robustly genotype 2,515 of these contigs in samples from the HapMap population. We found that 40% of the contigs are polymorphic. More than 97% were found in a single chimpanzee, supporting the conclusion that these sequences represent a combination of polymorphic human deletions and artifacts in the human genome reference assembly. To date, over a 100 of these regions have been sequenced. Several of these sequences map within transcribed regions and may contain additional spliced exon structures. We systematically analyzed unmapped fosmid clones for one individual (NA15510) by clone-based analyses, arrayCGH and fluorescent in situ hybridization. FISH mapping of these contigs indicates that the majority are single-copy sequences distributed throughout the genome with a particular enrichment in subtelomeric regions. We tested by FISH 69 insertions in 9 individuals predicted to be euchromatic based on paired-end sequence placement. Surprisingly, only 42% of these sequences mapped to the predicted location, with 45% mapping to the nucleolar organizer regions (NORs) on the p arms of acrocentric chromosomes. These results suggest that a subset of our contigs correspond to euchromatic sequences which have been copied into the short arm of acrocentric chromosomes. Taking into consideration the success of this analysis we applied this approach to paired-end sequence data from NGS. Based on a 42X sequence coverage from an African genome, we identified, mapped and partially characterized 1623 new insertions in one African individual ranging in size from 500bp to 6.2 kbp.

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### 1382/W/Poster Board #1040

**Copy number variations associated with developmental disorders in a Bronx minority population.** *M. Babcock[1], J. Samanich[2], R.W. Marion[2], P. Levy[2], A. Shanske[2], K. Pop[2], N. Yachelevich[2], D.T. Hsu[3], Q. Pan[4], C.K. Huang[4], L. Cannizzaro[4], K.H. Ramesh[4], C. Montagna[5], J. Flynn[6], B.E. Morrow[1].* 1) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY, USA; 2) Center for Congenital Disorders, Children's Hospital at Montefiore, Bronx, NY, USA; 3) Division of Pediatric Cardiology, Children's Hospital at Montefiore, Bronx, NY, USA; 4) Department of Pathology, Montefiore Medical Center, Bronx NY, USA; 5) Department of Pathology, Albert Einstein College of Medicine, Bronx, NY, USA; 6) Clinical Research Center, Albert Einstein College of Medicine, Bronx, NY, USA.

Over the last year, a pipeline to determine the genomic basis of developmental delay and multiple congenital anomalies has been created between Albert Einstein College of Medicine, Children's Evaluation Rehabilitation Center (CERC) and the Children's Hospital at Montefiore (CHAM). The goal has been to develop translational genomics research while simultaneously providing a clinical laboratory diagnosis. A total of 222 blood or saliva specimens from 96 probands and 126 parents were collected with their informed consent over the past year. Of these, 88 had developmental delay/multiple congenital malformations and 8 had cardiovascular malformations. Six of the probands had known translocations or inversions. The DNA was isolated and a targeted Agilent 44k microarray was used for aCGH (array comparative genome hybridization) testing at CHAM. Positive findings were followed up in the research lab. Of the 96 probands, 34 showed CNVs of which, 16 occurred in regions of known benign polymorphisms and 18 were in intervals of human diseases or in novel regions. One interesting finding was from a proband with skeletal anomalies who had a karyotype 46,XY, der(2)t(2;6)(q37.3;p25). The result of aCGH determined the presence of a 4Mb terminal deletion on 2q37.3, a 6 Mb duplication on 6p25.1-25.3, and a 0.8 Mb deletion on 2q13. Multiple genes for skeletal development were located in these intervals including, HDAC4, FOXC1, and FOXF2. Another example is a proband with obesity and family history of mental illness. The child had a normal karyotype and was negative for sub-telomere FISH, but had a 3.2 Mb deletion on 1p31.3 containing genes LEPR and PDE4B important for obesity and mental health, respectively. The pipeline developed can serve as a guide to other institutions embarking on translational genomics research.

### 1383/W/Poster Board #1041

**Copy number variation in subtelomeric regions in Brazilian male patients with intellectual disability.** *D.M. Christofolini, M.A.P. Ramos, S.I.N. Belangero, F.T.S. Bellucco, A.N.X. Pacanaro, M.I. Melaragno.* Genetics Division, Morphology, UNIFESP, SAO PAULO, SAO PAULO, Brazil.

INTRODUCTION: Chromosomal rearrangements affecting subtelomeric regions of chromosomes are believed to be responsible for 5-7% of moderate to severe mental retardation cases. However, this may be an underestimation because, due to the relative complexity and high cost of the screening methods used, only preselected patient populations, including mostly the more severely affected cases, are usually screened. Moreover, the presence of a small chromosome duplication, triplication or deletion is not easy to detect by routine chromosomal analysis. Recently, multiplex ligation-dependent probe amplification (MLPA) has been adapted for use in subtelomeric rearrangement screening, and has allowed characterizing larger patient samples. METHODS: In the present study, we used MLPA for the detection of microduplications/microdeletions in 120 male patients with intellectual disability, who presented normal karyotypes and negative results for the fragile-X syndrome. RESULTS: We detected five patients with copy number aberrations (4% of the investigated cases). MLPA probes showed duplications of regions inside genes FLJ22115, SYBL1, SECTM1, triplication of gene NDN, and deletion of the FBXO25 gene. These genes are located at 20p13, Xq28, 17q25.3, 15q11.2, and 8p23.3, respectively. Two of the probes (for genes SYBL1 and FBXO25) were located in regions with previously described copy number variations (CNVs). The clinical phenotypes of these patients were unremarkable. CONCLUSION: This study underscores the value of combining conventional karyotyping with novel technologies to unravel small chromosomal alterations and help understanding genomic mechanisms leading to disease. (Financial support: CAPES; FAPESP).

### 1384/W/Poster Board #1042

**A novel 1.5 Mb de novo non-recurrent deletion at 14q23 detected in Autism spectrum disorders.** *D.Q Ma[1], A. Griswold[1], R.H Chung[1], D. Salyakina[1], H.N Cukier[1], J.M Jaworski[1], I. Konidari[1], P.L Whitehead[1], D. Hedges[1], H. Wright[2], R.K Abramson[2], E.R Martin[1], J.P Hussman[3], J.R Gilbert[1], M.L Cuccaro[1], J.L Haines[4], M.A Pericak-Vance[1].* 1) Miami Inst Human Genomics, Univ Miami, Miller Sch Med, Miami, FL; 2) School of Medicine, University of South Carolina, Columbia, SC; 3) Hussman Foundation, Ellicott City, Maryland; 4) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN.

Autism spectrum disorders (ASDs) are a group of highly heritable neurodevelopmental disorders with a complex genetic architecture. Several lines of evidence have implicated the rare variants such as copy number variations (CNVs) in the etiology of ASDs. A genome-wide SNP-array mapping of CNVs was carried out on 602 ASD families genotyped on Illumina Human 1M Beadchip. Preliminary CNV detection yields 39,046 CNVs from 635 cases in 600 ASD families after sample quality control embedded in Penncnv. It includes 3767 homozygous deletions, 26703 hemizygous deletions, 8386 one-copy duplications and 190 two-copy duplications. Initial analysis identified 5276 possible de novo CNVs from 597 cases in 531 complete families using family-based CNV detection algorithm. The length of these de novo CNVs ranges from 40bp to 5Mb (the number of spanned SNPs: [3, 2427]) across 22 chromosomes. 52 of them have a size of more than 500Kb. Majority of these large CNVs falls into copy-number-rich regions. A novel 1.5 Mb-long non-recurrent deletion at 14q23.2-23.3 found in one case appears to be interesting. It completely deletes an autism candidate gene (MTFHD1) and has not been reported by any available CNV databases. In addition, we successfully replicated six previously reported autism-related CNV regions (CNVRs) (UBE3A, NRXN1, CNTN4, 22q11.21, 16p11.2 and SHANK3) and five newly reported common CNVRs over-represented in ASD patients (FBXO40, AK123120, UNQ3037, NLGN1 and GYPELOC441046). In conclusion, the novel de novo deletion at 14q23.2-23.3 disrupting MTFHD1 is implicated in ASD but warrant further validation experimentally.

### 1385/W/Poster Board #1043

**From chromosome to SNP-array, a new patient with 16p microdeletion syndrome.** *A. Perez-Juana del Casal[1], M. Artigas-López[1], A. Bengoa-Alonso[1], M. Nadal Sanchez[2], C. Harteveld[3], C. Ruivenkamp[3], A. Alonso Sanchez[1], MA. Ramos-Arroyo[1].* 1) Dept Genetics, Hosp Virgen del Camino, Pamplona, Spain; 2) Genetics, Inst Recerca Oncologica. L'Hosp, Barcelona, Spain; 3) Genetics, Clinical Molecular and Biochemical Geneticist. Leiden University,The Netherlands.

A one year-old mentally retarded boy with mild facial dysmorphism was referred to the Genetics Clinic for evaluation. The patient was the first child of a healthy, non consanguineous couple. Pregnancy was complicated by decreased fetal movements and IUGR. Delivery was at 37 weeks by C-section, birthweight 2310 g, length 45 cm and head circumference 31 cm. Microbrachycephalia, occipital cowlick, low set ears, downslanting palpebral fissures, narrow nose, small mouth, long philtrum, and tapering fingers were observed. Blood was drawn for conventional karyotype analysis and Fragile-X study. Fragile-X testing came back normal. A 16p deletion was suspected cytogenetically, but could not be demonstrated by any of the available techniques in our lab. FISH analysis (ToTelVysion, Vysis) with telomeric probes was normal. Next we tried to rule out an interstitial deletion with FISH of selected BAC clones, D16Z3, RP11-517F15 (16p13), CTD-2135D7 (16p13-12) and RP11-567P19 (16p12-11), again with normal results. Finally we contacted with an external laboratory who kindly performed a genome scan using a 250K SNP array (Affymetrix), this technique revealed a microdeletion 'popping-up' of at least 7.78 Mb (536 SNP probes) in the short arm of chromosome 16. This rearrangement has been defined as a syndrome, "microdeletion syndrome of 16p11.2p12.2", by Blake C Ballif, et al. (Nature Genetics, 2007), after screening 8789 patients with developmental disabilities and normal karyotypes. Five out of 8789 patients had different copy-number imbalances on 16p, and two of them had exactly the same microdeletion size and phenotype as the patient here presented.

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## 1386/W/Poster Board #1044

**Parental Origin Bias in *de novo* CNVs Detected in Autism Probands.** *N. Shah[1], R. Regan[2], J. Conroy[2], T. Magalhaes[4], J. Casey[2], R. Anney[5], A. Green[3,2], L. Gallagher[5], M. Gill[5], DC. Shields[1], A. Vicente[4], S. Ennis[2,3].* 1) UCD Conway Institute and UCD Complex and Adaptive Systems Laboratory, School of Medicine and Medical Science, University College Dublin, Dublin, Ireland; 2) Health Science, School of Medicine and Medical Science, University College Dublin, Dublin, Ireland; 3) National Centre for Medical Genetics, Our Lady's Hospital for Sick Children, Dublin, Ireland; 4) Istituto Nacional de Saúde, Lisboa, Portugal; 5) Trinity Centre for Health Sciences, Trinity College Dublin, Dublin, Ireland.

Genetic variation occurs in humans at both individual and population level. One such variation that contributes to human genetic diversity is copy number variation (CNV) of genomic segments. In addition to contributing to common variation among healthy individuals, CNVs are associated with a number of genetic disorders and the susceptibility to complex disorders. It has also been shown that there is an association between *de novo* CNVs and complex disorders including autism spectrum disorder (ASD). Such *de novo* mutations could occur in either maternal or paternal germ line or in developing embryos.

We are investigating whether there is a parental origin bias for *de novo* CNVs in ASD probands. Three hundred and eighty Irish and Portuguese autism trios were genotyped on the Illumina 1M beadarray. *De novo* CNVs in probands were predicted using PennCNV algorithm. To minimize false positive and false negative CNVs, genotype & intensity filters were applied. In our preliminary analysis of *de novo* CNVs, we observed a bias towards paternal origin (chi-square with continuity correction; P = 0.016) for *de novo* deletions with 2 or more SNPs informative and congruent for defining parental origin from SNP genotype and intensity data (maternal: paternal origin ratio is 2:12 deletions). We also observed that there is a bias towards maternal origin (chi-square with continuity correction; P = 0.041) for *de novo* duplication CNVs (maternal: paternal origin ratio is 31:16 duplications).

One theory regarding paternal origin bias in *de novo* deletions is that there are many more germ line cell divisions in the life history of a sperm relative to that of an egg and therefore greater opportunity for mutations to occur. Further studies on the relationship between duplication and deletion *de novo* CNVs and their parent of origin may provide further insights into the molecular mechanisms during meiosis. Replication of this study in a larger dataset and experimental validation is to be followed.

## 1387/W/Poster Board #1045

**Copy number variations analysis in 13 sporadic patients with mental retardation of unknown origin and normal karyotype.** *V. Stranecky[1], R. Ivanek[1], A. Baxova[2], H. Hartmannova[1], L. Noskova[1], A. Cizkova[1], S. Kmoch[1].* 1) Institute of Metabolic Disorders, Charles University, First Faculty of Medicine, Ke Karlovu 2, Prague 2, Czech Republic; 2) Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University and General University Hospital , Albertov 4, Prague 2,Czech Republic.

Microdeletions or microduplications (eg. losses or amplifications of DNA regions smaller then 5 Mb) that can not be detected by conventional cytogenetic analysis seem to be frequent among causes of sporadic syndromes associated with mental retardation. To estimate the frequency and eventual recurrence of such changes, we used Affymetrix SNP Array 6.0 and analysed copy number changes (CNVs) in 13 sporadic patients with mental retardation of unknown etiology associated with multiple (syndromology unspecific) congenital abnormalities suggestive of genetic origin and normal karyotype. Identified CNVs larger then 30 kb were tabled for each of the patient according to its sizes, characters (loss/gain) and overlaps with Database of Genomic Variations (DGV) and Database of pathologic CNVs (DECIPHER). Genomic content for each of the unique CNVs was assessed using UCSC browser and genotype - phenotype correlation was estimated based on gene expression patterns and its likely biological relevance to mental retardation and/or associated symptoms. In 4 patients we identified unique and clearly clinically significant changes ranging from 420 kb - 20 Mb. In 7 patients we identified unique deletions ranging from 35 kb to 150 kb and affecting genes coding for brain expressed transcription factors, regulatory proteins, regulatory elements or proteins of unknown function. In 2 patients no unique and biologically relevant changes were identified. Our data demonstrate that microdeletions are probably very frequent cause of sporadic syndromes and that high resolution copy number analysis provide clinically significant and scientifically useful data.

## 1388/W/Poster Board #1046

**Single Molecule Nanoarray Analysis for Genomic Structural Variation Mapping.** *M. Xiao, S. Vijayan, S. Das, p. Deshpande, S. Gallagher, M. Austin, H. Sadowski, M. Boyce-Jacino, H. Cao.* BioNanomatrix Inc, Philadelphia, PA.

We are developing a single molecule nanoarray platform for whole genome mapping. The system uses a highly miniaturized nano-channel device that enables rapid detection of single, labeled dsDNA molecules preserved in their long native linear state. Our method starts with sequence specific fluorescent labeling of high molecular weight genomic DNA. The labeled DNA molecules are then linearized inside the nano-channel device and imaged with a high resolution fluorescence imaging station. By determining the order of the fluorescent labels on each molecule, the distribution of specific sequence motifs can be inferred with great accuracy on fragments ranging in size from 50,000 to 350,000 base pairs in length, in a manner similar to reading a bar code. The motif maps generated from each long fragment are then re-assembled into a global genome map using proprietary software. We will present DNA mapping results on human BAC and fosmid samples with known structural variations as an example of the technology and describe more specifically the utility of the system in the discovery and confirmation of genome wide structural variations. Other potential applications for the system, such as molecular haplotyping and methylation analysis, will also be discussed.

## 1389/W/Poster Board #1047

**Epilepsy as an Endophenotype of Autism for Gene Discovery.** *A. Moreno-De-Luca[1], D. Moreno-De-Luca[1], S.L. Helmers[2], D.H. Ledbetter[1], C.L. Martin[1].* 1) Emory University School of Medicine, Department of Human Genetics, Atlanta, GA; 2) Emory University School of Medicine, Department of Neurology, Atlanta, GA.

Autism is a common and heterogeneous neurodevelopmental disorder characterized by a triad of impaired verbal communication, lack of reciprocal social interaction, and restricted or stereotypical patterns of behavior. It has one of the highest demonstrated heritabilities among developmental neuropsychiatric disorders: family studies have revealed a recurrence risk of 2-8% among siblings of affected probands and twin studies have shown a concordance of 60-90% among monozygotic twins as compared to ~10% in dizygotic twins. There is a close relationship between autism and epilepsy as ~30% of autistic individuals also have a history of seizures. We are using epilepsy as an endophenotype of the broader autism spectrum, with the aim of reducing phenotypic heterogeneity, to look for copy number variations (CNV) in patients with autism plus epilepsy. By using this strategy, we expect to identify underlying genetic causes shared by both conditions. Genome-wide array analysis is being carried out using a custom-designed 44K oligonucleotide array. To date, we have tested 20 patients and identified 6 cases (30%) with a CNV that could be responsible for the observed phenotype. Two deletions and 4 duplications were identified ranging in size from 452 kb to 1.6 Mb. One of the duplications involves the previously reported region on 16p11.2 which has been suggested to be associated with an increased risk for autism. The other changes are novel and we are interrogating their gene content for candidate genes. Interestingly, one case has a 452 kb de novo deletion involving only the SLC39A10 (solute carrier family 39A10) gene at 2q32.3. The proband presented with autism, developmental delay and absence seizures. This is the first case reported to have a CNV in this gene associated with autism and epilepsy and no common, benign CNVs of this region have been reported in normal control populations. The SLC39A10 gene belongs to a subfamily of proteins that show structural characteristics of zinc transporters. Extracellular zinc can interact with many different synaptic targets, including glutamate receptors and transporters. Therefore, this gene is a strong candidate since genes in the glutamate pathway have been implicated in autism spectrum disorders. The use of such rare CNVs for gene discovery in autism and epilepsy will provide insight into the underlying molecular mechanisms of these common disorders.

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## 1390/W/Poster Board #1048

**High-resolution cytogenetic analysis using customized oligonucleotide-based fluorescence *in situ* hybridization (FISH).** *N.A. Yamada[1], F. Antonacci[2], R.A. Ach[1], P. Tsang[1], E. Carr[1], A. Scheffer-Wong[1], N. Sampas[1], B. Peter[1], S. Laderman[1], J.M. Kidd[2], E.E. Eichler[2,3], L. Bruhn[1].* 1) Molecular Technology Laboratory, Agilent Laboratories, Santa Clara, CA; 2) Department of Genome Sciences, University of Washington, Seattle, WA; 3) Howard Hughes Medical Institute, University of Washington, Seattle, WA.

The comprehensive analysis of structural complexity within the human genome requires extension of cytogenetics methods to fine-scale resolution. Fluorescence *in situ* hybridization (FISH) offers unique abilities to analyze chromosome structures in the single-cell context, but current FISH methods have limitations that preclude its routine use in these studies. We have expanded FISH capabilities and utilities to meet the requirements of high-resolution cytogenetics.

We tested high complexity oligonucleotide libraries (94-194mers) to empirically determine the individual length of the oligonucleotides to be pooled. We then *in silico* selected probe sequences for the most informative elements within target loci. We maintained the downstream hybridization workflow to closely resemble those widely used for BAC-based FISH with improvements that eliminated the need for suppressive hybridization reagents.

Regions as small as 1.8 kb and as large as chromosome arms were successfully visualized in both metaphase and interphase cells without further iteration using our *in silico* probe design methodology and assay protocol. Because of the inherent flexibility in our probe design methods, we readily obtained specific and robust probes for regions rich in repeats and/or GC content. Simultaneous hybridization of probes labeled with three different fluorophores for multicolor visualization was achieved without any modifications to the assay workflow. Probe performance on interphase cells after immunocytochemistry treatment was also validated, providing evidence that protein-DNA co-detection can be achieved routinely.

We then applied our FISH technology to analyze a set of recently discovered "novel human sequences" that are absent from the current reference assembly. These sequences, identified by fosmid end-sequencing, ranged in sizes from 30 kb to 5 kb. Because we do not require physical starting template DNA for probe generation, we visualized these novel sequences and validated array-based copy number measurements using our routine workflow. Our results demonstrate that complex libraries of *in silico* selected oligonucleotides extend FISH capabilities to complement microarray- and sequencing-based discoveries and enable single-cell visualization of chromosome at fine-scale resolution to further our understanding of genome biology.

## 1391/W/Poster Board #1049

**An optimised fibre-FISH protocol for characterising genomic structural variation.** *F. Yang, N.P. Carter.* Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom.

Fluorescence in situ hybridisation (FISH) on stretched chromosomal DNA fibres is an indispensable tool for defining the size of gaps and overlaps between clone contigs in a given reference genome. There are many published protocols for fiber-FISH, but a robust protocol that allows high-throughput mapping by fibre-FISH is unavailable. Most fibre-FISH protocols involved the use of dual colour FISH of probes respectively labelled with biotin- and digoxigenin-dUTP by nick translation and detected indirectly with red and green fluorochrome conjugated antibodies. While being very informative in determining the genomic gap and overlap between clones, these protocols have limited use in physical ordering of multiple contigs and characterisation of complex arrangements and rearrangements that often require the simultaneous mapping of at least three probes (two test probes plus one anchor probe, each being visualised with a distinct colour). In response to the growing need of mapping genomic structural variations (copy number variation and inversions) by fibre-FISH from international community, we have further optimised the fibre-FISH protocol that is based on alkaline lysis of live cells. The main modifications include 1) the introduction of a modified whole-genome amplification kit for rapid generation of high-quality BAC and fosmid probes, and 2) the introduction of a third hapten in probe labelling (in addition to the widely used biotin- and digoxigenin-dUTP), and 3) the development of an appropriate antibody detection system suitable for the detection of three haptens. The modified protocol allows us to visualise simultaneously three or more probes in at least three distinctive visible fluorochromes (i.e. Texas red, Cy3 and FITC). We have successfully applied this protocol to the characterisation of genomic structural variations in normal human and great apes populations as well as those associated with human diseases. Our fibre-FISH results have led to a better interpretation and understanding of genomic structural variation identified by array-CGH and next generation paired-end sequencing. Fibre-FISH has also enabled a more definitive characterisation of multiallelic copy number variation that is difficult to validate by other approaches.

## 1392/W/Poster Board #1050

**Submicroscopic structural variation analysis in patients with mental retardation using SNP 6.0 array and combined data analysis.** *M. KOHDA[1], T. Hirata[1], E. Nakagawa[2,3], K. Kurosawa[4], Y. Goto[2,3], Y. Okazaki[1].* 1) Div. of Translational Research, Saitama Medical University, Hidaka, Saitama, Japan; 2) Natl. Ctr. Hospital of Neurology and Psychiatry, NCNP, Tokyo, Japan; 3) Natl. Inst. of Neuroscience, NCNP, Tokyo, Japan; 4) Div. Med. Genet., Kanagawa Child Med. Ctr., Kanagawa, Japan.

The genetic causes of mental retardation (MR) are quite heterogeneous. Recent studies have identified a series of genes involved in MR, however, there are still many patients whose causal genes are yet to be determined. On the other hand, copy number variations (CNVs) have been raised up as important genetic risk factors for developmental disorders, including neurodevelopmental disorders, schizophrenia, autism and MR. High density oligonucleotide array has emerged as a novel technology allowing one to detect DNA copy number changes that were previously unidentified by conventional cytogenetic techniques. In this study, 50 MR patients with normal karyotype were tested for CNV using Affymetrix Genome-Wide Human SNP Array 6.0. . The structural variations identified by SNP Array 6.0 were confirmed by qPCR and/or FISH. Approximately 20 DNA copy number changes per individual were detected. In about 10% of patients, suspicious disease-related imbalances were detected, ranging from 310 kb to 1.6 Mb in size. In some cases, the regions of the variations overlapped with those reported in the DECIPHER database. To identify individual variants that are pathogenic, we then combined our data with published (rare) CNVs from several studies in patients with such as autism, schizophrenia or MR and in normal populations. We will report candidate causal genes for these MR patients, and discuss about the challenges of analysing and interpreting the data generated using high density oligonucleotide arrays.

## 1393/W/Poster Board #1051

**Detection of copy number variations (CNV) in patients with mental retardation using high density SNP microarrays.** *N. Rivera-Brugues[1], M. Hempel[2], S. Spranger[3], B. Kazmierczak[3], T. Meitinger[1,2], T.M. Strom[1,2].* 1) Institute of Human Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg-Munich, Germany; 2) Institute of Human Genetics, Technische Univeristät München, Munich, Germany; 3) Praxis für Humangenetik, Bremen, Germany.

Chromosomal abnormalities are a major cause of mental retardation. Whole-genome array-based technologies have increased the detection rate of cryptic aneusomies among these patients up to 10-20%. DNAs from 109 children with MR and normal G-banded chromosomes and 1 mentally retarded child with a de novo balanced reciprocal translocation were evaluated for rearrangements by Illumina Human610-Quadv1_B arrays. Data quality was assessed with standard deviation (mean SD: 0.19) and mean absolute deviation (mean MAD: 0.12) of the log2 intensity ratios. In male samples, a signal-to-noise ratio (median log2 intensity ratio of the X-chromosomal SNPs minus median log2 ratio of the autosomal SNPs divided by MAD) was calculated (mean SNR: 3.82). CNVs were called using circular binary segmentation (DNAcopy). Conspicuous CNVs were compared with known variants as provided by the Database of Genomic Variants (DGV). After excluding known structural polymorphisms and the majority of CNVs within intronic and intergenic regions, we validated the microarray data and genotyped imbalances by qPCR. A total of 3,087 CNVs (2,431 losses, 656 gains) ranging in length from 118 to 13,386,172 base pairs (mean 85,149, median 24,352) were detected. 168 out of 3,087 candidate regions were investigated. Of these, 69 (41.1%) were confirmed, 99 (58.9%) were false-positive. 77 (77.8%) of the false positive were detected in regions defined by <10 SNPs, 18 (18.2%) were indicated by 10-20 SNPs and 4 (4%) by >20 SNP. We identified 16 de novo CNVs and one maternally inherited Xq13.1 deletion in a male patient, which correspond to 14 deletions and 3 duplications ranged in size from 3kb to 13.4Mb. The inheritance of 5 CNVs could not be established because of missing parental DNA. The resolution of Illumina microarrays showed to be sufficient to detect intragenic deletions. The identification of de novo CNV affecting only a single gene permitted us to consider that particular gene as a candidate for mental retardation. Mutational screening will follow based on a candidate gene approach in a large series of unexplained MR patients. Therefore, this study will lead to the identification of genes involved in MR, thus providing insights into the pathophysiology of mental retardation and into functional networks relevant for the development of the brain.

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## 1394/W/Poster Board #1052

**Analyses of 499 Cases of Multiple Congenital Anomalies with Mental Retardation using array-CGH for Investigation and Diagnosis.** *S. Hayashi[1], S. Honda[1], S. Mizuno[2], N. Okamoto[3], Y. Makita[4], A. Hata[5], I. Imoto[1], J. Inazawa[1].* 1) Dept. of Molecular Cytogenetics, Medical Resarch Institute, Tokyo Medical and Dental University; 2) Department of Pediatrics, Central Hospital, Aichi Human Service Center; 3) Department of Planning and Research, Osaka Medical Center and Research Institute for Maternal and Child Health; 4) Education Center, Asahikawa Medical College; 5) Department of Public Health, Chiba University Graduate School of Medicine.

In order to screen cryptic chromosomal copy-number aberration (CNA) in unknown congenital disorders, we performed array-CGH in patients with multiple congenital anomalies and mental retardation (MCA/MR) for four years, by using several types of in-house bacterial artificial chromosome (BAC)-based arrays. All cases showed normal karyotype by conventional cytogenetics. We first screened 499 cases using 'MCG Genome Disorder (GD) Array' containing BACs covering loci associated with known genomic disorders and subtelomeric regions of all chromosomes except short arms of acrocentric chromosomes, and detected CNAs in 51 of 499 cases (10.2%). We next performed the second screening using 'MCG Whole Genome Array-4500' harboring 4523 BACs throughout human genome in cases without any CNAs in the first screening. All detected copy-number variations (CNVs) were confirmed by FISH in patients and their parents as far as possible. Through those steps, we judged *de novo* CNVs as potentially pathogenic CNAs, and consequently we detected CNAs in 45 of 239 cases (18.8%). Among positive cases, two showed recurrent CNAs, allowing us to diagnose recently identified 1q41 and 16p11.2 microdeletion syndromes. In other two cases CNAs detected harbored genes closely associated with phenotypes in each patient; BMP4 for polysyndactyly and *CASK* for microcephaly with hypoplastic cerebellum, respectively. Subsequently, we investigated genomic aberrations of the *CASK* gene in eleven females with MR, microcephaly and hypoplastic cerebellum, and identified four mutations, two deletions and one duplication of *CASK*. Taken together, our trial could efficiently screen patients with MCA/MR to detect CNAs in about 30% in all cases showing normal karyotype by conventional cytogenetics, leading to the delineation of new syndromes and accelerating understanding of gene function.

## 1395/W/Poster Board #1053

**Genomic duplication identified by genome-wide array CGH: A multicenter study of 1,670 cases.** *M.M. Li[1], Y.S. Fan[2], P. Li[3], T.J. Chen[4], B. Xiang[2], X. Hu[1], H.C. Andersson[1], T.C. Narumanchi[1], Y. Wang[4].* 1) Hayward Gen Ctr, Tulane Univ Sch Med, New Orleans, LA; 2) Univ of Miami Miller Sch of Med, Miami, FL; 3) Yale Univ Sch of Med, New Haven, CT; 4) Dept Med Genet, Univ of South Alabama, Mobile, AL.

Genomic duplications have been well documented as the cause of many genomic disorders. However, the interpretation of genomic duplications uncovered by high resolution genome-wide array CGH (aCGH) has been a challenge due to the higher level of incomplete penetrance and variable expressivity compared to genomic deletions. We have collected and analyzed genomic duplications among 1,670 consecutive cases using Agilent 4x44K oligonucleotide arrays. Criteria for pathogenic duplications include following: 1) involvement of a region associated with a known microduplication syndrome; 2) presence of the same duplication in a similarly affected parent; 3) involvement of known dosage sensitive gene(s); or 4) association of multi-gene imbalances related to visible or cryptic cytogenetic alterations. Criteria for benign duplications include following: 1) involvement of a known copy number variation documented in multiple normal individuals; or 2) presence of the same duplication in phenotypically normal family members. Clinical indications for aCGH study include mild to severe mental retardation, multiple congenital anomalies, developmental delay, and autism spectrum disorders. A total of 613 duplications ranging from approximately 30 Kb to 61.5 Mb in size were identified. The incidence of genomic duplication in this cohort of patients was 23.7% (456/1760). Of these duplications, 17.5% (107/613) were considered pathogenic, 64.3% (394/613) were classified as benign, and 18.3% were variations of unknown significance. Among 107 pathogenic duplications, 26 were associated with described syndromes and 81 were private duplications presenting in individual families. Of the pathogenic duplications, 94% (100/107) were larger than 500 Kb, whereas the average size of benign CNVs was 270 Kb, with less than 10% larger than 500 Kb. While the majority of the pathogenic duplications were scattered across the genome, benign CNVs tended to be clustered in certain regions with known genomic variations. Our experience suggests that, with some exceptions, size and location should be used as the first-line criteria in classifying genomic duplications as pathogenic or benign. We also emphasize the importance of evaluating parental DNA samples to aid in the interpretation of novel genomic duplications.

## 1396/W/Poster Board #1054

**Multiple chromosome anomalies detected by microarray CGH, including deletion of the gene for familial adenomatous polyposis.** *M. Manning[1,2], A. Kwan[1], A. Cherry[2].* 1) Dept Peds/Div Med Gen, Stanford Univ Sch Med, Stanford, CA; 2) Dept Path, Stanford Univ Sch Med, Stanford, CA.

Microarray comparative genomic hybridization, aCGH, is a precise test at detecting submicroscopic chromosomal copy number changes and is becoming standard of care when evaluating patients with multiple congenital anomalies, dysmorphic features and developmental delay/mental retardation. Recent applications of aCGH have included evaluation of patients with autism and prenatal diagnosis of pregnancies with multiple anomalies. A new use for aCGH, that of determination of tumor susceptibility, has now been described. We highlight the latter application with the presentation of a case in which deletion of the APC gene on chromosome #5 was discovered serendipitously when aCGH was performed to further define breakpoints of a 2p deletion previously identified on conventional chromosome analysis. The patient was born at 30 weeks of gestation. A two-vessel cord, heart defect, microcephaly and polyhydramnios were detected by ultrasound in the 3rd trimester. Undescended testes and hypospadias were noted after delivery. Postnatal echocardiogram demonstrated a PDA, ASD and double aortic arch with coarctation of one of the arches. Chromosome analysis revealed an interstitial deletion of the short arm of chromosome #2 [46,XY,del(2)(p11.2p12)]. By age 3 years, the patient had developed hydrocephalus (s/p VP shunt placement), severe GE reflux and chronic lung disease. He was also growth retarded and developmentally delayed. Oligoarray was performed to clarify the deletion breakpoints. In addition to the 2p anomaly [arr 2p12(78,115,314-82,644,204)x1, at least 4.5 Mb], 2 other deletions were detected: an at least 7.4 Mb deletion of 5q [arr 5q21.3q23.1(108,464,047-115,906,031)x1] with breakpoints 5q21.3 to 5q23.1 and a 1.1 Mb deletion within 7p15.2 [arr 7p15.2(26,052,791-27,171,164)x1]. Of significance to the patient's future medical management was the 5q deletion which contained at least 21 genes, including the APC gene associated with familial adenomatous polyposis (FAP), the REEP5 (deleted in polyposis 1) gene and the MCC (mutated in colorectal cancers) gene. Literature reports of individuals with deletions of this region describe dysmorphic features and mental retardation in addition to FAP. This case illustrates the effectiveness of aCGH at precisely defining breakpoints of known chromosomal abnormalities as well as the effectiveness of uncovering tumor susceptibility genes, knowledge of which can directly affect patient management.

## 1397/W/Poster Board #1055

**Diagnosis of Cohen syndrome in twin brothers: Using array CGH to identify a deletion that unmasks a recessive condition.** *M.R. Rossi[1], E. Rooney-Riggs[1], H. Wang[2], M.P. Adam[1], P.M. Fernhoff[1], G. Richard[3], S.J. Bale[3], C.L. Martin[1], D.H. Ledbetter[1].* 1) Department of Human Genetics, Emory University, Atlanta, GA; 2) DDC Clinic for Special Needs Children, Middlefield, OH; 3) GeneDx, Gaithersburg, MD.

Cohen syndrome is a rare autosomal recessive condition characterized by moderate to severe mental retardation and typical facial features. Because the clinical features of Cohen syndrome may not be easily recognized in infancy or early childhood, many patients are diagnosed much latter in life. Here we describe the diagnosis of two year old twin boys with Cohen syndrome who carry both a heterozygous deletion and a nonsense mutation of the VPS13B (COH1) gene in trans. The diagnosis of Cohen syndrome was initiated after a clinical cytogenetic array performed by our laboratory identified a 173 kilobase (kb) interstitial deletion of chromosome 8 band q22.2 in one of the children. The heterozygous deletion removed exons 19-25 of the VPS13B gene, and was predicting to be an inactivating intragenic gene deletion. Subsequent resequencing of the entire coding region of the VPS13B gene in this same child, identified a heterozygous nonsense mutation, c.1141 C>T (p.Q381X). This heterozygous point mutation and the intragenic deletion were confirmed in the second twin. Parental testing identified that the boys' father was mosaic for the heterozygous 173 kb deletion, and their mother was a heterozygous carrier of the Q381X mutation. Although this case represents what may be a rare example of a cytogenetic array identifying a recessive disorder that was unmasked by deletion, it illustrates the imperative to interpret cytogenetic array findings within the clinical context of specific patients.

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## 1398/W/Poster Board #1056

**Reciprocal duplication of Sotos syndrome region (5q35) detected by oligo-array CGH in a patient with congenital anomalies.** *J. Lee, H. Zhang, M. Sun, J. Keeth, X. Lu, J. Mulvihill, S. Li.* Dept Pediatrics, OUHSC, Oklahoma City, OK.

The haploinsufficiency of the NSD1 gene due to 5q35 microdeletions or intragenic mutations is the major cause of Sotos syndrome, an overgrowth disorder characterized by large size, large hands and feet and poor coordination. The common ~1.9 Mb deletion breakpoints in Sotos syndrome are located at two flanking low copy repeats (LCRs), implying that non-allelic homologous recombination (NAHR) between LCRs is the cause of this deletion. This is a common mechanism of the recurrent microdeletion/duplication syndromes of 7q11.2, 17p11.2 and 22q11.2 regions. Using oligo-array CGH to detect genomic imbalances (gain or loss) of chromosomal segments that are too small to be seen by G-banding analysis is becoming a routine in patients with congenital anomalies. This new technology has resulted in the identification of many new syndromes, and has expanded our knowledge and understanding of known syndromes. Oligo-array CGH was used as a whole genome screening tool to detect copy number changes in a 2- year-old boy with microcephaly, seizures, delayed bone age and failure to thrive. Oligo-array CGH detected a duplicated fragment approximately 2.1 Mb in size located at 5q35.3 including the NSD1 gene. Reviewing the genomic database in the region, it showed the duplicated region likely arose by NAHR between LCRs as a reciprocal event of Sotos syndrome. Our case suggests that the gene dosage effect of the NSD1 gene causes a reversed phenotype of Sotos syndrome, i.e. microcephaly and delayed bone age.

## 1399/W/Poster Board #1057

**Chromosomal abnormalities detected in missed abortions occurring in Korean pregnant women.** *E.Y Choi[1], E.J. Seo[1, 2], K.R. Jun[1, 2], H.W. Yoo[1, 3], H.S. Won[4], J.Y. Lee[1].* 1) Medical Genetics Laboratory, Asan Medical Center, Seoul, Korea; 2) Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; 3) Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; 4) Department of Obstetrics and Gynecology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Causes of missed abortion are generally the same as those causing spontaneous abortion or early pregnancy failure causes, including anembryonic gestation (blight ovum), fetal chromosomal abnormalities, maternal disease, embryonic anomalies, placental abnormalities, and uterine anomalies. In this study, we performed to analyze the fetal karyotypes detected in missed abortions occurring in Korean pregnant women and compared the incidence of chromosomal abnormalities and causes of missed abortions with reports for other populations. We collected cytogenetic results of 217 cases of products of missed abortion over a period of 9 years (Jan, 2001 ~ May, 2009) and their clinical informations, including gestational age at the time of the pregnancy loss, maternal age, and previous reproductive history. The karyotyping was done by means of G-banding techniques after tissue culture of aborted products. Of the 217 cases, chromosomal abnormalities were identified in 89 cases (41.0 %). Of the chromosomal abnormalities identified, autosomal aberrations were predominant. Especially the most common abnormalities, autosomal trisomies, were found in 48 cases (53.9 % of the abnormal cases), including trisomy 16 (12 cases), trisomy 18 (7 cases), and trisomy 22 (8 cases). Sex chromosomal abnormalities were identified in 9 cases. Two cases with both autosomal and sex chromosomal abnormalities were founded. Five cases showed double trisomies, which had frequently chromosome 21 and advanced maternal age. Triploidy was identified in 10 cases and tetraploidy were found in 3 cases. Structural rearrangements were found in 10 cases; 4 cases with balanced translocations and 6 cases with unbalanced translocation or deletion. Our results detected skewing of sex ratio(M:F = 1:3.1) and low abnormal rate of fetal karyotype. This sex ratio may be likely to reflect the overgrowth of contaminated maternal cells in placenta culture. Cases of chromosomally normal spontaneous abortion will confirm by interphase FISH and array CGH. This study in Korean population may provide the valuable informations for genetic counseling.

## 1400/W/Poster Board #1058

**Novel Chromosome Xq28 Microduplication Mediated by the Homologous int22h-1 and int22h-2 Repeats in Four Cognitively Impaired Males.** *A. El-Hattab[1], V. Sutton[1], V. Enciso[2], E. Roeder[2], D. Grange[3], P. Stankiewicz[1], SW. Cheung[1], A. Patel[1].* 1) Dept Molec & Human Gen, Baylor Col Medicine, Houston, TX; 2) Genetics and Pediatrics, The University of Texas Health Science Center at San Antonio, San Antonio, TX; 3) Division of Genetics and Genomic Medicine, St. Louis Children's Hospital, St. Louis, MO.

During the last decade, about 80 genes on the X chromosome have been associated with X-linked mental retardation (XLMR). The recent expanded use of high resolution genome analysis by array comparative genomic hybridization (array CGH) has led to the identification of many cryptic microdeletions associated with XLMR. Chromosome X duplications has also been associated with XLMR with duplications including MECP2 gene being the most frequent. Here we report four cognitively impaired males with a novel ~ 0.5 Mb microduplication in Xq28 identified by array-CGH. Two male siblings aged 5.5 and 2.5 years who presented with developmental delayed and aggressive behavior. The older brother has minor facial features including broad forehead, down-slanting palpebral fissures, and broad nasal bridge. In addition, he has clinodactyly and tapered index fingers. The younger brother has failure to thrive, microcephaly, tapered distal fingertips, umbilical hernia, myopia and astigmatism, and minor facial features including, short forehead, poorly formed anterior helix, and broad nasal bridge. Their mother was found to carry the same Xq28 duplication. An 18-year-old male who presented at the age of 6 years with difficulties in walking that progressed to spastic diplegia with white matter hypomyelination noted in the brain MRI. In addition, he has scoliosis, bilateral syndactyly, and learning difficulties. A 12-month-old male infant presented with global developmental delay, microcephaly, and minor facial features including broad nasal bridge and deep seated eyes. Family history was significant for two maternal uncles with mental retardation. The proximal and distal breakpoints mapped to the directly oriented homologous low-copy repeat clusters known as int22h-1 and int22h-2 which, most likely, are responsible for mediating this duplication via non-allelic homologous recombination. The int22h-1 and int22h-2 in addition to int22h-3, mediate factor 8 gene inversion in about 50% of hemophilic patients. To our knowledge, similar duplications in this region have not been reported previously. However, an apparent reciprocal deletion has been reported in a family where females had skewed X inactivation and recurrent miscarriages, suggesting that such deletions may be lethal in males. In addition to the Factor 8 gene, there are six OMIM genes in the region. We propose this is a novel XLMR microduplication syndrome.

## 1401/W/Poster Board #1059

**Parental origin of *de novo* t(11;22)(q23;q11).** *T. Ohye[1], H. Inagaki[1], H. Kogo[1], M. Tsutsumi[1], T. Kato[1], M. Tong[1], M. Macville[2], L. Medne[3], E. Zackai[3], B. Emanuel[3], H. Kurahashi[1].* 1) Div Molecular Genetics, Fujita Health Univ, Toyoake Aichi, Japan; 2) Div Clinical Genetics, Univ Hospital Maastricht, Maastricht, Netherlands; 3) Div Human Genetics, Children's Hosp Philadelphia, Philadelphia, PA.

The constitutional t(11;22)(q23;q11) is a well-known recurrent non-Robertsonian translocation in humans. While translocations generally occur in a random fashion, the breakpoints of t(11;22)s are concentrated within several hundred base pairs on 11q23 and 22q11. These regions are characterized by palindromic AT-rich repeats (PATRRs), which could cause the genomic instability. *De novo* t(11;22)s are detected in sperm from healthy males at a frequency of $1/10^4$-$10^5$, but never in lymphoblasts, fibroblasts or other human somatic cell lines, suggesting that the generation of a t(11;22) is linked to meiosis. However, female germ cells have not been tested since the number of human oocytes that can be examined is limited. To investigate whether the translocation is meiosis-specific or male germ cell specific, we attempted to determine parental origin of *de novo* t(11;22) cases. We studied 7 carriers of *de novo* t(11;22)s and their parents. The PATRRs and flanking regions on chromosome 11, 22, der(11) and der(22) were amplified by PCR and the nucleotide sequences were determined. The highly polymorphic nature of the PATRRs allowed us to determine the parental origin of the *de novo* t(11;22)s. All of the seven translocations were found to be of paternal origin. This result indicates a novel mechanism of sperm-specific generation of t(11;22)s. It is proposed that replication errors during the numerous cell divisions in pre-meiotic spermatogenic cells or conformational changes of the DNA during chromatin remodeling in the post-meiotic stages of spermatogenesis might contribute to male-specific formation of *de novo* t(11;22)s.

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## 1402/W/Poster Board #1060

**Prenatal detection of a marker chromosome derived from chromosome 8 by array- CGH.** *N. Qin[1], R. Schultz[2], T. Freeman[1], T. Johnston[1], B. Huang[1].* 1) Genzyme Genetics, Orange, California; 2) Signature Genomic Laboratories, Spokane, Washington.

Marker chromosome detected during prenatal diagnosis pose a difficult situation for genetic counseling as the risk associated with different marker chromosomes varies. A precise identification of the genetic contents of the marker will therefore be helpful. We present a case a prenatally detected marker derived from chromosome 8 in a fetus with ventricular septal defect (VSD). Amniocentesis was performed on a 34-year-old, Hispanic woman due to an abnormal ultrasound finding of ventricular septal defect (VSD) and micrognathia at 19 weeks of gestational age. Cytogenetic analysis on amniocytes revealed four cell lines. Of the total 15 colonies, 5 from two independent cultures showed an extra small marker, 4 from two independent cultures showed an extra D-group size marker, 5 from two independent cultures showed both kinds of marker, and one colony had a normal 46,XX female karyotype. Fluorescence in situ hybridization (FISH) studies using probes specific for the centromeres of chromosomes 13, 14, 15, 18, 21, 22, X and Y were unable to identify the origin of both the marker chromosomes. Microarray CGH (a-CGH) was then employed and identified that both markers were derived from the peri-centromeric region of chromosome 8 with sizes of 2.3 and 12.4 MB respectively, containing both short arm and long arm material. FISH with chromosome 8 specific probes detected four cell lines, which was consistent with the cytogenetic findings. Maternal cytogenetic analysis showed a normal karyotype. Paternal chromosome study was not available. The pregnancy was then terminated. This study demonstrates that a-CGH is a powerful technique in identifying the origin of the unknown marker chromosome, which enables better karyotype-phenotype association.

## 1403/W/Poster Board #1061

**Efficacy of an Automated Metaphase Scanner in the Clinical Cytogenetics Laboratory.** *C.M. Higgins, W.G. Sanger, M.M. Hess, B.J. Dave.* Human Genetics Lab, Munroe Meyer Institute for Genetics and Rehabilitation, University of Nebraska Medical Center, Omaha, NE.

In the cytogenetics lab, microscopy has historically been a time-consuming part of chromosome analysis. Automated digital karyotyping systems have curtailed the cumbersome dark room tasks and replaced manual karyotyping with computerized digital versions. However, long, physically demanding hours are still required at the microscope to locate and analyze metaphases. More recently, automation has found its place in metaphase scanning to decrease the hours required at the microscope. An automated metaphase scanner locates the metaphases and captures digital images into specific case files which are then organized with a fully automated bar coding system. These cases can then be analyzed and karyotyped at individual computers. We have integrated the Genetix GSL 120® automated scanner into our laboratory work flow. To determine the efficacy of the system, we performed comparisons of work hours, abnormality rates, and turn-around times (TAT) for a period of 60 days before and after introducing the automated system. In the 60 days prior to the use of automated scanner, cytogenetic analysis of 173 consecutive bone marrow specimens from hematologic cancer patients, including diagnostic and follow-up studies, required 2,640 work hours from 5.5 cytogenetic technologists. The TAT was 2.9 days with an abnormality rate of 22%. In the 60 days after the implementation of the scanner, a total of 2,160 work hours from 4.5 technologists were used to analyze 169 bone marrow specimens with a reduced TAT of 2.57 days and the abnormality rate was maintained at 22.2%. With a work force reduction of 480 hours, the TAT was reduced by 0.3 days without compromising the abnormality rate. The integration of this system has thus increased the number of cases analyzed per technologist while organizing an entire study in the computer. This information can be easily accessed for a full review of the metaphases without the use of the microscope. The software has highly visual case summary capabilities that encapsulate the pertinent information for each case thus facilitating our goals for a “paperless” file. Most significantly, the system increases the learning capabilities and allows for cytogenetic analysis by technologists-in-training to be reviewed by experts. With the ever-increasing workload in the cytogenetics laboratory and smaller experienced workforce, the automated metaphase scanner is a welcome addition as an efficient system for timely reporting of cytogenetics results.

## 1404/W/Poster Board #1062

**Wide spectrum of Peters and Axenfeld-Rieger anomalies secundary to a 4q25 microdeletion encompassing the PITX2 gene.** *A. Hazard[1], M. Mathieu[1], G. Morin[1], B. Demeer[1], J. Andrieux[2], M.C. Vincent[3], A. Receveur[4], S. Kanafani[4], H. Copin[4], B. Devauchelle[5].* 1) Clinical Genetics, Amiens Unversity Hospital, Amiens, France; 2) Molecular Laboratory, Jeanne de Flandre Hospital, Lille, France; 3) Molecular Genetics, Toulouse University Hospital, Toulouse, France; 4) Cytogenetics, Amiens Unversity Hospital, Amiens, France; 5) Stomatology, Amiens Unversity Hospital, Amiens, France.

Many genes are involved in the ocular development. The alterations of some of them are responsible of the Peters or the Axenfeld-Rieger anomalies (PITX2, FOXC1, PAX6 ...). In theses conditions, angle anomalies are responsible of glaucoma in 50% of cases, usually congenital and difficult to manage. When they are associated with extra-ocular symptoms, the name of Rieger or Peter + syndrome can be used. In these entities, the mode of inheritance is usually autosomal dominant. We report a 21-year-old patient, third child of healthy non-consanguinous parents with a negative familial history. He presented a Peters anomaly of the right eye requiring an iridectomy at 3 months of age, and an Axenfeld-Rieger anomaly of the left eye associated with a glaucoma at the age of 10. During childhood, he beneficiated of several surgical interventions that concerned umbilical hernia, Meckel diverticulum, bifid uvula, posterior sub-mucous cleft palate, pharyngoplasty and tympanoplasty. He developed dysmorphic features including flat malar region and retrognathia requiring surgical repair at the age of 16. He also presented hypodontia and persistence of lacteal teeth. He had a normal mental development, a normal puberty and a normal stature (1m80), but secondary to his visual impairment, he studied in a special school. Genetic investigations were negative for the standard karyotype and the sequencing of the genes PITX2, FOXC1 and PAX6. The array-CGH exhibited a de novo 1.7 Mb deletion at the locus 4q25 encompassing 13 genes including PITX2.

## 1405/W/Poster Board #1063

**Exploration of genes related to X-linked mental retardation (XLMR) by BAC-based X-tiling array.** *S. Honda[1], S. Hayashi[1], I. Imoto[1], J. Toyama[2], N. Okamoto[3], K. Kurosawa[4], E. Nakagawa[5], Y. Goto[6], J. Inazawa[1].* 1) Dept Molec Cytogenetics, Medical Research Inst, Tokyo, Japan; 2) Okinawa Child Development Center, Department of Pediatrics, Okinawa, Japan; 3) Department of Medical Genetics, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan; 4) Division of Medical Genetics, Kanagawa Children’s Medical Center, Kanagawa, Japan; 5) Div. of Child Neurol. Musashi Hosp., Natl. Center of Neurol. and Psychiat., Tokyo Japan; 6) Dept. of Mental Retardation and Birth Defect Res., Natl. Inst. of Neurosci., Natl. Center of Neurol. and Psychiat., Tokyo Japan.

An estimated 10-12% of mental retardation (MR) is caused by mutation on the chromosome X. Although 82 X-linked mental retardation (XLMR) genes have been identified so far, many other XLMR genes have not been identified. We constructed a BAC-based X-tiling array which contains a total of 1001 BACs throughout chromosome X except pseudoautosomal regions. In order to identify novel XLMR-associated genes, we performed array-CGH in 147 families with XLMR or probable XLMR using ou X-tiling array. CNAs relevant to MR were judged when copy number changes detected were involved known XLMR genes or have not been registered as copy number variations (CNVs) in Database of Genomic Variants (http://projects.tcag.ca/variation/). As a result, CNAs were detected in 10 families (7%). Among them, 5 had CNAs in regions involved in known XLMR-associated genes. Dup(Xq28) including *MECP2* was detected in 3 families and this frequency (3/147=2.04%) is almost same in Europe and the United States. Each patient in the 3 families shows common phenotypes such as severe MR, muscular hypotonia, absent speech, recurrent respiratory infection and cerebral atrophy. Del(Xp11.22) including *SHROOM4* was detected in a boy with moderate MR in one family. Dup(Xp11.23) harboring *FTSJ1* and *PQBP1* was detected in a boy and his younger sister in another family. On the other hand, other CNAs in residual 5 families are novel.

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## 1406/W/Poster Board #1064

**Characterization of regulatory sequences essential for homologous pairing of chromosome 15q11-13.** *S. Horike[1], M. Meguro-Horike[1], M. Oshimura[2].* 1) Frontier Science Organization, Kanazawa University, Kanazawa,Ishikawa, Japan; 2) Department of Biomedical Science, Institute of Regenerative Medicine, Tottori University, Yonago, Tottori, Japan.

Autism is a common neurodevelopmental disorder characterized by abnormalities in social, communicative, and behavioral functioning. Although the etiology of autism remains largely unknown, cytogenetic and genetic studies have suggested that autism may be influenced by genomic imprinting of 15q11-13, through copy number gains of 15q11-13 occurring in 1-3% of autism cases. We focused on the homologous pairing of 15q11-13 in human neuronal cells, previously observed by Dr. Janine M. LaSalle (University of California, Davis). In addition, Dr. LaSalle observed that homologous pairing of 15q11-13 was deficient in Rett Syndrome (RTT), Angelmann Syndrome (AS), and autism brain. To model 15q11-13 maternal duplication in a neuronal cell line, we develop a systematic *in vitro* approach for studying the epigenetic consequences of cytogenetic disruptions to 15q11-13 using monochromosomal hybrids containing individual human chromosomes 15 of defined parental origin with and without targeted deletions of regulatory sequences. Modified paternal or maternal copy of human chromosome 15 will be transferred into the human SH-SY5Y neuronal cells by microcell fusion. Then, FISH analysis will be performed to compare how the homologous alleles of 15q11-13 are organized in human neuronal cells with and without modified human chromosome 15 copy number or sequence changes. The results of these studies are expected to be highly significant in 1) understanding how the homologous alleles of 15q11-13 are organized in the mammalian brain, and 2) designing better diagnostic tests and treatments for autism-spectrum disorders.

## 1407/W/Poster Board #1065

**Triplication of the *MECP2* gene causing a severe neurodevelopmental phenotype can be induced by the presence of low copy repeats (LCRs).** *C.M.B. Carvalho[1], M.B. Ramocki[2,3], L. Seaver[4,5], E. White[4], L. Friehling[6], P. Liu[1], S.W. Cheung[1], H. Zoghbi[1,2,3], J.R. Lupski[1,2,3].* 1) Dept Molecular Human Genetics, Baylor Col Medicine, Houston, TX; 2) Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Children's Hospital, Houston, TX; 4) Kapiolani Medical Specialists; 5) Departments of Pediatrics, John A. Burns School of Medicine, Honolulu, HI; 6) Children's Medical Associates, Alexandria, VA.

Duplication involving the *MECP2* gene is one of the most common genomic rearrangements identified in neurodevelopmentally delayed males. We recently published the molecular characterization study of 30 patients carrying *MECP2* duplications using a custom 4-Mb tiling-path oligonucleotide array in which we detected six patients with complex rearrangements characterized by a triplicated segment embedded within the duplication. We extended our cohort to 32 patients and identified one additional patient with a triplication, totaling 32 patients and seven triplications (22%). The complex rearrangements vary in size from ~430 kb to ~653 kb, whereas the triplicated regions range in size from ~33 kb to ~537 kb. Interestingly, despite the fact that the proximal rearrangement breakpoints are scattered, the distal breakpoints, for both duplications and triplications, map within the same regions: either low-copy repeat (LCR) K1 or K2. The LCR K1 and K2 are positioned in inverted orientation, have 99% sequence identity and are 11.3 kb in length. The triplicated region spans the *MECP2* and *IRAK1* genes in three patients who have much more severe phenotype when compared to patients carrying *MECP2* duplications. The complex rearrangements include duplication of the *FLNA* gene in all cases, which likely contributes to their phenotype severity. The common clinical features are a history of polyhydramnios, dysmorphic facial features, hypotonia, severe global developmental delays, recurrent infections, chronic lung disease, aspiration, requirement for gastric or gastric-jejunal tube feeds, constipation, gastroesophageal reflux in 2/3, intestinal pseudo-obstruction in 1/3, epilepsy/infantile spasms in 1/3, bilateral hearing loss in 2/3, and a history of acute life-threatening events requiring resuscitation in 2/3. In six out of seven cases we determined that the complexities were inherited from the mother. In conclusion, we provide evidence that the presence of LCRs in the *MECP2* vicinity can induce DNA strand lesions, such as a collapsed fork, and facilitate a Fork Stalling and Template Switching (FoSTeS) event producing the complex rearrangements involving *MECP2* and flanking genes.

## 1408/W/Poster Board #1066

**Cytological analysis reveals striking differences in crossover interference between males and females.** *J. Gruhn[1], T. Hansen[1], E. Cheng[2], T. Naluai-Cecchini[2], C. Rubio[3], K.W. Broman[4], T. Hassold[1].* 1) School of Molecular Biosciences, Washington State University, Pullman, WA; 2) Department of OB/GYN, University of Washington, Seattle, WA; 3) Instituto Valenciano de Infertilidad, Valencia, Spain; 4) Department of Biostatistics & Medical Informatics, University of Wisconsin-Madison, Madison, WI.

The number and placement of meiotic exchanges are highly controlled, with virtually all species exhibiting at least one crossover per chromosome and with the spacing of adjacent crossovers dependent on interference. Despite these tight controls, there are remarkable sex-specific differences in recombination in humans: linkage analysis indicates that females have over 1.5 fold as many exchanges as males, and the chromosomal location of exchanges varies between the sexes. In the present study, we used cytological methodology to visualize meiotic recombination events as they occur in human oocytes and spermatocytes, allowing us - for the first time - to directly compare crossover patterns and crossover interference in meiocytes of males and females. Specifically, antibodies against the meiotic crossover protein MLH1, the synaptonemal complex protein SYCP3, and centromere associated CREST, were utilized to identify the chromosomal location of recombination sites. Subsequently, fluorescence in-situ hybridization (FISH) was used to identify ten individual chromosomes (1, 6, 9, 13, 14, 15, 16, 18, 21 and 22), allowing us to generate chromosome-specific maps for each sex. Similarly, chromosome-specific interference levels were calculated by measuring distances between adjacent MLH1 foci and determining the deviation from the gamma distribution. Our analysis has identified striking differences between the sexes. Consistent with linkage studies, MLH1 foci were significantly elevated in females by comparison with males, and were more likely to be interstitially located. Especially remarkable was the difference in spacing of adjacent MLH1 foci. For each chromosome examined, females displayed narrower inter-focus distances, correlating with weaker levels of interference. These observations provide the first cytological insights into sex-specific differences in cross-over control and suggest that differences in interference underlie the variation in recombination levels between human males and females.

## 1409/W/Poster Board #1067

**High Levels of Meiotic Recombination in the Absence of Synapsis and Homolog Pairing in Drosophila Oocytes.** *H.E. Hall[1], J.P. Blumenstiel[2], J.K. Jeffress[3], S.L. Page[4], R.S. Hawley[1,5].* 1) Stowers Institute for Medical Research, Kansas City, MO; 2) Department of Ecology & Evolutionary Biology, The University of Kansas, Lawrence, KS; 3) Institute of Molecular Biology, University of Oregon, Eugene, OR; 4) Comparative Genomics Centre and School of Pharmacy and Molecular Sciences, James Cook University, Townsville, Australia; 5) Department of Physiology, Kansas University Medical Center, Kansas City, KS.

In Drosophila melanogaster oocytes, the synaptonemal complex (SC) forms between homologous chromosomes and facilitates both the maintenance of euchromatic pairing and the formation of reciprocal crossovers. The C(3)G protein, which composes the transverse filament of the SC, is comprised of a central coiled-coil domain flanked by N- and C-terminal globular domains. Null mutants in C(3)G display a failure to produce mature SC, a near complete absence of crossingover, and failure to maintain euchromatic pairing. These data have supported a model where long regions of synapsis and/or pairing are required for reciprocal crossingover. To identify functional domains of C(3)G, we have constructed in-frame deletion constructs that remove both a small region of the coiled-coil domain and the entire coiled-coil region. We expressed these constructs both by using the nanos-GAL4::VP16 over-expression system and by using the genomic promoter (via generation of site-directed transgenic flies). Preliminary studies of oocytes using the over-expression construct missing a small region of the coiled-coil domain as their only source of C(3)G display near-normal levels of meiotic recombination. However, these same oocytes show defects in synaptonemal complex formation, homologous chromosome pairing, and exchange distribution. This implies that neither complete homolog pairing nor synapsis are required for high levels of meiotic recombination, though they may be required for proper exchange distribution (interference). These data suggest that the role of C(3)G in crossover formation is functionally separate from its role in pairing and synapsis.

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## 1410/W/Poster Board #1068

**Regulation of Polo kinase by Matrimony is critical for faithful segregation of non-exchange homologs and cell cycle progression in *Drosophila* female meiosis.** *S. Smith[1,2], Y. Xiang[1], J. Griffiths[1], R. Hawley[1,2].* 1) Stowers Institute for Medical Research, Kansas City, MO USA; 2) University of Kansas Medical Center, Kansas City, KS USA.

Recent studies by Oliver et al., 2008 estimate that chromosome 21 is non-exchange in approximately 20% of human euploid oocytes, suggesting that a system exists to ensure faithful segregation of non-exchange homologs in human female meiosis. One model for such a system is segregation of non-exchange homologs in *Drosophila melanogaster* females. Utilizing this model, we have identified a gene called *matrimony (mtrm)*, which is essential for faithful segregation of non-exchange homologs and for proper timing of nuclear envelope breakdown (NEB) during *Drosophila* female meiosis. Moreover, a series of genetic and biochemical studies have shown that the Mtrm protein specifically regulates Polo kinase (Polo) in order to properly execute both of the meiotic processes mentioned above. Polo-like kinases are increasingly recognized as universal regulators of many meiotic and mitotic events, thus, a close examination of the mechanism by which Mtrm regulates Polo is essential. Here we utilize the site-specific ϕC31 integrase system to generate a series of transgenic *Drosophila* lines expressing mutant versions of the Mtrm protein. We examine the ability of mutant versions of Mtrm to rescue meiotic defects observed in oocytes containing compromised levels of the endogenous *mtrm* (and thus, mis-regulated Polo). Furthermore, we examine the ability of these mutant versions of Mtrm to physically bind Polo. We find that several mutant versions of the Mtrm protein including mutations that convert two evolutionarily conserved serines to alanine (MtrmS48A and MtrmS52A) are unable to rescue the meiotic defects observed in oocytes containing compromised levels of endogenous *mtrm*. While further work is necessary to confirm whether these residues are critical for the direct interaction of Mtrm and Polo in the fly, initial yeast two-hybrid analysis of these interactions suggests that this is the case. Overall, our work utilizing *Drosophila* as a model provides new insights into the mechanism by which Mtrm regulates Polo kinase to ensure faithful segregation of non-exchange homologs and proper cell cycle progression in female meiosis.

## 1411/W/Poster Board #1069

**Preimplantation Microarray Analysis using a SNP-based Whole Genome Platform allows for Simultaneous Screening of 24 Chromosome Aneuploidy, Unbalanced Translocations, and Single Gene Disorders in Human Embryos: First Application of Comprehensive Triple Factor PGD.** *B. Levy[1,2], X. Tao[2], J. Su[2], L. Northrop[2], M. Kamani[2], K. Miller[2], N. Treff[2,3], R.T. Scott[2,3].* 1) Dept Pathology, Columbia University Medical Center, New York, NY; 2) Reproductive Medicine Associates of New Jersey, Morristown, NJ; 3) UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ.

Targeted aneuploidy screening, detection of unbalanced translocations (unbal-t/locations) & prevention of single gene disorders represent the major areas of PGD & simultaneous screening for all 3 disorders in human embryos has hitherto been impossible. We have previously validated a novel SNP-based microarray method for aneuploidy screening of all 24 chromosomes (24-chr-AS) & demonstrated its superiority over current FISH-based methods. Here we apply this technique together with translocation & single gene disorder screening in the 1st triple factor PGD case ever performed. First, we validated our ability to detect unbal-t/locations using microarrays by evaluating discarded IVF embryos diagnosed by FISH to have unbal-t/location derivatives. Second, the ability to accurately determine the inheritance of single gene disorders was established by evaluating single cells from embryos discarded after a PCR-based diagnosis of a single gene disorder as well as from cell lines "affected" with Mendelian disorders like Aarskog syndrome (FGD1), dwarfism (FGFR3), & cystic fibrosis (CFTR). Finally, triple factor PGD was applied to a 27 year old patient with Alagille syndrome & a "balanced" translocation - 46,XX,t(2:20)(q21;p12.2) that also included a 2.36MB microdeletion at the breakpoint region on chromosome 20, including the JAG1 locus. We established a multiplex real-time PCR assay of informative SNPs for inheritance of the microdeletion based on the parental SNP microarray profiles. Trophectoderm (TE) biopsies from 6 morphologically normal blastocysts were obtained for analysis from the Alagille patient. The embryos were cryopreserved so that genetic analysis could be performed with the intent of thawing & transferring euploid & unaffected embryos. Half of the TE biopsy was evaluated by FISH for the microdeletion, unbal-t/location derivatives, & aneuploidy of chromosome 18 & the sex chromosomes. The remaining TE was evaluated by real-time PCR for the microdeletion, & by microarray analysis for the microdeletion, unbal-t/location derivatives, & 24chr-AS. Thirteen arrested embryos were also evaluated and none of them were genetically normal. Two cryopreserved embryos were diagnosed with Alagille syndrome (carriers of the same maternal "balanced" translocation), & 4 were unbalanced &/or had aneuploidy for one or more chromosomes. Real-time PCR, microarray, & FISH based microdeletion, translocation, & aneuploidy analyses were 100% consistent.

## 1412/W/Poster Board #1070

**Comparison of genome-wide array technologies for the detection of copy-number variants.** *A. Montpetit[1], T. Tucker[2], E. Lemyre[3], S. Chenier[3], P. Eydoux[4], D. Vincent[1], A. Delaney[5], J.L. Michaud[3], J.M. Friedman[2].* 1) McGill University and Genome Quebec Innovation Centre, Montreal, PQ, Canada; 2) Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 3) Division of Medical Genetics, Hôpital Sainte-Justine, Montreal, Quebec, Canada; 4) Department of Pathology and Laboratory Medicine, Children's and Women's Health Centre of British Columbia, Vancouver, BC, Canada; 5) Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, BC, Canada.

Copy-number variation (CNVs) is a widespread and common phenomenon among humans and represents the majority of the sequence differences at the base level between two individuals. They also likely play a major role in phenotypic diversity, disease susceptibility and evolution. Although next-generation sequencing can provide single-base resolution for CNV identification, this method is not yet cost-effective for clinical diagnostic or for biomedical research. Many CGH- or SNP-array technologies can identify CNVs throughout the genome, but it is not well-known what are their respective experimental false positive and false negative rates and how well they compare to one another. In this study, we sought to identify pathogenic CNVs in 30 children with mental retardation by comparing the data from 4 different technologies (Agilent, NimbleGen, Affymetrix, Illumina). By using both parents as reference samples, our experimental design was specifically set-up to identify de novo CNVs. Overall we have identified an average of over 100 CNVs per child, including 5 occurring de novo. However, only 21% of the calls made with one technology did overlap with at least one other technology. Moreover, only the de novo CNVs identified by 2 or more technology could be validated by FISH or MLPA suggesting that the majority of the calls are false positives. This study demonstrates the importance of an appropriate validation method before reporting CNVs linked with diseases. An investigation of the effect of various algorithms for CNV detection will also be presented.

## 1413/W/Poster Board #1071

**Detection of two novel chromosomal deletions associated with autism using array-based comparative genomic hybridization.** *W. Chien[1,5], Y. Wu[2], S. Gau[3], Y. Huang[2], C. Chen[1,4,5].* 1) Institute of Medical Sciences, Tzu-Chi University, Hualien, Taiwan; 2) Department of Child Psychiatry, Chang-Gung Children Hospital, Kewi-Shan, Taiwan; 3) Department of Psychiatry, National Taiwan university Hospital, Taipei, Taiwan; 4) Graduate Institute of Clinical Medicine, Tzu-Chi University, Hualien, Taiwan; 5) Division of Mental Health and Addiction Medicine, Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan.

Autism is a childhood-onset neurodevelopmental disorder with a strong genetic basis in its etiology. Conventional karyotype analysis has revealed that approximately 5-12% of ASD patients are associated with chromosomal structural aberrations, including translocation, inversion, deletion and duplication. Recently, several groups reported that submicroscopic deletion and duplication (collectively known as copy number variations, CNVs) are significantly associated with ASD using array-based comparative genome hybridization (aCGH) technology, suggesting genomic rearrangement is an important genetic mechanism of ASD. We screened for genomic DNA in a sample of patients with ASD using an oligonucleotide-based whole genome CGH array from Taiwan. Confirmations were performed using fluorescent in situ hybridization (FISH) and real-time quantitative PCR. Two boys were found to have a de novo subtelomeric deletion of ~ 6.8 Mb at 4q35.1-35.2 and a de novo terminal deletion of ~2.4 Mb at 8p23.2-pter, respectively. These two deletions were verified using fluorescent in situ hybridization (FISH) and real-time quantitative PCR, and they were not detected in another 282 patients with autism. To our knowledge, these two mutations have not been reported to be associated with ASD in the literature. Our data support that rare genomic variants may contribute to the genetic etiology of autism, and genes mapped to the deleted 8p and 4q regions as found in these two patients can be further considered as plausible candidate genes of autism in future study.

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## 1414/W/Poster Board #1072

**CGH analysis in a cohort of 121 patients with a malformation of cortical development.** *L. Villard[1,2], C. Mignon-Ravix[1,2], P. Cacciagli[3], B. El-Waly[1,2], M. Milh[1,2,3], N. Girard[3], B. Chabrol[1,2,3], N. Philip[1,2,3].* 1) INSERM, U910, Marseille, France; 2) Aix-Marseille University, Marseille, France; 3) Marseille University Hospital, La Timone, Marseille, France.

We have collected clinical details and DNA samples for 121 patients with a malformation of cortical development in a national prospective study performed in France since 2004. The cohort is composed of the following broad categories : 36 patients with lissencephaly, 37 patients with polymicrogyria, 26 patients with grey matter heterotopia and 22 patients belonging to two different categories. All the patients were genotyped for known genetic causes allowing the identification of 15 new mutations (12%). All negative cases (n=106) were analyzed with the Agilent 44K array CGH platform. In this group of patients, we have identified 27 genomic imbalances not previously described in the database of genomic variants (25%) in addition to several hundred non pathogenic CNVs. Among these cases, 21 were confirmed using quantitative PCR (77%). In 6 cases, we were able to show that the rearrangement was inherited from one parent. These cases were not included in further analysis. In the 15 remaining cases, we found defects on chromosomes 3p24 (200kb), 5q21 (150kb), 6q14 (1Mb), 6q22 (460kb), 7q11 (2Mb), 7q33 (120kb), 9p13 (140kb), 16p13 (900kb), 17p12 (1,5Mb), 17p13 (90kb to 300kb), 17q11 (300kb) and 19p13 (350kb). Interestingly, we detected LIS1 microdeletions in three patients previously analyzed with the commercial probe designed to detect chromosome 17p13.3 deletions. In the same 17p13.3 region, we identified a patient with nodular heterotopias and a small deletion involving the YWHAE gene as the only gene expressed in the brain. An unexpected 17p12 duplication involving the PMP22 gene was detected in a patient with nodular heterotopias and cerebellar vermis hypoplasia. On chromosome 16p13, we identified a patient with periventricular nodular heterotopias and a duplication of the CBP gene. In the other genomic regions involved, we performed expression analysis in search for genes expressed in the brain. Interestingly, several microrearrangements contain one or very few genes. These genes are currently being investigated for their putative role during the development of the cerebral cortex and are screened in patients with the same clinical picture.

## 1415/W/Poster Board #1073

**Unusual Clinical Presentation of the 3q29 Microdeletion Syndrome: Autism and Psychotic Symptoms.** *F. Quintero-Rivera[1], J.A. Martinez-Agosto[2].* 1) Pathology & Lab Medicine, David Geffen Sch Med UCLA, Los Angeles, CA; 2) Department of Human Genetics and Division of Medical Genetics, Department of Pediatrics, David Geffen School of Medicine, University of California, Los Angeles, CA.

The screening of individuals with minimal dysmorphic features and mental retardation as the predominant feature has resulted in the delineation of several new microdeletion syndromes. Interstitial deletions of 3q29 have been recently described as one of these new syndromes. The clinical phenotype is variable despite an almost identical deletion size. Here, we report on a new individual to further expand the clinical presentation of this rare disorder and compare with previous reports to further define the phenotype. The proband is a 10 year-old female with a history of autism and schizophrenia versus bipolar disorder presenting with increasing suicidal ideation, and dysmorphic features including: epicanthal folds, a broad nasal bridge with a prominent metopic suture and micrognathia, asymmetric face with the right face and lips drooping, and myopathic face, almond-shaped eyes, mandibular hypoplasia, and tapered fingers. SNP array CGH detected a 1.629 Kb de novo chromosome 3q29 interstitial microdeletion, arr cgh 3q29(197194059-198823098)x1. These findings were confirmed by standard chromosome analysis (550- band resolution) and FISH with the 3p/3q subtelomere Vysis probe, 46,XX,del(3)(q29).ish 3p(TELx2),3q(TELx1). This 1.6 Mb deletion encompasses 5 known genes (PCYT1A, PAK2, MFI2, DLG1, BDH), and 17 uncharacterized transcripts. Our patient expands the phenotypic features of 3q29 deletion syndrome. The unusual clinical presentation reflects a general trend towards the classification of mental retardation syndromes associated with fine chromosomal abnormalities on the basis of similar molecular abnormalities, and not on grouping of clinical dysmorphology features alone.

## 1416/W/Poster Board #1074

**Precise identification of a unique psu dic(15;15)(q13;q13) by array cgh, FISH and classical cytogenetics.** *S.D. Batish[1], M.J. Macera[2], J. Breshin[2], F. Cohen[2], D. Thakkar[2], M. Ito[1], C. Towne[1], C. Braastad[1], J. Koch[1], A. Babu[2].* 1) Athena Diagnostic, Worcester, MA; 2) Div of Molecular Medicine and Genetics, Dept of Medicine, Wyckoff Heights Medical Center, Brooklyn, NY.

An eighteen month-old male was referred with severe developmental delay/mental retardation, encephalopathy, infantile spasms, hypotonia and dysmorphic features. Chromosome micro array analysis (CMA) revealed a gain of copy number on chromosome 15q11.2-q13.3, arr cgh 15q11.2-q13.2(20,277,049-28,157,051)x3; 15q13.2-q13.3(28,157,051-30,707,020)x3, which was detected by 988 probes. The size of the duplication is approximately 10.3 Mb and includes 35 genes. Cytogenetic analysis with GTG banding revealed the addition of a small satellited marker chromosome. FISH analysis for D15Z1 and SNRPN located at 15q13.3, confirmed chromosome 15 as the origin of the marker. These probes labeled two pericentromeric regions located at the distal ends and two copies of SNRPN, located in the interstitial section of the marker. One unique feature of this marker chromosome, as revealed by GTG banding, was the presence of two morphologically different satellites. Both stalks of the marker chromosome are positive by AgNOR staining. The karyotype is 47,XY,+psu dic(15;15)(q13;q13).ish psu dic(15;15)(D15Z1++,SNRPN++). Clinical presentation of a duplicated 15q11-q13 region, generally described as the inv dup (15) or idic (15) syndrome, is frequently of maternal origin. Features include developmental delay, central hypotonia, epilepsy, intellectual disability and autistic behavior. Microsatellite analysis on parental DNA or methylation analysis on the proband DNA can determine the parent of origin of the additional material. However, neither of these molecular methods routinely clarifies if the material is from a single chromosome or from homologous chromosomes. In this case, because of the identifiable variation in satellite/stalk of chromosome 15, we were able to determine that what would have been classified as an inv dup (15) was actually derived from two homologues and could be more correctly designated as psu dic. The phenotype of this individual case strongly suggests that the material is maternal in origin. The identifiable nature of the satellites will likely allow us to determine parental origin using classical cytogenetics karyotype analysis on the parents. This work is underway. This case emphasizes the importance of the use of all available methodologies to ensure a correct diagnosis and more precisely define the idic/inv dup (15) syndrome.

## 1417/W/Poster Board #1075

**Interstitial Deletion of 8q in a girl from Unbalanced Segregation of a Paternal Deletion/ring Karyotype.** *R.D. Burnside[1], J. Ibrahim[2], C. Flora[2], S. Schwartz[1], J.H. Tepperberg[1], P.R. Papenhausen[1].* 1) Cytogenetics, LabCorp, Durham, NC; 2) St. Joseph's Children's Hospital, Paterson, NJ.

The McClintock mechanism of chromosome breakage and centromere misdivision to create a deleted chromosome with its concomitant excised ring chromosome has been described in approximately one dozen reports. We report a case of a 6 year old girl with a prenatal history significant for IUGR and a two-vessel umbilical cord. She was diagnosed neonatally with a VSD that was surgically repaired. At 4 years of age, she was referred to genetics for short stature and noted to be developmentally delayed with dysmorphic facial features, including narrow facies, protuberant ears and a beaked nose. Her skeletal exam was significant for bilateral fifth finger clinodactyly, broad thumbs and great toes. Chromosome analysis and aCGH testing were recommended at that time, but not performed due to insurance issues. Two years later at a follow-up visit chromosome analysis and Affymetrix 6.0 SNP microarray was ordered and performed on cultured blood lymphocytes, and revealed a cytogenetically visible deletion in proximal 8q that was further defined by microarray as a 10.6 Mb deletion from 8q11.1->q12.1. Fluorescence in situ hybridization using an alpha- satellite probe demonstrated that the deletion included a significant portion of the pericentromeric repeat sequences, as well as 73 genes in the proximal q arm. The deleted chromosome 8 appeared to have a new constriction at 8p22, suggesting the formation of a neocentromere, even though some alpha-satellite sequences still appeared at the normal location. Chromosome analysis of the phenotypically normal father revealed a mosaic karyotype of 47,XY,del(8)(q11.1q12.1),+r(8)(q11.1q12.1).ish del(8)(q11.1q12.1)(D8Z2 dim),+r(8)(D8Z2+)[18]/46,XY[2]. His deleted chromosome 8 also demonstrated the presence of an apparent neocentromere at 8p22. FISH studies revealed that the majority of the chromosome 8 alpha-satellite DNA was excised from the del(8) and resided in the ring chromosome. This excision likely necessitated the formation of a neocentromere to stabilize the chromosome during mitosis. This case clearly illustrates the utilization of classic cytogenetics, FISH, and array technologies to better characterize chromosomal abnormalities and to provide the proper recurrence risks for these aberrations. It also indicates a rare case where a neocentromere can form on a chromosome even in the presence of existing alpha-satellite DNA, consistent with the "McClintock mechanism".

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## 1418/W/Poster Board #1076

**Xq-Yp translocation? - Cytogenetic characterization of a rare 46,XX individual with SRY on Xq.** *M. Fang, S. O'Hara-Larrivee, J. Eck, P. Peterson, K. Starr, K. Kroeger, C. Kellum, S. Pereira.* Seattle Cancer Care Alliance, Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA.

Translocations involving the X and Y chromosomes are often associated with abnormalities of sex differentiation. Aberrant translocations between the pseudoautosomal region on the short arms of the human X and Y chromosomes (Xp-Yp translocation) is the most frequent cause of 46,XX maleness when the testis-determining SRY gene is transferred to the distal Xp. However, Xq-Yp translocations are extremely rare. To our knowledge, only one case has been reported in a 46,XX true hermaphrodite. Here, we report a possible second case of Xq-Yp translocation. A male post-bone-marrow-transplant patient with a female donor was evaluated for chimerism using short tandem repeat (STR) analysis, which demonstrated 100% donor engraftment, but the sequence at the Amelogenin locus that distinguishes the X and Y chromosomes suggested that the donor was a male. G-banding cytogenetic analysis of the bone marrow demonstrated a 46,XX female karyotype with a potential subtle Xq deletion of uncertain clinical significance. Further characterization of the X chromosome with fluorescence in situ hybridization using probes specific to the SRY gene and the X chromosome centromere revealed that the SRY sequence was located on the long arm of the X chromosome with the potential deletion. Chromosome microarray analysis is underway to determine the exact nature of the rearrangement. This is likely another rare case of Xq-Yp translocation. The SRY gene appeared to be on the inactive X-chromosome exclusively, which may explain the apparent femaleness of the donor. Additional clinical phenotypic evaluation of the donor and a molecular study of the X-inactivation pattern in this individual will provide further insight into the phenotype-genotype correlation.

## 1419/W/Poster Board #1077

**Characterization of novel copy number changes in the clinical diagnostic setting using customized targeted cytogenomic arrays.** *M.E. Gorre[1], J. Kim[1], C. McCaskill[1], R. Shah[1], D. Suciu[2], M. Caraballo[2], M.S. Mohammed[1].* 1) Combimatrix Molecular Diagnostics, Irvine, CA; 2) Combimatrix Corporation, Mukilteo, WA.

Clinical use of microarrays for assessment of genomic copy number changes is becoming increasingly common for diagnosis of mental retardation and congenital abnormalities. Although most diagnostic arrays were initially designed to target a defined set of disorders, the trend toward enhancement of these targeted arrays with additional non-disease associated probes has provided advantages such as data normalization controls and the ability to scan the patient's genome for unexpected structural changes at higher resolutions. However, as array platform probe densities increase, so too does the complexity and challenge of data interpretation, clinical reporting, and confirmatory testing of abnormal findings, particularly in light of our limited understanding of copy number variations and their pathological significance. Here we describe the development and utility of a strategy for ultra high-density characterization of copy number aberrations discovered in routine clinical array CGH testing that limits the potential liabilities of performing whole genome ultra high-density array analysis. Using completely customizable long oligonucleotide microarrays of 12,000 probes, we were able to synthesize case-specific personalized arrays with uniquely-designed probes to refine breakpoints at the various probe densities and redundancies within a turnaround time of 10 days. With this strategy, we demonstrate the ability to confirm and further characterize or "zoom in" on discovered structural abnormalities for a set of 10 patients with mental retardation of unknown etiology within a timeframe that makes clinical implementation possible. As the complexity of array-based copy number analysis technology rapidly increases, strategies that utilize these advancements while mitigating the accompanying complications will be important for broadening utilization of microarrays in routine clinical diagnostics.

## 1420/W/Poster Board #1078

**Detection of mosaicism and UPD14 in a baby with Prader-Willi like features.** *L. Jenkins[1], H. Levy[2], E. Chen[2], X. Li[1], J. Jones[3], J. Schoof[4], J. Johnson[4].* 1) Kaiser Permanente Regional Cytogenetics Laboratory, San Jose, CA; 2) Kaiser Permanente Genetics Department, Oakland, CA; 3) Molecular Diagnostic Laboratory, Greenwood Genetics Center, Greenwood, NC; 4) Department of Medical Genetics, Shodair Children's Hospital, Helena, MT.

A 12 month baby boy was referred for a genetics evaluation for congenital hypotonia, severe vomiting, anal ring requiring dilation, small penis and deceleration in growth curves for weight and more severely for height. All newborn screening tests were reported to be normal. Brain MRI revealed a normal pituitary but narrow infundibulum. At 12 months, his was just below the 5th percentile, and height was much below the 5th percentile. He had forehead bossing, small mouth, penis, and feet. There was very faint hyperpigmentation variably on the extremities. He had mild motor delays. Growth hormone (GH) deficiency was observed and he was started on GH and testosterone. He subsequently was diagnosed to have food-protein-induced-enterocolitis syndrome. Prior high resolution cytogenetics, methylation study for Prader-Willi syndrome and molecular fragile X testing all showed normal results. To rule out a possible genomic imbalance or uniparental disomy (UPD) for chromosome 14, whole genomic microarray (aCGH; Agilent 44K design) analysis and UPD 14 tests were performed. aCGH revealed a level of 40% mosaicism for a trisomy 14 cell line. Further analysis for mosaic trisomy 14 was performed counting additional metaphase cells (50) and interphase FISH analysis was performed using the IGH probe at 14q32 (Abbott; Vysis, Inc.). These analyses revealed 10 and 60% mosaicism for trisomy 14, respectively. Interphase FISH analysis of a buccal sample showed an 18% level for trisomy 14. Microsatellite UPD14 testing showed biparental inheritance for all informative loci. One informative locus showed three alleles present, 2 maternal and 1 paternal. These results supported the prior cytogenetic and aCGH findings of mosaicism for trisomy 14. The samples were assessed for maternal UPD by quantitative PCR, showing a 4:1 ratio of mat:pat alleles , consistent with presence of a paternal copy only in the trisomic cell line, with the disomic cell line showing two copies of the maternal chromosome 14, assuming roughly equal populations of the two cell lines. The observed maternal allele also showed that the disomic cell line was presumably maternally heterodisomic for chromosome 14. CONCLUSION: A diagnosis of maternal UPD14 should be considered in a young child with a clinical picture of Prader-Willi like findings including hypotonia, feeding difficulties, poor growth, micropenis, and mild delays.

## 1421/W/Poster Board #1079

**Identification of 16p13.11p13.3 duplication on amniotic fluid using arrayCGH.** *K. Jänkälä[1], M. Kaare[1], H. von Koskull[1], C. Saloranta[1,2], T. Alitalo[1,3].* 1) Dept of Clinical Genetics, Helsinki University Central Hospital, Helsinki, Finland; 2) Dept of Obstetrics and Gynaecology, Helsinki University Central Hospital, Helsinki, Finland; 3) Dept of Medical Genetics, University of Helsinki, Helsinki, Finland.

Trisomy 16 is one of the most common trisomies found in prenatal samples, but structural rearrangements of chromosome 16 are less common. A total of 42 cases of 16p duplication have been published to date, of which 41 have been detected postnatally. A 16p11.2p12.1 duplication is the only prenatally diagnosed case so far. Since euchromatic variants 16p+ have also been described, interpretation of intrachromosomal changes in 16p can be problematic in prenatal samples. A patient was referred to our clinic because increased nuchal translucency was detected in the ultrasound. Amniocentesis was performed at the 15th week of gestation. Both *in situ* and long term cell cultures were established. Cytogenetic studies on amniotic fluid presented extra material in the short arm of one chromosome 16. The karyotypes of the parents were normal, which indicated that the chromosome 16 rearrangement had arisen *de novo*. Since it was impossible to determine exactly which region of the 16p was duplicated, if any, we continued to analyze the sample using microarrays (Agilent, human 244K). Cells from one small culture bottle were used for DNA extraction. The analysis revealed a 12.4 Mb duplication, which covered the bands 16p13.11p13.3, excluding the most telomeric region of the band 16p13.3. Although no other fetal sonographic anomaly than nuchal translucency was detected, other studies had previously shown that the presence of the region 16p13.1-p13.3 in triplicate gives rise to a defined syndrome. Phenotypic features of this syndrome include mental retardation, growth retardation, microcephaly, hand anomalies and a special facial appearance. After genetic counseling, the parents decided to opt for termination of the pregnancy. The fetus had a round face and both slender shoulders and body. The autopsy results are still pending. All the results were obtained within four weeks. The aCGH method was crucial in the identification of the 16p13.11p13.3 duplication.

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## 1422/W/Poster Board #1080

**Array-CGH and FISH analysis in characterization of marker chromosome.**
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Supernumerary marker chromosome [SMCs] is structurally the part of extra chromosome material that poses a major challenge of diagnostic dilemma to the geneticist for its precise nature and role in disease identification and development. Recent advancements in array-CGH have made it possible to substitute the chromosome target with DNA spotted on a glass slide. Technological progress accomplished in these last years has increased the resolution of entire genome from 1Mb to few Kilobasepair. We hereby report four different patients who were found to have SMCs during conventional cytogenetic [CC] study and investigated further by oligonucleotide based array using 44K probes. Case-1 was a young woman having Bad Obstetrical History (BOH) and array-CGH has shown 8.6 Mb duplication of 5p13.2p11 region. Case-2 also had BOH and array-CGH could not characterize de-novo marker chromosome, which has ruled out euchromatic region involved in the imbalance. Further study by multi-centric FISH confirmed the marker to be of #3 centromeric region. Case-3 was a 15yrs old girl with short stature and primary amenorrhea. Three markers of varying size were observed by CC and array-CGH has confirmed 10.9 Mb of 2q24.33, 3.5 Mb of 3p12.1p11.1 and 1.1 Mb of 7p11.21p11.1 duplication. Case-4 was 13 months old girl with MCA. Array-CGH revealed 14.9Mb 18p11.32p11.31 duplication. This shows that array-CGH enhance the identification and origin of SMC of euchromatic region that helps in providing SMC/phenotype correlation for better genetic counseling and future course of action for the affected family members. Supported by DBT-India.

## 1423/W/Poster Board #1081

**Small supernumerary marker chromosomes (sSMC) characterized by Molecular Cytogenetic technique: FISH.**
*H. Sheth[1,2], F. Sheth[2], J. Andrieux[3], A. Weise[4], E. Ewers[4], J. Sheth[2], T. Liehr[4].* 1) Dept. of Biomedical Sciences, Newcastle University, Newcastle Upon Tyne NE2 4HH, United Kingdom; 2) FRIGE's Institute of Human Genetics, FRIGE House, Satellite, Ahmedabad-380 015. Gujarat, India; 3) Laboratoire de Génétique Médicale, Hôpital Jeanne de Flandre CHRU de Lille, France; 4) Jena University Hospital, Institute of Human Genetics and Anthropology, Kollegiengasse 10, D-07743 Jena, Germany.

Small supernumerary marker chromosomes (sSMC) form a heterogeneous group of chromosomes originating from any part of the chromosome either inherited/de novo, satellited/non-satellited seen with various incidences. The incidence in general population is 0.044% that increases to 0.075% in prenatal cases to 0.125% in sub-fertile group to 0.288% in mentally challenged population. This suggests that sSMC influences the clinical manifestation and need proper characterization. Majority of the sSMC are derived from acrocentric chromosomes having satellited/bisatellited constriction and around half are derived from chromosome 15. Non-acrocentric sSMC are comparatively rare and 30% are coupled with phenotypic effects. sSMC are parentally inherited/de novo and linked with vast clinical features from normal to extremely mild to severe. We hereby report five subjects who were found to have sSMC during conventional cytogenetic study and investigated further using variety of FISH probes such as: centromeric-FISH, centromeric M-FISH, subcentromeric M-FISH, subtelomeric-FISH, multicolor banding, microdissection and reverse FISH. Marker chromosomes were de novo in all the cases. Case-1 was a prenatal study and marker chromosome was diagnosed in amnitoic fluid analysis. Microdissection and reverse FISH characterized the sSMC as a neocentromeric Y - 47,XX,+mar.ish der(Y)inv dup(Y)(pter→Yp11.2::Yp11.2→pter). Second case with MCA and FISH analysis confirmed it as tetrasomy 18p - 47,XX,+mar.ish der(18)i(18)(pter→q11.-1::q11.1→pter). For case 3 with bad obstetric history, FISH study confirmed it as a partial trisomy of 5p - 47,XX,+mar.ish der(5)min(5)(:p13.1→q11.1:). Case 4 presented with typical features of Turner syndrome and sSMC was a 46,X,+mar.ish der(14)min(14)(pter→q11.1:). The last case portrayed with Down syndrome features and FISH analysis confirmed it to be 47,XX,der(14,21)(q10;q10),+mar.ish t(14;21)(q11.1;p11.2),+21. Besides cases 2 - 3 were also characterized by array-CGH (Agilent platform). This shows presence of sSMC in various patient groups needs further precise characterization by the application of modern molecular cytogenetic techniques such as FISH which not only help to characterize quickly the sSMC with an accurate genetic diagnosis and also can further help in obtaining accurate genotype-phenotype correlation leading to better informed counselling. Supported in parts by DBT-India, Prochance 2008 and 2009 of the Friedrich Schiller University Jena.

## 1424/W/Poster Board #1082

**A Strategy For Precisely Mapping Chromosomal Translocation Breakpoints.**
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Background: The identification of chromosomal translocations by conventional cytogenetics has implications for diagnosis, prognosis, and recurrence risk. For some chromosomal regions the banding pattern is repetitious, making it hard to determine with accuracy the specific segments involved. FISH analysis with BACs can help localize breakpoints, but it is time-consuming and the limit of resolution is approximately 100-200 Kb. In our study, we used PCR for mapping breakpoint regions by amplifying the isolated derivative chromosomes with primers sets specific for each chromosome. Objective: To determine the breakpoint positions in a patient with a balanced translocation between chromosomes 3 and 17. Methods: Chromosome sorting was performed at Mayo Clinic using conversion technology. Initial primer sets for chromosomes 3 and 17 were designed based on cytogenetic data. Primers were placed in low homology regions and tested using in silico PCR application with human and mouse DNA. Primers were used only if in silico testing did not give a mouse DNA product. Successive primer sets were designed to span the region between a known deleted and non-deleted region to further localize the breakpoints. Results: Cytogenetic examination localized the breaks to chromosome 3p21.1 and chromosome 17q23.3. Subsequent PCR analysis showed the der17 breakpoint between chr3:54,367,097 and chr17:65,484,251 separated by 18bp whose origin is unknown. Within this 18 bp segment are several short overlapping regions (5-9 bp) of homology to both flanking regions. The der3 breakpoint occurred between chr3:54,367,099 and chr17:65,484,249. A "CT" pair is present on both normal homologues and can not be assigned to either chromosome. The molecular data demonstrates that the breakpoint on chromosome 17 is actually in q24.3, ~6 Mb away from initial position determined cytogenetically. Conclusions: It is important to perform molecular genetic studies in patients with translocation to accurately determine the breakpoints and the corresponding genes disrupted or whose expression is altered. It is only by characterizing patients at this level that genotype-phenotype correlations will be possible. Utilizing conversion technology service to obtain chromosome sorted DNA and PCR for mapping makes this an easy, cheap and fast method that can be done in virtually every molecular biology lab. Each localization round, including primer design and PCR, takes 2-3 days.

## 1425/W/Poster Board #1083

**Utility of Array CGH for the Detection of Congenital Trisomy 8 and Trisomy 9 Mosaicism.**
*E. Thorland, T. Gliem.* Dept. of Lab Med & Pathology, Mayo Clinic, Rochester, MN.

The phenotype of patients with mosaicism for trisomy 8 or trisomy 9 is well described. However, the postnatal detection of these mosaic trisomies in peripheral blood specimens can be cytogenetically challenging. The abnormal cell line may not compete well with normal cells in the PHA-stimulated cultures necessary for conventional chromosome analysis and the trisomic cells can rapidly disappear over time. In older patients, the trisomic cells can sometimes only be detected in fibroblast cultures after skin biopsy. Herein, we describe our experience with the detection of mosaicism for trisomy 8 and trisomy 9 using array CGH. Through clinical array CGH analysis of approximately 3000 cases, 3 patients with mosaic trisomy 8 and 3 patients with mosaic trisomy 9 were detected. The age of the patients at the time of sample collection ranged from 1 day to 5 years of age. In addition to the array CGH studies, chromosome studies were performed in 3 of the cases. In two cases (trisomy 8 at age 3 and trisomy 9 at age 3 weeks), no trisomic cells were detected after the analysis of 20 metaphases and in one case (age 10 days) trisomy 8 was detected in 5 of 20 metaphases. Interphase FISH studies were performed on both unstimulated direct preparations and PHA-stimulated cultures using the appropriate centromere probe and a locus-specific probe (c-MYC and p16 for chromosomes 8 and 9, respectively) in all 6 cases. In each case, the trisomic cells were present at an equivalent or greater percentage in the unstimulated cells than in the PHA-stimulated cells. Trisomic cells ranged from 20.5% to 65% in unstimulated cultures and correlated closely with the observed deviation in the array CGH studies, indicating that array CGH has the ability to detect mosaicism for trisomy 8 or 9 down to at least 20%. Overall, these data provide evidence that array CGH is a sensitive method for the detection of mosaic trisomy 8 or trisomy 9 and may be preferable to chromosome studies on PHA-stimulated peripheral blood lymphocytes. In addition, if sufficient clinical suspicion for either of these syndromes remains following normal chromosome studies, array CGH studies should be considered before initiating chromosome studies on fibroblasts derived from an invasive skin biopsy procedure.

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## 1426/W/Poster Board #1084

**Characterizing genomic imbalances of chromosome 15 by microarray-based comparative genomic hybridization(aCGH).** *S. Yu, M. Kielt, A. Stegner, S. Fiedler, D. Zwick.* pathology, Children's Mercy Hosp, Kansas City, MO.

Chromosome 15 is one of a few human chromosomes prone to structural rearrangements as the underlying causes of some genomic disorders. This chromosome is also one of a few chromosomes containing known imprinted regions. Therefore, the clinical phenotypes in individuals with chromosome 15 abnormalities depend on both genetic content and parental origin of the rearranged chromosome 15. Application of aCGH techniques has revolutionized our understanding of human genomic architecture and is becoming a new standard method for assessing genomic imbalances with high-resolution and high-throughput. In this report, we present a CNV profile of chromosome 15 derived from aCGH-244K testing to evaluate 670 consecutive pediatric patients with diverse abnormal phenotypes and 59 additional individuals with a variety of structural genomic abnormalities. In addition to 745 benign CNVs, 16 abnormal CNVs on chromosome 15 were identified in our group of 670 samples representing a 2.4% detection rate of genomic abnormalities in this cohort of patients. Four abnormal CNVs on chromosome 15 were defined in the other 59 samples. Some of the abnormal CNVs includes: 15q11.2q13.2 (18657188-26829699)x3, a 8.2 Mb duplication with BP1 as proximal breakpoints in 2 cases; 15q11.2q13.1(21246327-26208802)x1, a 4.96 Mb deletion between BP2 and BP3 in one case with PWS; 15q11.2q13.1(20249686-26365196)x1, a 6.12 Mb deletion between BP1 and BP3 in one case with AS; 15q11.2q13.3(19879461-32163873)x1, a 12.28 Mb deletion between BP2 and BP5 in one case with atypical PWS; 15q11.2q12(22,289,073-24,136,116)x1, a 1.85 Mb deletion in one case with PWS; 15q13.1q13.2(26738880-28441369)x1, a 1.7 Mb deletion between BP3 and BP4 in two cases representing a possible novel genomic disorder; 15q22.31(64430492-64542830)x1, a 112 kb deletion containing MAP2K1 gene in one case, representing a novel genetic mechanism of MAPK1 gene mutation leading to Noonan syndrome; 15q21.1q22.2 (44,586,481-57,997,910)x3, a 13.41 Mb duplication in one case. Three cases with 15q13.3 microdeletion syndrome with breakpoints between BP4 and BP5 were identified with an estimated ~1/12,000 population prevalence. Two of them have hemizygous deletions as previously reported. The remaining patient has a homozygous deletion in a boy with severe clinical phenotype, inherited from non- consanguineous parents each carrying a hemizygous deletion of the15q13.3 region.

## 1427/W/Poster Board #1085

**Case Report and Further Characterization of the 12q14 Microdeletion Syndrome.** *R.E. Pyatt[1,2], A.K. Bailes[1], A. Brock[1], C. Deeg[1], J.G. Gordon[1], A. McKinney[1], C. Astbury[1,2], S. Reshmi[1,2], K.P. Shane[4], D. Lamb Thrush[1], J.M. Gastier-Foster[1,2,3], A.M. Sommer[4].* 1) Dept. of Laboratory Medicine, Nationwide Childrens Hospital, Columbus, OH; 2) Dept. of Pathology, Ohio State Univ., Columbus, OH; 3) Dept. of Pediatrics, Ohio State Univ., Columbus, OH; 4) Dept. of Human and Molecular Genetics, Nationwide Childrens Hospital, Columbus, OH.

The 12q14 microdeletion syndrome is a rare condition characterized by pre and postnatal growth retardation, failure to thrive, developmental delay, and proportionate short stature. Osteopoikilosis, or multiple areas of dense calcification producing a mottled bone appearance, has also been identified in some children due to haploinsufficiency of the LEMD3 gene. Deletions at 12q14 range from 1.83 to 8.95 Mb and contain the LEMD3 gene in a majority of reported cases. The HMGA2 gene is deleted in all reported cases and is a candidate gene for the proportionate short stature associated with this syndrome. We describe here a case which further characterizes the features of this syndrome. The patient was born to a 22 year old primigravida mother in whom intrauterine growth retardation was noted at 5 months gestation. The child was delivered at 33 weeks by elective C-section due to oligohydramnios and was small for gestational age with a weight of 1.4 kg and a length of 40 cm (measurements below the fifth percentile). The infant was hospitalized for 3 weeks and fed by nasogastric tube. Skeletal surveys, MRI, chromosome analysis, and metabolic screens were normal. Physical examination at 5.5 years of age revealed her weight, height, and head circumference measurements below the third percentile. She was attending preschool along with speech therapy for apraxia, but otherwise had normal development and was generally healthy. A skeletal survey showed only minor, non-diagnostic findings including small iliac wings, minimally gracile long bones, and a slight flare to the distal half of the clavicles. The family history was non-contributory, and the parents and a 3 month old brother were of average height. Microarray analysis was conducted using the SignatureSelect OS 105K v1.1 oligonucleotide microarray platform which identified a 4.17 Mb deletion at chromosome 12q14.3 through 12q15 and confirmed by FISH. The deletion was confirmed by FISH and contained 25 genes which did not include the LEMD3 gene (as in most previous cases). The deleted region included the 3' coding portion of the HMGA2 gene. As a partial deletion of HMGA2 would support its role as a candidate gene for proportionate short stature, additional characterization of the HMG2A deletion will be conducted. This case refines the genotype-phenotype correlation in the 12q14 microdeletion syndrome and narrows the critical region to an approximately 1.42 Mb region at 12q14.3.

## 1428/W/Poster Board #1086

**Array CGH detection of UBE3A single gene deletion associated with Angelman syndrome.** *B. Xiang[1], PJ. Benke[2], H. Zhu[1], YS. Fan[1].* 1) University of Miami Miller School of Medicine, Miami, FL; 2) Pediatric genetics, Joe DiMaggio Children's hospital, Hollywood, FL.

Angelman syndrome (AS) occurs in about 6% of children presenting with severe mental retardation and epilepsy. UBE3A with maternal allele specific expression in brain is the critical gene of the syndrome. Approximately 70% of the patients have a ~4Mb deletion in a maternally inherited chromosome 15 at the 15q11-13 region. Paternal uniparental disomy (UPD) of chromosome 15 (3%), mutation in the imprinting center (1%) and mutation of the UBE3A gene (6%) are the other known causes of the syndrome. The remaining 20% of patients have no detectable genetic abnormality. We have recently studied 16 patients with mental retardation or developmental delay using a customized genome-wide oligonucleotide array 44K platform (Baldwin et al, Genet Med, 2008) with enriched probes for the known microdeletion syndrome regions and detected in a 2-year girl a small deletion with a size of 216 Kb that encompasses only the UBE3A gene. The deleted region is covered by 19 probes in this platform versus 4 probes by the standard Agilent 44K platform. The 2 year old female proband had development delay, speech delay, a small head, minimal epicanthic folds, borderline low set ears, and thin upper lip. The diagnosis of Angelman Syndrome was not made prior to the array CGH detection. Our case demonstrates advantages of a customized array in detection of single gene deletions associated with a known genomic disorder not detectable with other methodologies.

## 1429/W/Poster Board #1087

**18q12 deletion detected by CGH-array in a boy with inv(18) presented dysmorphia, mental retardation and epilepsy.** *M. Minz[1,3], L. Telvi[1], C. Sevin[2], P. Clement[3], P. Aubourg[2].* 1) Cytogenetics Laboratory, Hospital St Vincent de Paul, Paris, France; 2) Department of Pediatric Endocrinology, Hospital St Vincent de Paul, Paris, France; 3) Clement Laboratory, Blanc Mesnil, France.

A 5 years-old boy who presented dysmorphia and mental retardation showed a karyotype with a pericentric inversion of chromosome 18. The karyotype of the mother was normal, however the karyotype of the father with normal phenotype could not be done. The patient showed severe mental retardation, dysmorphic features and high frequency of myoclonic seizures rebel to treatment. The frequency of the myoclonic seizures is about 5 to 10 times in a day. The treatment of the seizures include Urbanyl, Valium and Epitomax. The CGH array analysis showed a large deletion at 18q12 chromosome region. The size of the deletion is large as 8.66 Mb and include many genes. Some of them are described as related to pathology of epilepsy. The FISH analysis do not detect any deletion with the Tuple1 and Shank3 probes. Epilepsy is commonly observed in patients with chromosomal aberrations. The association of seizures to chromosome 18 abnormalities was reported by many authors, and many of the loci was defined as a potential candidate susceptibility gene for epilepsy. The seizures reported with 18q-syndrome are very variable as status epilepticus (Kanazawa & al. 1989), autonomic seizures (Sturm & al. 2000, Stephenson, 2005), idiopathic generalized epilepsy (Durner & al. 2001, Lucarelli & al. 2007), febrile seizures (Baulac & al. 2001, Nakayama & al, 2003, Winawer & Hesdorffer, 2004, Nabbout & al. 2007) apneic seizures (Kumada, 2003), benign focal epilepsy (Verrotti & al. 2004), adolescent-onset idiopathic generalized epilepsy (Greenberg & al. 2005). Most of the authors suggest that the haplo-insufficiency of genes located on the chromosome 18 could be associated with epilepsy. McLin & al (2008) reported a study in inbred mouse providing the evidence that the genes of the distal part of chromosome 18 are important determinants of vulnerability to hippocampal neurodegeneration. While further EEG/clinical/genetical investigations are needed to validate these observations.

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## 1430/W/Poster Board #1088

**COMPREHENSIVE PREIMPLANTATION GENETIC DIAGNOSTICS (PGD) BY SINGLE CELL SNP MICROARRAYS.** *W.G. Kearns[1], A. Benner[1], R. Pen[1], E. Widra[2], R. Leach[1].* 1) Shady Grove Center for Preimplantation Genetics, 15001 Shady Grove Rd., Ste 220, Rockville, MD 20850; 2) Shady Grove Fertility RSC, 15001 Shady Grove Rd., Ste400, Rockville, MD 20850.

We successfully amplified DNA from a single embryonic cell to perform preimplantation genetic diagnostics (PGD) and aneuploid screening (PGS) for all 23-pairs of chromosomes.

We used modified whole genome amplification (WGA) protocol on 565 single blastomeres from 61 embryos and 34 known cell lines. We used invariant DNA genomic loci to ensure the entire genome was amplified and TaqMan PCR to ensure heterozygous allele amplification. The Illumina HumanHap370 microarray was employed to determine chromosome aberrations and genotype data for 370K SNPs. Data was analyzed with deCODE genetics Disease Miner Professional and Illumina BeadStudio and KaryoStudio software.

Analyses of 565 blastomeres (n=61 embryos) and 34 cell lines showed in many cases, a genomic coverage > 98%, a heterozygous allele detection rate > 90% and a microarray detection rate and genotype call rate > 90%. Our DNA amplification protocol permits us to successfully identify ploidy in all cells without having to determine parental DNA genotypes. Sixty-nine percent (42/61) of the embryos were mosaic diploid/aneuploid, 25% (15/61) were mosaic aneuploid and 7% (4/61) were complex mosaics. Structural chromosome imbalances were identified from all cytogenetically abnormal cell lines. Genotype information was also obtained for 370K SNPs from each cell. These genome wide scans can also identify genetic disorders such as DiGeorge syndrome, some deletion forms of Prader-Willi and Angelman syndrome, uniparental disomy and some single gene disorders. By determining parental, embryonic and fetal genotypes, we can also identify which partner provided the extra chromosome in aneuploid embryos and which embryo implanted.

We successfully obtained genetic information from all chromosomes from single embryonic cells using a modified WGA protocol and microarray analyses. These types of analyses may also be successful on other cell types such as polar bodies or trophectoderm cells.

## 1431/W/Poster Board #1089

**Partial Deletion of Neuroligin 1 by aCGH in a Child with Microcephaly, Seizure Disorder and Severe Mental Retardation.** *A. Millson[1], M.J.H. Willis[2], M.E. Aston[3], S.T. South[1,4,5], E. Lyon[1,4], D.M. LaGrave[3].* 1) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; 2) Clinical Genetics, Naval Medical Center, San Diego, CA; 3) Cytogenetics and Molecular Cytogenetics, ARUP Laboratories, SLC, UT; 4) Pediatrics, Univ of Utah, SLC, UT; 5) Pathology, Univ of Utah, SLC, UT.

Array CGH is used to evaluate chromosomal gains or losses in patients with mental retardation and/or dysmorphic features at a much higher resolution than is possible by standard cytogenetic means. Results of uncertain significance are not uncommon. Parental studies can clarify these results, demonstrating that the imbalance is either *de novo* or inherited, and therefore is more or less likely to be causative. However, the possibility that even inherited deletions and duplications may play a role in the phenotype of the proband cannot be excluded. NLGN1 is a member of the neuroligin gene family and may represent a candidate gene for developmental delay as members of this gene family are involved in formation and remodeling of central nervous system synapses. NLGN1 forms a heterotetramer with Nrx1. Together; they promote cell adhesion between dendrites and axons, forming a functional synapse. We present a 6.5 year-old girl born at 37 weeks gestation by C-section due to failure to progress. Developmental delay was noted at 5-6 months of age. Evaluation identified abnormal eye movements, and she was diagnosed with infantile spasms. EEG showed hypsarrhythmia. Parents report loss of milestones: that she used to smile (likely non-specific), sit in a highchair, and eat by mouth. Currently she is completely bed or wheelchair bound, has no meaningful interaction, no facial expression, and is G-tube dependent. Physical exam was significant for severe microcephaly, short stature, and unusually large fat pads on her fingers and toes. She has low central tone with increased tone and increased reflexes in lower extremities. She withdraws from stimulation, but has no other purposeful movement. Brain MRI at 9 months of age showed bilateral non-specific regions of increased T2 signal in the frontal periventricular white matter. Extensive biochemical, cytogenetic and molecular evaluations were normal. Blood was sent for aCGH and a 2.1 Mb deletion spanning 28 oligonucleotides was identified at 3q26.31. Findings were confirmed by FISH. DNA from the mother was subsequently analyzed with the same FISH probe and a deletion was detected. Quantitative real-time PCR targeting multiple exons in NLGN1 showed disruption of the gene, confirming the heterozygous loss of several coding exons in both the proband and her mother. Although the deletion is inherited, it is difficult to conclude the deletion of NLGN1 benign in consideration of its role in neural development.

## 1432/W/Poster Board #1090

**Variability in interpreting and reporting copy number changes detected by array-based technology in clinical laboratories.** *K.D. Tsuchiya[1], L.G. Shaffer[2], S. Aradhya[3], J.M. Gastier-Foster[4], A. Patel[5], K. Rudd[6], J. Sanford Biggerstaff[7], W.G. Sanger[8], S. Schwartz[9], J.H. Tepperberg[9], E.C. Thorland[10], B.A. Torchia[2], A.R. Brothman[11].* 1) Dept of Laboratories, Seattle Children's Hosp, and Dept of Lab Med, Univ of Washington Med Ctr, Seattle, WA; 2) Signature Genomic Laboratories, Spokane, WA; 3) GeneDx, Gaithersburg, MD; 4) Dept of Path and Lab Med, Nationwide Children's Hosp, and Depts of Path and Pediatrics, Ohio State Univ, Columbus, OH; 5) Dept of Molec and Hum Genet, Baylor College of Med, Houston, TX; 6) Dept of Hum Genet, Emory Univ School of Med, Atlanta, GA; 7) Idaho Cytogenet Diag Lab, Boise, ID, and PAML, Spokane, WA; 8) Dept of Pediatrics, Munroe-Meyer Inst, Univ of Nebraska Med Ctr, Omaha, NE; 9) Lab Corp of America, Research Triangle Park, NC; 10) Dept of Lab Med and Path, Mayo Clinic, Rochester, MN; 11) Depts of Pediatrics, Hum Genet and Path, University of Utah, and ARUP Laboratories, Salt Lake City, UT.

The use of microarrays for the assessment of copy number changes (CNCs) in individuals with constitutional disorders is becoming widespread in clinical cytogenetic laboratories. Although this technology has significantly increased the detection of chromosome imbalances that are known to be associated with abnormal phenotypes, the finding of CNCs in healthy individuals (also known as benign copy number variants or CNVs) has created new challenges for laboratorians and clinicians who must interpret the clinical significance of previously undescribed CNCs. The purpose of this study was to assess the variability in interpretation and reporting of copy number changes detected by array-based technology in the clinical laboratory. Thirteen copy number changes that were detected by array comparative genomic hybridization (six by BAC array and seven by oligonucleotide array) in one clinical laboratory were evaluated by directors from ten other clinical laboratories to determine how they would interpret and report the findings. The CNCs that were chosen for the study were not associated with an abnormal phenotype in the literature. The thirteen CNCs were chosen because they potentially posed an interpretive challenge in terms of their clinical significance. None of the thirteen copy number changes showed complete agreement amongst the eleven laboratories in the interpretation of the clinical significance of the change. For some cases, the interpretations ranged from normal to abnormal. Some laboratories appear to use a binary reporting system (normal vs. abnormal), whereas other laboratories utilize three or more categories for reporting. Some laboratories have absolute size cut-offs below which they do not report CNCs, whereas other laboratories will potentially report any size CNC depending on other variables. This study demonstrates the need for guidelines for interpreting and reporting copy number changes detected by array-based technology in order to clearly and more consistently communicate the clinical significance of these findings to ordering providers.

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## 1433/W/Poster Board #1091

**Classification of pathogenic imbalances detected by genome-wide array CGH in patients with mental retardation and genetic counseling.** *Y.S. Fan[1], B. Xiang[1], B.L. Wu[2], Y.P. Shen[2], P. Li[3], M. Li[4], T.J. Chen[5].* 1) University of Miami Miller School of Medicine, FL; 2) Children's Hospital Boston and Harvard Medical School, MA; 3) Yale University School of Medicine, CT; 4) Tulane University School of Medicine, LA; 5) Dept Med Genet, University of South Alabama College of Medicine, AL.

We have collected the array CGH results on1499 patients with mental retardation or developmental delay from five academic diagnostic laboratories where a genome-wide oligonucleotide array, Agilent 44K has been used. We have also reviewed genome-wide array CGH results on 2501 patients published recently with different platforms used. Pathogenic imbalances have been reported in 461 of the total 4,000 patients reviewed. The diagnostic yields varied from 6.25% to 24% with an overall diagnostic yield of 11.53%. The majority of imbalances were larger than 500 Kb in size (95%) and 300Kb in size (99%). We classified the pathogenic imbalances into 6 groups according to their structures: Class 1, a deletion or duplication of a single DNA segment; Class 2, a deletion of the terminal region of one chromosome and a duplication of the terminal region of another chromosome; Class 3, a deletion in one chromosomes and a duplication in another with at least one interstitial alteration; Class 4, a deletion and a duplication involving a contiguous genomic region within the same chromosome arm; Class 5, a deletion and a duplication in different arms of the same chromosome; Class 6, other types of imbalances including complex abnormalities and mosaicism. Approximately 87% (400/461) of the abnormal cases had a simple single segment deletion or duplication whereas the remaining 13% (61/461) had a compound genomic imbalance involving two or more DNA segments. A single deletion or duplication could be the result of the segregation of a balanced insertion. A deletion and a duplication in the same patient could be derived from a balanced translocation. The finding of both a deletion and a duplication on the same chromosome can be caused by an inversion in a parent. We have recommended a conventional karyotyping for the parents to rule out a balanced translocation, inversion or insertion when an alteration was likely to be chromosomally visible or FISH when an alteration involved subtelometic regions or known microdeletion regions. Classification of the pathogenic imbalances and discussion of the possible mechanism in the report can provide useful information for genetic counseling.

## 1434/W/Poster Board #1092

**Identification of carrier status as a consequence of whole-genome microarray analysis: dilemmas regarding clinical obligation, confirmation and reporting.** *L.R. Rowe[1], A. Millson[1], J. Swenson[2, 3], E. Lyon[1, 2, 3], E. Aston[3], D. LaGrave[3], S. Shetty[3], A.R. Brothman[1,2, 3, 4, 5], S.T. South[1,2,3,4].* 1) Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT; 2) Department of Pathology, University of Utah, Salt Lake City, UT; 3) ARUP Laboratories, Salt Lake City, UT; 4) Department of Pediatrics, University of Utah, Salt Lake City, UT; 5) Department of Human Genetics, University of Utah, Salt Lake City, UT.

Although carriers of mutations resulting in autosomal recessive disorders are not usually affected phenotypically, nor are they symptomatic, identifying heterozygous deletions for genes in which homozygous deletions have clinical consequences has merit. For example, identification of carrier status allows an individual to make informed decisions regarding child bearing. We discuss heterozygous findings involving three genes in which homozygotes are clinically affected. Nephronophthisis (NPH) is an autosomal recessive nephropathy with chronic tubulointerstitial involvement which represents the leading cause of end-stage renal disease in children and adolescents. The most frequent genetic abnormality found in NPH is a large homozygous deletion of the NPHP1 gene. Homozygous deletions of NPHP1 have also been identified in a subset of patients with Joubert syndrome. The most common cause of renal tubular Fanconi syndrome, cystinosis, is a lysosomal storage disorder which can be caused by homozygous deletion of the CTNS gene which encodes the lysosomal cystine carrier cystinosin. Alpha thalassemia, the most prevalent worldwide autosomal recessive disorder, is a hereditary anemia. Homozygous deletion of both HBA1and HBA2 genes results in prenatal or early neonatal death. We have identified heterozygous deletion of NPHP1, CTNS, or HBA 1 and HBA2 genes in routine clinical samples submitted for array comparative genomic hybridization (aCGH) analysis in pediatric patients referred for developmental delays and/or multiple congenital anomalies. Although these findings are likely not pertinent to the patient's indication for testing, we aim to perform confirmatory testing and include the findings of such testing in the clinical report. Using a combination of molecular tests, we have confirmed heterozygous deletions in each of these cases. While it is important to develop a robust test for confirmation and carrier status detection in heterozygous cases such as these, conveying of this information, and how it is done requires careful education and explanation. We believe these three examples are likely to be representative of multiple additional genes where clinical interpretation of aCGH results needs to be carefully presented through a health care provider such as a genetic counselor.

## 1435/W/Poster Board #1093

**Detection of Long Contiguous Stretches of Homozygosity using the Affymetrix Cytogenetics Research Solution Microarrays.** *B.M. Bolstad.* Affymetrix, Inc, Santa Clara, CA.

Affymetrix® Cytogenetics Research Solution microarrays contain both non-polymorphic markers for measuring copy number variation and markers for measuring single nucleotide polymorphisms (SNPs). On the Whole-Genome 2.7M array there are 400,103 markers for measuring SNP variability. These allow the detection of loss of heterozygosity (LOH), uniparental disomies (UPD), and regions of the genome that are identical-by-descent. The polymorphic markers were selected for the array based on performance and maximizing genomic coverage. An algorithm was developed to detect the occurrence of LOH events based on allelic intensity values rather than genotype calls. The ratio of allelic intensities can be used to summarize a single marker, and a reference set is used to improve these allelic ratios. Information from adjacent contiguous markers is combined to determine the presence of any LOH. The algorithm compensates for differences in individual marker performance by assigning each a value measuring its information content. These are used to decide how many markers should be considered at any one time in a window and weight markers appropriately. For each window a test is carried out to decide whether or not there is evidence to support the presence of underlying LOH. Windows are moved along the genome and overlapping LOH windows produce segments. Sample quality, and hence data quality can vary; to account for these differences, the algorithm dynamically selects optimal parameterization of the test statistic and window size for each sample. Using this algorithm, the Whole-Genome 2.7M array is able to robustly detect LOH events spanning 3Mb or larger and some events smaller than 3Mb.

## 1436/W/Poster Board #1094

**Development and performance of a simple assay for high resolution copy number analysis on the Affymetrix 2.7M Cytogenetics Research Array.** *Z. Hu, T. Berntsen, J. Chen, D. Flaucher, V. Huynh, S. Nautiyal, N. Ngo, D. Pankratz, D. Abdueva, B. Bolstad, V. Carlton, B. Liu, J. Veitch, J. Collins.* Affymetrix Inc, 3420 Central Expy, Santa Clara, CA.

The research field of molecular cytogenetics is transitioning from the use of classic techniques such as chromosome banding analysis and fluorescent in-situ hybridization to DNA microarray-based virtual karyotyping, which offers distinct advantages such as greatly enhanced resolution and less objective assessment of aberrations. However, broad adoption of currently available microarray tools is often hindered by laborious and complex assay workflows, an inability to detect certain aberration types, complex data analysis tools or insufficient resolution for subtle cytogenetic changes. To overcome these hurdles, we have developed a whole-genome assay that, when combined with the new high-resolution Affymetrix 2.7M Cytogenetic Research arrays and data analysis software suite, has enabled reliable detection of a broad range of chromosomal abnormalities. Starting with as little as 100 ng of genomic DNA, this assay uses a PCR-free whole genome amplification approach to amplify the sample, which is then purified, fragmented and labeled before hybridization to microarrays. The assay is fast, simple and robust, requiring less than three hours of hands-on processing time and merely 30 hours from DNA to result. The 2.7M Cytogenetic Research arrays provide the highest-available density of 2.7 million copy number markers along with 400,000 single nucleotide polymorphisms (SNPs) markers, with increased coverage of genes and regions of cytogenetic interest. Microarray data are analyzed using the Chromosome Analysis Suite software package. We present data demonstrating the successful detection of very small single copy gain and loss events (<50 kb). We also demonstrate the utility of SNP markers in enabling the reliable detection of copy number neutral loss of heterozygosity (LOH) events such as those associated with uniparental disomy (UPD) and identical-by-descent (IBD). Data are presented from a variety of clinical sample types commonly encountered in cytogenetics research, including amniotic fluid, blood, cell lines, chorionic villi, fresh frozen tissue, and saliva.

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## 1437/W/Poster Board #1095

**Mosaic monosomy 7 in a male infant with ambiguous genitalia and multiple congenital anomalies.** *D.M. LaGrave[1], C.W. Booth[2], D.A. Rita[2], M.E. Aston[1], K.B. Geiersbach[1, 4], S. Shetty[1, 4], L.R. Rowe[5], S.T. South[1, 3, 4].* 1) Cytogenetics and Molecular Cytogenetics, ARUP Laboratories, Salt Lake City, UT; 2) Lutheran General Hospital, Park Ridge, Ill; 3) Department of Pediatrics, University of Utah, Salt Lake City, UT; 4) Dept of Pathology, University of Utah, Salt Lake City, UT; 5) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT.

Array comparative genomic hybridization (aCGH) was ordered and blood received on a three-month old, premature Hispanic male infant (corrected age 3 weeks) for the indication of ambiguous genitalia and multiple congenital anomalies, including bilateral club feet, small kidneys with pelviectasis, and hyperpigmentation. Chromosomes were normal, 46,XY by amniocentesis and peripheral blood at birth. The infant had been delivered at 27 4/7 weeks by emergency C-section due to IUGR, decreased fetal movement, and bradycardia. Perineoscrotal hypospadias and bifid scrotum were noted at birth. Low cortisol levels were found, and treated with steroids. Growth continued to lag and there were repeated episodes of sepsis as well as frequent episodes of apnea and bradycardia. There were no cardiac anomalies other than a PDA. Chest films revealed a small thymus. Absolute lymphopenia and thrombocytopenia were persistent. Chronic anemia of unclear etiology required repeated transfusions. DEB-induced breakage studies were normal. Array CGH on uncultured blood using the Cyto6000 array platform (Human Genome build: hg 18) demonstrated a mosaic monosomy for chromosome 7. Findings were confirmed using the CEP7 FISH probe (Abbott Molecular) in both a direct, unstimulated blood smear, and in cultured interphase cells where 84% of cells demonstrated monosomy 7. Further examination of this sample demonstrated two CEP7 signals in metaphase cells and two normal-appearing chromosome sevens by karyotype analysis. FISH examination using CEP7 and D7S486 (7q31) was performed on buccal cells at another laboratory and demonstrated the presence of two signals for each probe in 200/200 cells examined. The discrepant findings between aCGH and cytogenetic studies may be partially explained by the presence of sepsis and a high neutrophil count causing an overrepresentation of myeloid lineage cell in the patient at the time of aCGH. At 5-months of age, the infant developed hypernatremia and became unresponsive. The parents elected to discontinue extraordinary measures, and the infant died. Parents declined postmortem examination. We describe the identification of mosaic monosomy 7 by aCGH in an infant with an unusual constellation of findings, compare this case to one other similar case found in the literature and suggest a possible etiology explaining clinical and cytogenetic findings.

## 1438/W/Poster Board #1096

**Analysis of a Variety of Deletions and Duplications within the Dystrophin Gene Using the Affymetrix SNP Array 6.0 and Multiplex Ligation Dependent Probe Amplification.** *L. Toji[1], L. Kalman[2], J. Tarleton[3], C. Bridges[3], N. Gerry[1], C. Beiswanger[1], J. Leonard[1].* 1) Coriell Inst Med Res, Camden, NJ; 2) Centers for Disease Control and Prevention, Atlanta, GA; 3) Fullerton Genetics Center, Asheville, NC.

Mutations in the Dystrophin gene result in muscular dystrophy varying from the severe Duchenne type to the much milder Becker type. Males affected with Duchenne muscular dystrophy (DMD) have several mutation types. Approximately 65% have deletions,10% have duplications and the remainder have point mutations. To identify and characterize the causative mutations in DMD cell lines in the National Institute of General Medical Sciences Human Genetic Cell Repository (NIGMS), we studied DNA samples from 35 DMD cell lines using two methods to assess copy number; the Affymetrix SNP Array 6.0 and Multiplex Ligation-dependent Probe Amplification (MLPA). Samples were selected in order to confirm prior DNA analysis or to identify the causative DMD mutation. The sample set included 24 different probands. Both fibroblast and lymphoblastoid cell cultures were available for 5 probands; for 3 probands samples from affected brothers were available and for 1 proband a sample was available from the carrier mother. Eighteen different deletions and duplications were detected with the Affymetrix SNP Array 6.0. The assay identified duplications or deletions of identical size and location in the paired samples. These samples were further characterized using MLPA analysis. In two samples, the array identified a deletion that was one exon smaller than was determined with the MLPA analysis. The basis for these differences is under investigation. Neither method detected copy number changes in five of the DNA samples. These samples will be sequenced to identify potential point mutations. The results from these analyses provide accurate descriptions of the DMD mutation in the samples and identified for the first time, DMD exon duplications in the NIGMS collection. These publicly available samples are described on the Coriell Cell Repositories web-site (http://ccr.coriell.org/) and provide the genetics community with highly characterized cell lines and DNA samples for use as reference materials for genetic testing or for biomedical research.

## 1439/W/Poster Board #1097

**Simulation of detection of fetal cells in the maternal blood: Evaluation of the efficiency of the automatic scanning in retrieval of rare events.** *A. Emad[1], E. Bouchard[1], K. Krabchi[1], A. Dutta[2], U. Klingbeil[2], R. Drouin[1].* 1) Div Genetics / Dept Pediatrics, Univ Sherbrooke, Sherbrooke, PQ, Canada; 2) MetaSystems, 300 Bear Hill Road, Waltham, MA 02451.

Introduction- Presence of fetal cells in the maternal blood during pregnancy provide an opportunity for developing non-invasive prenatal diagnosis. Accurate identification of fetal cells is essential due to their extreme low frequency of 2-6 cells/ml. They could be identified by targeting specific genetic marker exclusive for the fetal cells; for example, male fetal cells can be located through the unique presence of Y-sequence using XY-Fluorescence In Situ Hybridization (XY-FISH). The manual scanning process used in their retrieval in very cumbersome and time-consuming. Validation of its efficiency and automation will be required before its widespread application. Objectives- Determining the efficiency of automatic scanning to detect rare XY cells amongst thousands of XX cells using FISH technique and compare it with the efficiency of manual scanning. Hybridization efficiency was also determined. Methodology- Spreading of 1-10 XY cells was done, followed by staining with Giemsa and imaging. On top, a drop of XX cells was spread, followed by FISH using XY probes. The slides were then automatically scanned using the Y-chromosome probe serving as marker. Pictures taken of XY cells were compared with the ones taken before with Giemsa. The data were recorded. Results- Results obtained by automatic scanning shows an overall detection rate of 88% (117/133) of XY-cells distributed on 60 slides with low false positive rate of 4%. A cell was considered false positive for the Y-probe hybridization by reverse color re-FISH. By evaluating the hybridization of the Y-probe in the missed events, it was found that 1.5% (2/133) were not hybridized, 3% (4/133) were poorly hybridized and the hybridization was adequate in the remaining 7.5% (11/133). The overall efficiency of the FISH technique using the index of Youden was 0.88. Conclusion- Evaluation of the results obtained by manual and automatic scanning shows that although the average time required per slide was slightly longer in automatic scanning (4 hours vs 3 hours), the automatic scanning was more efficient in comparison to the manual one (88% vs 84%). Furthermore, it alleviates the burden required for manually scanning the slides and more productive as it can work non-stopped 24 hours per 24 hours.

## 1440/W/Poster Board #1098

**Partial Trisomy 2p Arising from Inverted Duplication of 2p with Terminal Deletion of 2pter: Cytogenetic and Molecular Characterization.** *S.A. Henderson[1], S. Iyer[1], R.H. Collins[3], E. Stewart[2], I. Ratner[2], A. Holdridge[1], J. Doolittle[1], S. Patel[1], N. Uddin[1], D. Payne[1], C.A. Tirado[1].* 1) Pathology, University of Texas Southwestern, Dallas, TX; 2) Pediatrics, Univeristy of Texas Southwestern, Dallas, TX; 3) Internal Medicine, University of Texas Southwestern, Dallas, TX.

First outlined by Francke and Jones in 1976, partial trisomy 2p is associated with multiple distinctive congenital anomalies including psychomotor delay, dysmorphic facial features, and congenital heart disease. Here we present a case of partial trisomy 2p arising from inverted duplication of 2p with terminal deletion of 2pter. Clinically, this patient had microcephaly, frontal bossing, hypertelorism, low-set ears, and a mild increase in lower extremity tone. In addition, pulmonary stenosis, atrial septal defect, nasopharyngeal reflux, and a relatively undersized right kidney were also present. At about 2 months the patient demonstrated significant growth retardation and developmental delay. Conventional cytogenetics showed a complex karyotype: 46,XX,del inv dup(2)(qter->p25.1::p25.1->p22.2:). ish del inv dup(2)(qter->p25.1::p25.1->p22.2:)(VIJyRM2052-,ALK++). FISH studies using a telomere probe specific for the terminal short arm of chromosome 2 and a DNA probe specific for the band 2p23 (VIJyR2052, ALK) revealed that the additional material on 2p is derived from chromosome 2 and that the 2p-specific telomere sequence is deleted. Comparative genomic hybridization (CGH) helped to refine the break points. The duplicated region was approximately 34 Mb in length while the deleted sub-telomere region was estimated to be 2.3 Mb. Many of these clinical findings overlap with those found in previously documented cases of partial trisomy 2p. Maternal cytogenetic evaluation was normal and chromosome studies from the father was not possible. To the best of our knowledge, this is the fourth reported case of an inverted duplication of 2p with an associated terminal deletion. The phenotypic effects of both the inverted duplication as well as the terminal deletion are discussed.

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## 1441/W/Poster Board #1099

**The Orientation of Unique Genetic Sequences in the Human Genome.** *F. Ray[1,2], E. Zimmerman[2], M. Cornforth[2,3], J. Bedford[1,2], E. Goodwin[2], S. Bailey[1,2].* 1) Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO. 80523; 2) KromaTiD Inc., 515 E. Laurel St., Fort Collins, CO 80524; 3) Department Radiation Oncology, University of Texas Medical Branch, Galveston, TX 77555.

CO-FISH is a strand-specific *in situ* fluorescence hybridization technique that allows determination of the 5'-3' orientation of DNA sequences along the length of chromosomes. The technique requires that cells be cultured through a single S phase to allow incorporation of BrdU into newly replicated DNA strands. The nascent strands are then photo-enzymatically digested, leaving complementary single stranded target sequences on each chromatid. Thus, for a single stranded probe, hybridization can occur only on one sister chromatid at the same locus. CO-FISH has been used to study the orientation of repetitive sequences associated with telomeres and paracentric DNA but, to date, it has not found application for orientation analysis of unique sequences, largely because of difficulties surrounding the preparation of single-stranded probes of adequate length. Unique single-stranded DNA probe sets were designed to be complementary to a single contig on chromosome 3q. In order to eliminate interspersed repetitive sequences, DNA sequences were entered into CENSOR (www.girinst.org) and masked. Unmasked, non repetitive sequences totaling approximately 10kb were then analyzed using tiling software. The result was an ordered series of short oligonucleotides designed to hybridize to a complementary target of ~10Kb. These oligonucleotides were synthesized and then fluorescently labeled using a conventional tailing reaction. Probes were hybridized to metaphase chromosome spreads that had been previously prepared for CO-FISH. Bright punctate fluorescent probe signals were detected at the expected target loci, and were confined to a single sister chromatid (on each of the two homologs). Additional probe sets targeted to sequences of the same orientation, but further down the same contig, also hybridized to the same chromatid. Thus, our results show that CO-FISH may be used to determine the orientation of unique sequences. We can imagine a few interesting applications for this approach. For example, consider the situation whereby many such oligo probe pools were used to effectively "paint" completely one chromatid of a metaphase chromosome. In that case small inversions would appear as a microscopic segment of labeled chromatin that "jumped" across the chromosome, from one chromatid to its sister. The ability to construct probes for all 23 pairs of chromosomes simultaneously would allow the detection of even small inversions (e.g., <0.5 Mb) on a genome-wide basis.

## 1442/W/Poster Board #1100

**Automating the Workflow of aCGH Tests.** *M. Herrler, W. Ke, J. Stanchfield.* Product Development, SciGene, Sunnyvale, CA.

The current sample preparation process for aCGH tests is tedious; leading to errors that confound clinical results and increasing re-test rates and costs. To overcome this problem, we have developed three specialized instruments that work together to automate benchwork from DNA labeling to array scanning. A robotic liquid handling and incubation system (ArrayPrep® Target Preparation System) automates the labeling and magnetic bead purification of 8 to 96 genomic DNA samples then loads them onto microarrays for hybridization using a rotating incubation system (Mai Tai® Hybridization System). Arrays are then post-processed using a robotic instrument (Little Dipper® Processor) for washing and drying. In this study, we compared the reproducibility of aCGH test results of samples prepared using the automated aCGH platform to those prepared manually. Over 100 genomic DNA samples were processed using both methods. We measured technician bench time needed for processing typical numbers of patient samples. We also measured the yield and purity of labeled DNA and the degree of fluorescent dye incorporation. Samples were then analyzed using Agilent 4x44K arrays and software. The results of this study show that the automated platform reproducibly generated labeled samples of high yield (5.3±0.6μg) and purity (OD260/280=1.83±0.03) with consistently high dye incorporation (AF-3= 2.9±0.46pmol/μl; AF-5=4.2±0.46pmol/μl). Arrays showed high signal intensities and low background noise with DLR values ≤0.2 (avg. 0.16). Compared to samples processed on the automated platform, manually prepared samples showed somewhat higher but more variable yields (6.9±1.0μg) with comparable dye incorporation and purity. Arrays showed similar signal intensities but higher background and slightly higher and more variable DLRs. Time and motion studies showed that the automated aCGH platform requires a fixed technician time (~1 hour) independent of batch size. In comparison, manual processing required a varying amount of technician time, up to 4.5 hours. In conclusion, the automated aCGH platform reproducibly provides high quality labeled genomic DNA with yields sufficient for aCGH-based tests. It provides consistently high quality microarray results with less variability than that seen with manually prepared samples. Finally, the system reduces the amount of technician hands-on time by as much as 75%, thereby reducing sample handling errors for improved overall test reliability.

## 1443/W/Poster Board #1101

**Genomic imbalances detected by array-CGH in patients with syndromic congenital eye malformations.** *A. Delahaye[1,2], E. Pipiras[1,2], N. Chassaing[3], M. Gérard-Blanluet[4], A. Toutain[5], L. Faivre[6], C. Francannet[7], L. Van Maldergem[8], C. Baumann[4], ML. Jacquemont[4], A. Verloes[2,4], A. Aboura[2,4], S. Drunat[4], C. Dupont[1,4], ML. Maurin[4], AC. Tabet[2,4], P. Gressens[2], J. Elion[4,9], B. Benzacken[1,2,4], P. Bitoun[1].* 1) Histology-Embryology-Cytogenetics Department and Medical Genetics, AP-HP-University Hospital Jean Verdier, Paris XIII, UFR SMBH, Bondy, France; 2) UMR 676 Inserm-Paris VII et Paris XIII, France; 3) Service de Génétique Médicale, Hôpital Purpan, University Hospital of Toulouse, Toulouse, France; 4) Genetics department, AP-HP-University Hospital Robert Debré, Paris VII, Paris, France; 5) Genetics department, Hôpital Bretonneau, University Hospital of Tours, Tours, France; 6) Genetic Center, University Hospital of Dijon, Dijon, France; 7) Service de Génétique Médicale, University Hospital of Clermont-Ferrand, Univ Clermont1, UFR Médecine, Clermont-Ferrand, France; 8) Genetics department, AP-HP-University Hospital Henri Mondor, Paris XIII, Créteil, France; 9) UMR 763 Inserm-Paris VII.

Genomic imbalances are a major cause of malformations, mental retardation and developmental delay. With the advent of whole genome array-CGH analysis, the number of especially interstitial genomic imbalances described increased dramatically. Hereditary diseases of the eyes are a frequent cause of blindness in early childhood with complex etiology. The exact frequency of chromosomal deletions and duplications causing congenital eye abnormalities is unknown. Considering the high frequency of genomic imbalances associated with eye disorders we hypothesize that the sensitivity of array-CGH could improve the genetic diagnosis in patients with idiopathic eye anomalies associated with mental retardation.

Fifty patients with unexplained congenital eye malformations and other malformation or mental retardation were analyzed using 105K oligonucleotide arrays (Agilent). CNV polymorphism were excluded using on line databases.

We detected 8 possibly pathogenic *de novo* imbalances (16%) among analysed patients. Seven are deletions and 1 is duplication. No recurrent abnormality was identified. FISH analyses confirmed the chromosomal abnormalities in 5 cases and real time quantitative PCR in the remaining cases. In 5 patients, the genetic imbalance was shown inherited from a phenotypically normal parent (1 deletion and 4 duplications). Further analyses to confirm chromosomal abnormalities suspected using CGH array are still under investigation in 13 cases.

These results demonstrate that array-CGH is able to provide a high diagnostic yield in patients with congenital eye malformation and mental retardation. Interestingly, only 2 of the 8 possibly pathogenic de novo imbalances encompassed genes already known to be involved in eye development (*FOXC1* and *OTX2*). Besides their importance for diagnosis and genetic counselling, these data may pave the way to the identification of genes involved in eye development.

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### 1444/W/Poster Board #1102

**"Classical" Cytogenetics: Not dead yet.** *D.A.S. Batista[1,2], E.C. Lisi[3], E. Wohler[2], M. Walsh[3], A. Hoon[2], R. Cohn[3], J.E. Hoover-Fong[3,4].* 1) Dept Pathology, Johns Hopkins University, Baltimore, MD; 2) Kennedy Krieger Institute, Baltimore, MD; 3) McKusick Nathans Institute, Johns Hopkins University, Baltimore, MD; 4) Greenberg Center for Skeletal Dysplasias, Johns Hopkins University, Baltimore, MD.

Microarray CGH and SNP analysis have revolutionized cytogenetics leading to the discovery of new chromosomal abnormalities and providing mechanistic and detailed insight into previously recognized syndromes. The excitement generated by these advances prompted the thought that "classical" cytogenetics would become obsolete, substituted by microarray testing. Hundreds of clinical cases were processed by microarray in our laboratory and we share the excitement and wonder. However, much emphasis has been placed on what array detects beyond karyotype without much published data pertaining to what it misses. We summarize three cases from our laboratory in which the correct diagnosis would not be achieved without karyotype. Case 1: A newborn with features of cleidocranial dysplasia, had normal RUNX2 gene mutation analysis and high resolution BAC array CGH. Karyotype showed a de novo, presumably balanced translocation between chromosomes 2q22 and 6p12.3, the band where RUNX2 is mapped (UCSC Genome Browser, Mar 06). FISH showed that the breakpoint on 6p12.3 occurred within a 110 kb region that includes RUNX2, whereas SNP analysis showed no loss or gain. Further FISH analysis and allele-specific sequencing are underway to define the breakpoint, which most likely affects the RUNX2 protein product, creating the clinical phenotype. Case 2: Array showed gain of the short arm of chromosome 18, possibly tetrasomy, and also suggested loss of chromosome Y. Karyotype confirmed both abnormalities, however in two distinct cell lines, each one containing only one of the abnormalities. The karyotype was therefore, 47,XY,i(18)(p10)/45,X. This finding has implications for the origin of these cell lines since it was a twin pregnancy. Case 3: Karyotype showed a small ring chromosome in 10% of the cells. Software analysis of array data however, was normal. Given the karyotype result, we closely inspected the raw array data from the regions around all centromeres. Chromosome 1 had a slight increase in copy number in this region but it was well below the established cutoff for anomalies. FISH confirmed the origin of the marker from the pericentromeric region of chromosome 1 including euchromatin. This accurate diagnosis allowed for the appropriate prognostic counseling. It is therefore important to emphasize that G-banded karyotype, FISH and microarray are complementary to each other and when used together provide the most accurate diagnosis of genomic copy number variations.

### 1445/W/Poster Board #1103

**Diagnosis of recessive disorders using a genome-wide SNP microarray.** *M. Crowley[1], L. Conlin[1], M. Berman[1], B. Thiel[2], C. Haldeman-Englert[1], D. Clark[1], L. Ernst[2], J. Ganesh[1], C. Ficicioglu[1], X. Gai[3], H. Hakonarson[4], I. Krantz[1], N. Spinner[1,2].* 1) Pediatrics, CHOP, Philadelphia, PA; 2) Pathology, CHOP, Philadelphia, PA; 3) Bioinformatics Core, CHOP, Philadelphia, PA; 4) Center for Applied Genomics, CHOP, Philadelphia, PA.

Utilization of genome-wide SNP arrays has proven highly successful in the identification of disease causing genomic deletions and duplications. Commonly, heterozygous deletions are identified that include genes associated with clinical abnormalities when present in only a single copy (haploinsufficiency). We used the Illumina Quad 610 array to study over 1,000 patients with various clinical features ranging from multisystem syndromes to apparently isolated single organ involvement. In general, we consider deletions and duplications as potentially pathogenic when they involve 20 or more SNP probes, are larger than 200 kb, and contain at least one gene that is not commonly deleted or duplicated in control individuals. In some cases, smaller deletions may be considered pathogenic when they involve one or more genes that are known to be dosage sensitive. In addition, homozygous deletions can be differentiated from heterozygous deletions, allowing diagnosis of recessive disorders. To date we have identified five genes with homozygous deletions whose known disturbance results in an autosomal recessive disorder, consistent with the referring diagnosis of the patient. The homozygous deletions were all relatively small, called by 2 to 28 SNPs, and to date 4 of the 5 have been validated by an alternative technique. The first validated diagnosis was non-ketotic hyperglycinemia caused by homozygous deletion of at least 8 exons (6 SNPs) of the glycine decarboxylase gene. Cockayne syndrome was diagnosed by a 28 SNP, 203 kb deletion involving both alleles of the ERCC8 gene. A 5 SNP homozygous deletion of the otoferlin (OTOF) gene caused hearing loss in one patient, and a 4 SNP, homozygous deletion of the holocarboxylase synthetase gene (HLCS) caused multiple carboxylase deficiency. In addition, a 2-6 SNP deletion within the stereocilin (STRC) gene is associated with hearing loss, although validation has not yet been carried out for this patient. These copy number abnormalities highlight the evolving utility of high-density, genome-wide SNP microarrays to obtain diagnoses in patients with various clinical features. Additionally, we identified heterozygous deletions of several genes associated with recessive disease, and sequencing of the non-deleted allele resulted in molecular diagnosis of the associated disorder. This data highlights the importance of evaluating each copy number abnormality in relation to patient phenotype and gene content.

### 1446/W/Poster Board #1104

**UPD detection by homozygosity profiling using a SNP based microarray.** *P.R Papenhausen, S. Schwartz, R. Burnside, V. Jaswaney, H. Risheg, I.K. Gadi, R. Pasion, K. Friedman, J.H. Tepperberg.* Dept Cytogenetics, Labcorp America, Res Triangle Park, NC.

Polymorphic SNP targeting in the Affymetrix 6.0 microarray easily provides sufficient allele differentiation (>900,000 SNPs) to study patterns of homozygosity (HZ). Specific HZ patterns can be seen for consanguinity, reduced gene pools and uniparental disomy (UPD). Targeting a primarily DD/MR patient base (6,000 cases), we have now prospectively ascertained and molecularly confirmed 16 cases of UPD that in addition to nine retrospectively studied cases, allow an improved HZ pattern correlation with UPD. Ten of the 25 cases showed complete chromosomal HZ consistent with de facto isoUPD and a post zygotic derivation. The remaining 15 cases showed single chromosomal LCSH (long contiguous stretches of HZ) that varied in length from 13.5-76 Mb, consistent with meiotic recombination in the homologues of the disomic gamete preceding post-zygotic trisomy rescue. Since the smallest LCSH found in UPD retrospective analysis was 13.5 Mb (4 times the average longest LCSH in our control study), we used that size for all reporting of potential UPD. In all, fifty-eight cases showed LCSH of 13.5 Mb or greater in a single chromosome. Using cases that only contained a single chromosome with LCSH, eliminated >100 cases of identity by descent which show multiple chromosomal LCSH delineating regions of recessive gene risk. Molecular follow-up of the 58 cases was obtained in 21 cases of which 15 were confirmed as hetero/isoUPD and one segmental isoUPD. Most of the cases molecularly pursued involved imprinted chromosomes, although the risk of occult trisomy effects from incomplete rescue in UPD cases may be considerable. In the five false positive cases there were no terminal LCSH found while 11 of the 16 confirmed UPD cases (including the segmental case) had at least one terminal block, a finding that fits well with UPD origin. The loci of the five false positive LCSH appear to cluster near recombinational cold spots, suggesting the need to account for these to improve predictive values. True segmental UPD secondary to mitotic recombination as a cause of a positive (done in three of the five cases) should be investigated in molecular follow-up studies. Although all retrospective cases of UPD were LCSH "positive" in these studies, clearly more need to be studied to determine whether this signature evidence of recombination is present in all cases.

### 1447/W/Poster Board #1105

**Customized oligonucleotide FISH probes offer increased sensitivity in characterizing abnormalities detected by array CGH.** *L.S. Rector[1], N.A Yamada[2], M.E. Aston[1], R.A. Ach[2], P. Tsang[2], E. Carr[2], A. Scheffer-Wong[2], N. Sampas[2], B. Peter[2], S. Laderman[2], L. Bruhn[2], A.R. Brothman[1,3].* 1) ARUP Laboratories, Salt Lake City, UT; 2) Agilent Laboratories, Agilent Technologies, Santa Clara, CA; 3) Deparments of Pediatrics, Human genetics and Pathology, University of Utah School of Medicine, Salt Lake City, UT.

Array comparative genomic hybridization (aCGH) is now an accepted methodology for detection of genomic copy number changes. FISH is often used to confirm and further characterize abnormalities detected by aCGH. It is not unusual for abnormalities detected by aCGH to be in a genomic region or of a genomic size which prohibits traditional FISH using large probes such as bacterial artificial chromosome (BAC) vectors. Our goal was to establish a robust method for visualizing FISH probes on metaphase chromosomes to further characterize small abnormalities which would not be readily seen by conventional FISH techniques. Oligonucleotide FISH (oFISH) is an alternative to BAC FISH because it allows for higher resolution visualization of smaller loci. In this study, libraries of long oligonucleotides (194mers) with high complexity (~>150 oligonucleotides per locus) were chemically synthesized. Probe generation involved PCR amplification, followed by the introduction of fluorescent labels by chemical modification. By directing oFISH probes at the most informative elements of the region under investigation, we achieved high specificity and eliminated the need for Cot-1 or other suppressive hybridization reagents. Upon the initial evaluation of 14 oFISH probes, 12 of these (86%) gave strong signals with good specificity and sensitivity. The two oFISH probes which did not hybridize well initially included regions at distal 4p and Xq which both contain a high frequency of repeat sequences. When a modified *in silico* oligo selection method was used to enhance signal while maintaining specificity, easily detectable signal was achieved at the expected sites in the 4p and Xq regions even though as little as 10% (6.5 Kb) of the initial region was included in the probe sequences. Ongoing evaluation of additional oFISH probes indicates that this method will be powerful for routine analysis of previously difficult genomic regions. The ease, reproducibility and high level accuracy of this technique suggest that it may prove invaluable to the human genetics community.

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### 1448/W/Poster Board #1106

**Two Mosaic Ring Cases with Evidence of Structural Evolution Determined by SNP Microarray.** *H. Risheg[1], W. Chung[2], I. Gadi[3], R. Pasion[3], S. Schwartz[3], J. Tepperberg[3], P. Papenhausen[3].* 1) Laboratory Corporation of America, Seattle, WA; 2) Childrens Hospital of New York, New York, NY; 3) Laboratory Corporation of America, Research Triangle Park, NC.

We report two cases with supernumerary mosaic ring chromosomes identified by SNP microarray. Case 1 is a 6 month old female with mild dysmorphic features, severe congenital microcephaly and constant ataxic movements. Case 2 is a newborn male with a prominent forehead, plagiocephaly, hypertelorism and low set ears. Chromosome analysis in Case 1 revealed a 45,X karyotype in 24/ 30 cells and 6/30 with 46,X,+r. High resolution SNP analysis revealed the presence of 1.2 copy dosage from Xp11.2 to q25 (bp: 54,908,000-128,500,000) consistent with approximately 20% mosaicism of the apparent *XIST* (Xq13) bearing ring X. Furthermore, the X chromosome copy dosage was 1.1 at the long arm distal extent of the dosage gain, suggesting that the ring reduced in size to the Xq24 (bp: 117,400,000) in about half of the cells with the ring. The reason for the apparent severity of the phenotype was not clear in a case that should have an inactivated X ring, however there could have been a problem with the function of the *XIST* gene. Microarray analysis consistent with a supernumerary ring chromosome in Case 2 again showed different size and dosage gains of chromosome 8. Cytogenetic/FISH analysis showed two supernumerary ring chromosomes which included the 8 centromere. The array showed different distal q11.22 dosage gain and are 4.44 Mb and 4.8 Mb in size. This agrees with the cytogenetics studies which showed mosaicism of the different sized supernumerary rings. Eight of 20 cells demonstrated one extra ring chromosome, while the remaining 12 showed two ring chromosomes. An extended region of allele homozygosity observed on chromosome 8 was also noted, suggestive of UPD. Subsequent chromosome 8 microsatellite analysis confirmed UPD8 of maternal origin and an apparent partial rescue from zygotic trisomy. Although there have been no confirmed imprinted genes reported for chromosome 8, the homozygotic stretch of genes may be subject to disorders due to pairing of recessive genes. There may also be residual effects of an early developmental presence of trisomy during the usual rescue etiology to UPD. Determining the exact structure of supernumerary ring chromosomes has always been problematic. These cases demonstrate the precision of high resolution SNP array in identifying small mosaic supernumerary chromosomes and the mechanistic insights of supernumerary ring formation which would not have been recognized by conventional cytogenetics.

### 1449/W/Poster Board #1107

**De novo 21q22.1q22.2 deletion including RUNX1 mimicking a congenital infection.** *L. Faivre[1], P. Callier[1], C. Tauvin-Robinet[1], I. Desguerres[2], A. Masurel-Paulet[1], N. Mejean[3], S. Falcon-Eicher[4], S. Bidot[5], F. Huet[1], M. Beri-Dexheimer[6], P. Jonveaux[6], F. Mugneret[1].* 1) Département de Génétique et Centre de Référence Anomalies du Développement et Syndromes Malformatifs, Hôpital d'Enfants, CHU Dijon, Frances, Dijon, France; 2) Neurologie Pédiatrique, Hôpital Necker-Enfants Malades, Paris, France; 3) Radiologie Pédiatrique, Hôpital d'Enfants, Dijon, France; 4) Cardiologie Pédiatrique, Hôpital d'Enfants, Dijon, France; 5) Ophtalmologie, CHU Dijon, France; 6) Génétique moléculaire et cytogénétique, Nancy, France.

Patients with partial monosomy 21 exhibit various phenotypes dominated by prenatal and postnatal growth restriction, severe developmental delay, abnormal muscle tone, a variety of dysmorphic facial features, and heart defects. Patients with deletions encompassing the RUNX1 gene on 21q22.12 can also present thrombocytopenia and a predisposition to acute myeloid leukemia. To date, molecular cytogenetic characterisation has been reported in only 4 patients with this syndromic thrombocytopenia. Here we report on a sporadic case of a 7-year-old boy who presented at birth with growth retardation, thrombocytopenia, atrial septal defect with non-obstructive, hypokinetic, hypertrophic cardiomyopathy, facial dysmorphic features and poor eye-contact. There was no history of infection during pregnancy and analyses for infection and immune cells were normal. Investigations revealed normal bone marrow, normal cytogenetic and metabolic investigations. Corpus callosum dysgenesis was evidenced, with absence of the central region, in favour of a clastic origin. At 4 months of age, the cardiomyopathy regressed and the platelet count spontaneously improved but he still needed nasogatric feeding. At one year of age, he could not sit and had poor eye contact. Hypermetropia microcornea, goniodysgenesis of the left eye with iridocapsular synechies and astigmatism were diagnosed. At 2 years of age, enamel dysplasia was evidenced. He walked at 6 years of age, but had no speech. Epilepsy was diagnosed. Growth hormone therapy was started but stopped because of unsatisfactory results on growth. The association of ocular synechies, central corpus callosum agenesis, enamel dysplasia, transient cardiomyopathy and fluctuating thrombocytopenia with normal results for infection analysis led to the conclusion that an undiagnosed congenital infection was the most likely diagnosis. Nevertheless, array-CGH 105K was performed and revealed a 2.8 Mb deletion on chromosome 21q22.1-q22.2 encompassing the RUNX1 gene. This finding correlates well with the presence of thrombocytopenia, facial dysmorphism, growth retardation and psychomotor delay, but the additional clinical and imaging features contribute further to the description of this new syndrome. This observation further emphasizes that the 21q22 microdeletion could be a novel cause of mental retardation, in which a platelet disorder would be a distinctive phenotypic manifestation.

### 1450/W/Poster Board #1108

**Determining X Chromosome Inactivation Patterns.** *B. Bertelsen, Z. Tümer, K. Ravn.* Kennedy Center, Glostrup, Denmark.

In X-linked recessive inherited disorders, only males manifest the disease features, whereas the female relatives are usually asymptomatic carriers. However, it is known that female carriers may also display clinical symptoms. In these cases, skewed X chromosome inactivation (XCI) has been considered to correlate with the manifestation of the symptoms. It is therefore important to investigate the XCI patterns in these females for clinical assessment and genetic counselling. To define the XCI pattern usually the androgen receptor (*AR*) gene residing at the Xq12 is used as a genetic marker. In this approach, a region within the first exon of the *AR* locus, containing two methylation sensitive *Hpa*II sites and a highly polymorphic CAG repeat, is used. The two X chromosomes are differentiated by the polymorphic CAG repeat, and their inactivation status is determined by their methylation difference at the *Hpa*II sites.This simple, fast and elegant method has been used successfully since 1992 to determine the XCI status of many females. However, the method has its limitations, as some females are not informative for the CAG repeat. In these cases, another polymorphic locus located at Xq28 (*FMR1*) can be used for investigation as it exhibits the same features as the *AR* in regards to methylation. The aim of the present study is thus to identify other differentially methylated polymorphic loci across the X chromosome. Through in silico screening of the Xchromosome we have identified several polymorphic loci with nearby sequences, which may be differentially methylated. These loci are currently being investigated for their methylation status in active and inactive X chromosomes. The most appropriate sites will be validated in families with known X-linked disorders where the female carriers are protected from the disease due to full inactivation of the mutated X-linked gene.

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### 1451/W/Poster Board #1109

**Chracterization of SRY deletions in female horses with a 64,XY karyotype.** *T. Lear, R. McGee, E. Bailey.* Veterinary Sci, Univ Kentucky, Lexington, KY.

Disorders of sexual development afflict horses, as well as humans and other mammals. Although no cases of XY sex-reversal have been reported in dogs and cats, it is the most common form of sex-reversal reported in the horse. The phenotype of affected horses ranges from a feminine female (mare) with a reproductive tract within normal limits to a masculine mare that is large in stature. Affected horses have normal female genitalia but hypoplastic ovaries and uterus and are infertile. This condition has been found in many horse breeds and can occur sporadically or within families. Four mares, three Thoroughbred and one Standardbred, were presented for cytogenetic analysis due to the lack of estrous cycles and small, inactive ovaries. All four mares had a 64,XY karyotype. Subsequent FISH studies with a horse BAC containing the SRY gene were positive for three mares while PCR for SRY was negative in all four mares. Further studies by PCR determined the entire SRY gene was deleted from the Y chromosome in all four horses. The deletion encompassed approximately 33 Kb and occurred in a similar but not identical position on each horse Y chromosome. Sequencing across the deletion was unsuccessful suggesting a structural rearrangement had occurred on the Y chromosome. Since the deletions were similar in all four horses it is possible this region may be a hotspot for mutation leading to the deletion of the SRY gene. One thousand female horses under two years of age are being assessed to determine how prevalent this SRY deletion may be in the Thoroughbred horse population and if it has a familial component.

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## 1452/T/Poster Board #1

**"Universal" Carrier Screening of Recessive Genetic Disorders by Next Generation Sequencing.** *S. Kingsmore, D. Dinwiddie, C. Bell, N. Miller, J. Crow, E. Ganusova.* Natl Ctr Genome Resources, Santa Fe, NM.

While individually quite rare, recessive genetic diseases together are a major medical burden and cause significant morbidity and mortality. 20-30% of infant deaths and 11% of pediatric hospital admissions are related to genetic disorders. Widespread carrier screening was first adopted for Tay-Sachs disease (TSD, #272800), resulting in greater than 90% decrease in incidence in North American Jewish populations. In collaboration with the Beyond Batten Disease Foundation, we are developing a carrier screening test for approximately four hundred autosomal and X-linked recessive (XLR) disorders causing high morbidity or mortality in childhood, such as Juvenile Batten Disease (#204200). Proof-of-principle studies of this "universal" carrier test have demonstrated accurate identification of XLR mutations: Genomic DNA samples from 18 patients with Pelizaeus-Merzbacher disease (#312080), Lesch-Nyhan syndrome (#300322), and Menkes disease (#309400), comprising different mutations, were evaluated. The mutation spectrum consisted of SNPs, nucleotide indels, and deletions of entire exons. Illumina sequencing libraries from the 18 samples were multiplexed, enriched for chromosome X exons and sequenced. Overall, 50 +/- 9% of sequences aligned to chromosome X exons, reflecting an average enrichment of 379-fold. A median of 627 megabases of sequence corresponded to at least 20-fold coverage of 61% of target sequences; depth of target coverage varied with depth of sequence (r2 0.96). Bioinformatic analysis using the Alpheus software system correctly identified the mutations and discovered two new mutations previously mischaracterized. A prototype "universal" carrier screening test is anticipated to be available in 2010 and to be available at similar cost to current molecular tests for single disorders.

## 1453/T/Poster Board #2

**Health Technology Assessment of Ashkenazi Jewish Carrier Screening Panels.** *D.J. Allingham-Hawkins, A.P. Lea, S. Levine.* Hayes Inc, Lansdale, PA.

Objectives: Carrier screening is performed to identify couples in which both members are carriers of alleles that cause a specific disorder so that affected couples can make informed reproductive choices. Several genetic laboratories currently offer genetic testing to screen for carriers of up to 12 autosomal recessive disorders known to occur in the Ashkenazi Jewish (AJ) population. This health technology assessment focused on defining an evidence-based set of criteria for inclusion of a test for a disorder in an AJ carrier screening panel (AJCSP) and determining which disorder tests meet these criteria. Methods: A literature search was performed to identify studies of genetic tests for disorders appropriate for successful screening in the AJ population. This evidence base was reviewed to identify characteristics associated with clinical utility of the screening tests using the ACCE model that was developed by the Centers for Disease Control and Prevention (CDC) specifically to evaluate genetic tests. Fifteen autosomal recessive disorders were evaluated with this approach. Results: The minimal criteria found to be most relevant to determining if inclusion of a test for a disorder in an AJCSP is warranted were: disorder causes significant mortality and/or morbidity; an allele frequency of 1:70 or greater; a relatively clear relationship between genotype and phenotype with high penetrance; and a screening test that detects at least 90% of alleles that cause the disorder. Tests for only 4 of the 15 disorders were found to meet all of these criteria: Canavan disease, cystic fibrosis, familial dysautonomia, and Tay-Sachs disease. Several societal and ethical factors were also found to be necessary in order for an AJCSP to be successfully implemented: voluntary participation of the AJ population; adequate access to educational materials and genetic counseling; and an informed consent process that allows couples to opt out of testing for any individual disorder based on personal beliefs. Conclusions: Although several genetic laboratories provide AJCSPs that include tests for up to 12 disorders, there is currently sufficient evidence of clinical utility to support the inclusion of testing for only 4 of these disorders. Furthermore, because of the ethical requirement of allowing couples to opt out of testing for individual disorders, genetic laboratories must be able to specifically select the tests to be performed based on patient request.

## 1454/T/Poster Board #3

**Early pregnancy hemoglobinopathy diagnostics in the Dutch city of The Hague. Provisional results and a new hemoglobin variant : Hb Den Haag [β45 CD4 (Phe →Tyr)].** *J.O. Kaufmann[1], C.L. Harteveld[1], M. Bakker-Verweij[1], A. Selles[2], S.G.J. Arkesteijn[1], P.W. Wijermans[3], J.L. Kerkhoffs[3], P.C. Giordano[1].* 1) Clinical Genetics, Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands; 2) Midwife practice Anno, The Hague, The Netherlands; 3) Dept. of Hematology HAGA city Hospital, The Hague, The Netherlands.

To implement country wide carrier diagnostics for hemoglobinopathy in The Netherlands at the early pregnancy level a universal pilot is ongoing in the Dutch city of The Hague. We report here the provisional figures and a new abnormal hemoglobin found in 3 members of a family of Scandinavian origin. During the first pregnancy control HPLC based Hemoglobinopathy carrier diagnostics is offered in additional to the regular test for anemia analysis. Hb separations are done on Variant 2 and when needed molecular analyses is done by Gap-PCR, by direct sequencing and or MLPA. Carriers of frequent abnormal Hb (S, C, E, D) and β-thalassemia are regularly found in the immigrant population at risk and partner analysis follows rapidly to exclude or confirm risk and to offer prevention. Thus far of the 436 women included in this study, 6 were found to be carriers of HbS, 5 β-thalassemia, 4 α-thalassemia, 4 δ-thalassemia and 3 of a Hbvariant. This has lead to the discovery of 2 couples at risk of a child with a hemoglobinopathy (HbSS and HbSC) who were referred to genetic counseling. In this cohort a 34 years old woman of Scandinavian descendant presented with chronic hemolytic anemia. HPLC analysis revealed a partially separated fraction following HbA. Molecular diagnostics disclosed a TTT→TAT transversion at HBB:c.137 causing a Phe→Tyr single amino acid substitution at position 45 of the beta globin gene. Similar abnormalities previously described at the same position were reported associated with mild chronic hemolysis. We describe the hematological picture of the 6 members of the family, the biochemical and molecular data and we discuss a possible risk in association with the β°-thalassemia and HbS traits, very common in this country.

## 1455/T/Poster Board #4

**Prenatal diagnosis of rare δβ-thalassaemia following successful identification of deletional mutation by Multiplex Ligation-dependent Probe Amplification (MLPA).** *H.Y. Law[1,2], Y. Zhao[3], R.S. Roch[1], Y.M. Tan[2], A.H.M. Lai[1], I.S.L. Ng[1,2].* 1) Dept Pediatrics, KK Women's & Children's Hosp, Singapore, Singapore; 2) National Thalassaemia Registry, KK Women's & Children's Hospital, Singapore; 3) Dept Clinical Research, Singapore General Hospital, Singapore.

**Purpose:** A couple with history of β-thalassaemia was referred to National Thalassaemia Registry (NTR) for risk assessment. The wife had typical β-thalassaemia indices and DNA analysis identified IVSInt5 (G→C) mutation in β-globin gene. The husband's indices (MCV=73.5%, $HbA_2$=2.4, HbF= 19.1%) were indicative of δβ-thalassaemia carrier. However, routine analysis for deletions associated with δβ-thalassaemia and hereditary persistence fetal haemolgobin (HPFH) in local population were negative. To determine if the couple was at risk for thalassaemia intermedia/major, Multiplex Ligation-dependent Probe Amplification (MLPA) analysis was carried out to establish if the husband was a carrier of a large deletion in the β-globin gene cluster. **Methods:** MLPA kit (MRC-Holland SALSA P102HBB) for detection of deletion in β-globin gene cluster was used to establish the extent of gene deletion in husband and was subsequently used for prenatal diagnosis to confirm presence of the paternal δβ-thalassaemia in the chorionic villus sample (CVS). **Results:** MLPA found deletion of exon 3 in $\gamma^G$ and δ-globin genes, and the entire β-globin gene in husband's sample. Between the 2 partially deleted $\gamma^G$ and δ-globin genes was an intact region that included the entire $\gamma^A$ and pseudo-β globin gene. Prenatal analysis using reversed dot blot analysis found presence of maternal IVSInt5 (G→C) mutation and absence of normal allele in the fetus. MLPA confirmed presence of paternal deletion. This predicted the fetus to be a thalassaemia intermedia with hemizygous IVSInt5 (G→C)genotype. PCR using published protocol confirmed the deletion to be "Asian-Indian inversion-deletion" that caused δβ-thalassaemia. **Conclusion:** The MLPA kit is useful for detection of novel/rare deletion and is thus useful for risk assessment in couples present with δβ-thalassaemia or HPFH. It is particularly useful for prenatal diagnosis in a mixed ethnic population, as prior knowledge of the breakpoint or the extent of deletion is often not available in view of the wide spectrum of deletion reported in the β-globin gene cluster.

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## 1456/T/Poster Board #5

**Factors influencing uptake of cascade carrier testing for cystic fibrosis.**
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In Victoria, Australia, carrier testing for cystic fibrosis (CF) is performed by a state-wide laboratory and counselling service. CF carrier testing is offered free to relatives of babies diagnosed with CF through newborn screening. Although cascade testing is known to detect carriers for CF, its effectiveness has been questioned because most babies with CF are born to couples who do not have a family history. Uptake of cascade carrier testing was audited by examining pedigrees of newborns diagnosed with CF between 2000 and 2004, and performing data linkage to the laboratory database records. Uptake of carrier testing amongst adult relatives was adjusted for clustering within families; uptake was less than 20%. The majority of relatives have not had cascade carrier testing despite being at high risk. We identified factors influencing uptake of CF carrier testing by administering a multi-generational survey to family members of children with CF. The questionnaire assessed knowledge of CF and carrier testing, attitude towards carrier testing, factors that influence a decision about carrier testing, and demographic information (age, sex, education, parity, future reproductive plans). The questionnaire was sent to 284 family members, with a response rate of 79%. Respondent demographics were: male:female (40%:60%); tested:non-tested (37%:43%). Of those tested, the proportions were: 24% aunts, uncles; 23% grandparents; 20% parents; 13% second cousins; 11% great aunts, uncles; 7% first cousins; 1% siblings (≥18yr). The majority of respondents (77%) answered ≥9 knowledge questions correctly (max. score 12 correct). The tested group had a higher knowledge score and more positive attitudes compared with the non-tested group, although overall both groups were positive about having carrier testing. The most commonly cited factors that may influence a decision to have carrier testing were influences of another person (having a carrier partner, partner's wishes and doctor's suggestion) and having a person in the family with CF. There was no major reason cited for not having testing. Open-ended responses from family members other than parents indicate that the main barrier to being tested was a lack of awareness that testing was available for them through the clinical service. Findings provide insight into the characteristics of family members who are tested and those who are not and inform clinical service delivery around offering carrier testing to family members.

## 1457/T/Poster Board #6

**Is reflex testing for the CFTR 5T/7T/9T polymorphism needed in R117H heterozygous cases?**
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In 2001, the American College of Medical Genetics and American College of Obstetrics and Gynecology recommended a panel of cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations and variants be tested as a part of population screening for couples planning a pregnancy or for those already pregnant. Included in these guidelines were: 1) reflex testing for the 5T/7T/9T polymorphism should be performed in R117H positive samples and 2) the reproductive partner of a carrier should be tested for CFTR gene mutations. The purpose of this study was to determine whether the 5T/7T/9T reflex test ever changed patient management. Thirty R117H heterozygous cases submitted for pregnancy carrier screening were identified. Electronic medical records were searched for CFTR mutation results in the reproductive partner. If this information was unavailable, letters were sent to the ordering physicians. Information was obtained in 17/30 cases (11 via electronic medical records, 6 responses to letter). In 11 cases, the father tested negative. In 5 cases, the father was not tested (the father refused testing (n=1), the woman refused to have the father tested (n=2), the father could not pay (n=1), unknown (n=1)). In one case, simultaneous screening of the husband and wife was performed: the husband was R117H heterozygous and the wife was negative; 5T/7T/9T reflex testing was not done in the husband. When physicians were contacted via letters, they were asked if the 5T/7T/9T testing provided helpful information. No responses to this question were received. 5T/7T/9T testing was done in 16/17 R117H positive cases (7T/7T (n=11), 5T/7T (n=4), 7T/9T (n=1)). No cases were found where the 5T/7T/9T results altered outcome. 5T/7T/9T testing should be done only if the partner of a R117H heterozygous individual is also a carrier and if 5T/7T/9T test results would alter decisions regarding prenatal testing or pregnancy termination. Eliminating upfront 5T/7T/9T reflex testing for R117H heterozygotes would save unnecessary test charges and potential confusion in interpreting and explaining the results to patients. For cases where the reproductive partner of a known R117H (or other CFTR gene mutation) carrier refuses testing, prenatal testing can be offered.

## 1458/T/Poster Board #7

**Outcomes and Experience of Spinal Muscular Atrophy (SMA) Carrier Screening on a Semen Donor Population.**
*P. Callum[1], R.E. Falk[2], J. Iger[1], M. Ray[1], C.A. Sims[1].* 1) Genetics, California Cryobank, Inc., Los Angeles, CA; 2) Cedars Sinai Medical Center, Los Angeles, CA.

Donor eligibility at our facility is determined in part by a genetic risk assessment: 3-generation pedigree, chromosome analysis, hemoglobinopathy and cystic fibrosis carrier screening. Ashkenazi Jewish carrier testing is completed if indicated. SMA carrier screening was added to our donor screening protocol in August 2008. Testing was performed on 258 semen donors and donor applicants. We identified 5 carriers, all of Caucasian, non-Jewish ancestry, representing 3% (5/172) of this population; 7 (4%) had 3 copies of SMN1. Three copies of SMN1 were detected in 5/36, 0/17, 2/9, 2/10, and 0/14 men of Jewish, Hispanic, African American, Asian, and other ancestries, respectively. Specimens from 4 of the 5 carriers were already available for use. Clients who purchased these specimens were informed of the donors' positive test results, clinical features of SMA, risk to offspring, and option of carrier screening. Formal genetic counseling was recommended. Some clients were appreciative; others were angry that this information was not identified during the donors' genetic evaluations. Surprisingly, of those who were most upset, many had not attempted to conceive a pregnancy while some who received the news more calmly were pregnant at the time of contact. Many clients pursued carrier screening, but were upset by the high cost of testing. One client was found to be a carrier for SMA. General population carrier screening for SMA is controversial, in part due to differing guidelines published by ACMG and ACOG and the limitations of this test. Given the high carrier frequency and severity of the disorder, SMA screening on gamete donors is useful for assessing donor eligibility and reducing risks for medical problems in offspring, despite the limitations. Our sample is too small to assess allele frequencies but other data indicates that counseling about SMA may be revised based on variable allele frequencies and residual risks for individuals of different ethnicities. While concern about increased medical risks to their offspring is understandable, the clients' reactions suggest that they do not comprehend the limitations of genetic screening. This finding supports the need for preconceptual genetic counseling for clients using donor semen yet these services do not appear to be well utilized. Further studies are indicated to determine if this patient population is unaware of the availability or utility of this service or if they are resistant to seeking counseling.

## 1459/T/Poster Board #8

**Spinal muscular atrophy carrier screening in a diverse population: pitfalls and promise.**
*K. Bajaj, P. Reingold, J. Powers, S. Klugman.* Montefiore Medical Center Division of Reproductive Genetics, Bronx, NY.

**Background:** Prenatal carrier screening for cystic fibrosis has proven to be a cost-effective, widely accepted practice. Spinal muscular atrophy (SMA) is a disorder characterized by degeneration of motor neurons. With a carrier frequency of 1 in 40, SMA is the second most common fatal autosomal recessive disorder after cystic fibrosis. The American College of Medical Genetics recommends universal prenatal SMA carrier screening because the disorder is severe, has a high carrier frequency, and can be diagnosed prenatally. Recently, the American College of OB/GYN released a committee opinion contradicting the ACMG stance, stating there is not enough data on patient counseling and utility measures to support universal SMA carrier screening. **Aim:** To ascertain patient understanding of SMA carrier screening after genetic counseling. **Methods:** All English or Spanish speaking women receiving genetic counseling at two of our outpatient centers were asked to participate in a 25 question survey. The survey assessed demographic data, had 5 questions about clinical features and prenatal diagnosis of SMA, and investigated the motivation behind patient screening choices. The responses were analyzed using standard statistical software. **Results:** 70 women completed the survey. The mean age was 32 years and 57% of respondents identified themselves as African-American or Hispanic. 74% were privately insured and 39% had a college degree or higher. 60% of subjects had not heard of SMA prior to their visit. 46% of subjects answered 4 or 5 post-counseling questions correctly; two-thirds of these women had a college degree or higher. 14% of patients declined SMA carrier screening, of which 80% answered 4 or more post-counseling questions correctly. The bulk of those that declined perceived the disorder as "too rare" or "didn't want to know" their carrier status. Of those opting for screening, 50% identified the desire to know their carrier status as the main reason for testing. Another 15% chose screening because they were having their blood drawn for other reasons. **Conclusion:** After formal genetic counseling, the vast majority of patients will opt for SMA carrier screening. In our experience, only half of women have an adequate understanding of the clinical features and prenatal diagnostic options related to the disease. Regarding SMA, there is great opportunity for development of educational tools to provide patients with a complete range of reproductive options.

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## 1460/T/Poster Board #9

**Managing the demands of the increasing disease burden in NF1.** *A. Bergner[1], L. Jordan[1], A. Belzberg[2], J. Blakeley[1,3].* 1) Department of Neurology, Johns Hopkins University, Baltimore, MD; 2) Department of Neurosurgery, Johns Hopkins University, Baltimore, MD; 3) Brain Cancer Program, Johns Hopkins University, Baltimore, MD.

Background: Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant conditions affecting the nervous system. NF1 is defined as a progressive condition, and the increasing burden of disease with NF1 has been demonstrated by both the objective measurement of symptom prevalence and the subjective experience of patients and care providers. However, it is not clear how this increasing burden impacts the daily care coordination for patients with NF1 or whether the current delivery of healthcare services for these patients is adequate given the well-demonstrated progressive nature of their disease. Objectives: This study aimed to quantify the disease burden of NF1 by utilizing the number of referrals to ancillary studies and medical specialists that were required by pediatric and adult patients with NF1 as an objective marker of disease burden. Methods: Chart review was undertaken for 42 pediatric and 40 adult patients with NF1 who were seen consecutively for routine annual outpatient Neurology appointments within the Johns Hopkins NF Center between January 1 and December 31, 2008. The number of referrals and the services to which patients were referred were separately tallied for pediatric and adult patients and then compared. Results: 22 (55%) adult NF1 patients required 5 or more referrals at the time of their visit, while only 6 (14%) pediatric patients required the same (Chi square 13.6, p=0.0006). The mean number of referrals for a pediatric patient was 3.4 (SD=1.17) and for an adult patient was 5.13 (SD=1.81) (Student's test, t=5.1, p<0.0001). Conclusions: On average, adult patients with NF1 required significantly more ancillary studies and medical specialists to manage complications related to their diagnosis than pediatric patients. These results provide further support for the understanding that the burden of disease with NF1 increases over time. Medical providers that work with adult patients with NF1 are likely to experience the increasing burden of this disease through the greater need for ancillary studies, procedures and medical management. This information can assist medical providers in planning for the comprehensive medical care that is needed by the majority of adult patients with NF1. It also has further implications for health systems utilization and costs, and is important to recognize as new technologies are developed to better diagnose and treat patients with NF1.

## 1461/T/Poster Board #10

**Expanding the Multidisciplinary Care of CDH1 Carriers.** *D. Patel, K.M. Shannon, M. Jacobs Gabree, G. Chan-Smutko.* CCRA, MGH, Boston, MA.

Hereditary diffuse gastric cancer (HDGC) syndrome is a genetic disorder that increases an individual's risk of developing diffuse gastric cancer and female breast cancer. Approximately one third of families with a clinical diagnosis of HDGC harbor germline mutations in the E-cadherin gene, also known as CDH1. Females with CDH1 mutations have an 83% risk of developing diffuse gastric cancer as well as a 39-52% risk of developing lobular breast cancer. Male carriers are at a 68% risk for diffuse gastric cancer. Given the limited effectiveness of screening, prophylactic total gastrectomy is considered to be the only definitive intervention to prevent death from gastric cancer. Our experience at Massachusetts General Hospital is that the majority (4/6) of unaffected individuals who tested positive for a familial mutation in CDH1 have undergone prophylactic gastrectomy. Through post-operative genetic counseling we have identified various issues that extend beyond genetic testing and risk assessment for the family and require additional specialty services outside of the MGH Center for Cancer Risk Assessment. We have developed a multidisciplinary, comprehensive care program to address the needs of individuals with CDH1 mutations. Female carriers are referred to a breast cancer genetics specialist to discuss the options of increased breast screening and risk-reducing bilateral mastectomy. Parents are referred to the MGH PACT (Parenting at a Challenging Time) team who has been helpful in assessing parents' motivation for testing minors and has been able to discuss with parents ways to approach the topic of genetic testing with their children. To address the issue of behavioral management with respect to eating post-gastrectomy, individuals are referred to the MGH Weight Center. The Weight Center is comprised of experts including physicians, nutritionists, and psychologists who understand weight problems and eating habits and can offer guidance to patients with respect to nutrition, meal planning, and expected behavioral changes post-gastrectomy. Given their experience in treating patients with food dependency, this team will be better equipped to offer long-term strategies for emotional adjustment after surgery. We expect that the integration of breast health care services, child psychiatry, and weight center clinicians will provide the necessary comprehensive approach to the longitudinal care of CDH1 carriers.

## 1462/T/Poster Board #11

**Cost Savings through Molecular Diagnosis for HHT.** *R.E. Pyeritz[1], D. Asch[2], C. Zayac[1], S. Keddem[1], B. Bernhardt[1].* 1) Penn Center for the Integration of Genetic Healthcare Technologies, University of Pennsylvania, Philadelphia, PA; 2) Leonard Davis Institute of Health Economics, University of Pennsylvania, Philadelphia, PA.

Hereditary hemorrhagic telangiectasia (HHT) is an age-dependent, autosomal dominant disorder that can cause major morbidity and premature death. Early diagnosis enables preventive measures that greatly improve outcomes. Mutations in at least six genes can cause HHT, but clinical testing is available for only 3. Patients with clinically verified HHT have a detectable mutation in ENG or ALK1 in ~80% of cases. We have found mutation analysis useful for confirmation of diagnosis and screening relatives who are at 50-50 risk. However, patients, providers and insurers often question the need for DNA testing. One previous study found a cost-savings of 1915 Canadian dollars per HHT family by pursuing mutation detection; however, the cost structure of many aspects of health care is different from the U.S. We conducted a cost-minimization analysis comparing the costs of screening at-risk relatives by accepted clinical methods (clinical evaluation for signs of HHT, brain MRI and contrast echocardiogram followed by chest CT if positive) vs. determining the proband's mutation followed by testing at-risk relatives for the proband's mutation if found, and then performing clinical screening only on relatives who have the mutation. We modeled 100 probands with 400 relatives and assumed full compliance. Costs were actual Blue Cross/Blue Shield reimbursements for 2009 in our region. We assumed the relatives were young, which means that negative or equivocal clinical testing requires periodic follow-up until either the diagnosis is established, or the person reaches young adulthood and is likely to be truly unaffected because of lack of clinical involvement. We assumed that genetic testing would detect a pathogenic mutation in 80% of probands. The clinical model resulted in total costs of $8,426,920, while the molecular diagnosis model resulted in total costs of $3,556,228. Because of the assumptions made, we are conducting sensitivity analyses to set upper and lower bounds on these costs. Nonetheless, the more than two-fold cost savings of mutation detection of probands provides strong evidence for cost savings through DNA testing.

## 1463/T/Poster Board #12

**Closing the Loop and Opening Vistas: A Conference to Explore the Genetic Counseling Needs and Experiences of *BRCA* Carriers and Their Families.** *C.R. Huizenga[1], D.J. MacDonald[1], S.R. Sand[1], K.R. Blazer[1], K. Hurley[2], J.O. Culver[1], K.C. Banks[3], J.N. Weitzel[1].* 1) Clinical Cancer Genetics, City Hope Comprehensive Cancer Ctr, Duarte, CA; 2) Clinical Genetics Service, Memorial Sloan-Kettering Cancer Ctr, New York, NY; 3) Cancer Genetics Program, St. Joseph Hospital, Orange, CA.

Patients seen for genetic cancer risk assessment services (GCRA) in our institution indicated on long-term follow-up surveys a need for ongoing information and support after testing is complete. A key challenge is that dissemination of updates through a newsletter or other media would not provide opportunities for questions or support, and limited resources preclude additional visits. To address this challenge, we invited patients back for an all-day conference. Information was delivered through podium presentations, expert and patient panel discussions and interactive breakout sessions to: 1) explore GCRA-related experiences and needs and 2) share medical advances in risk assessment, management and treatment, current research, and information and resources for individuals genetically predisposed to breast and ovarian cancer. Invitees were individuals who completed GCRA at City of Hope or collaborating regional facilities between 1998-2008 and carried a *BRCA1/2* mutation or variant of uncertain significance (VUS), and their family members. Mixed methods research (surveys and discussion directed by Audience Response System (ARS) interaction) was used to explore participant GCRA experiences and needs. All 79 participants answered ARS questions and 70 (89%) completed a voluntary survey. Most survey responders were female (84%; mean age, 48 years); 64% were *BRCA* positive, 4% had a VUS, and 32% were non-carrier relatives, spouses, or friends. Fifty-seven percent had a personal history of cancer, primarily breast (86%) and ovarian cancer (20%). The majority of participants expressed that at least some information provided during GCRA was overwhelming (68%) and that the GCRA process evoked anxiety or worry (59%), particularly regarding unexpected parental transmission guilt and concern for relatives. Needs for an additional appointment (62%) and updates on cancer risk (71%), screening and prevention (74%), and cancer genetics (60%) were expressed. Participant ideas for GCRA process improvement included: 1) sending more information prior to the appointment; 2) placing more emphasis on bringing a spouse or significant other to the appointment; and 3) allowing more time to digest test results before discussing risk management. Ongoing information needs beyond *BRCA* result disclosure and risk management recommendations can be effectively met through large group format. Clearly, the Closing the Loop conference concept is an effective way to improve the GCRA process.

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### 1464/T/Poster Board #13

**Use of X-linked Short Tandem Repeats Loci to Confirm Mutations in Parentage Caseworks.** *D.P. CHEN[1,2], C.P. TSENG[2,3], S.H. TSAI[1], M.C. WANG[1], S.C. LU[1], T.L. WU[1], P.Y. CHANG[1], C.F. SUN[1,4].* 1) Department of Laboratory Medicine, Chang Gung Memorial Hospital, Tao-Yuan, Taiwan; 2) Department of Medical Biotechnology and Laboratory Science, Chang Gung University, Taoyuan County, Taiwan; 3) Graduate Institute of Medical Biotechnology, Chang Gung University, Taoyuan County, Taiwan; 4) Department of Pathology, School of Medicine, Chang Gung University, Taoyuan County, Taiwan.

Background: Analysis of short tandem repeat (STR) has become widespread in routine for parentage test. However, the accuracy of STR is sometimes interfered by the presence of microsatellite mutations. Analysis of other DNA markers such as the HV1 and HV2 hypervariable regions of mitochondrial DNA or the Y-STR becomes essential to settle the noncongruence. Owing to the limited polymorphism of mitochondrial DNA and the time-consuming nature of the test, we investigated here the use of X-chromosome STR (X-STR) to resolve the maternity disputes. Methods: At first, the STR mutation frequencies among 4758 Taiwanese individuals were analyzed. Population data were obtained from randomly selected 99 females and 101 males to setup the X-STR database. Two families with a mismatch of one allele in autosomal STR analysis were subjected to the X-STR test to explore its clinical application. Results: The STR mutations occurred in all 15 autosomal STR loci with the exception of TH01 and TPOX. The mutation rates could reach as high as 0.106% for the D8S1179 and D18S51 loci. As to the X-STR frequencies, the p-values for DXS7132, DXS981, DXS6789, DXS101, and HPRTB were 0.1471, 0.0019, 0.0025, 0.1427, and 0.1167, respectively. By linkage analysis, 33 and 34 different haplotypes were revealed for DXS101-DXS6789 and DXS7132-DXS981, respectively. Furthermore, two cases with one allele mismatch in routine parentage test were resolved by performing X-STR analysis. Conclusion: Typing of X-STR markers is recommended for parentage test when one or two alleles mismatch is present or when the samples are difficult to be analyzed.

### 1465/T/Poster Board #14

**The impact of 'uninformative' BRCA1/2 genetic test results on health professionals, caring for affected and unaffected women with a significant breast cancer family history.** *A. Ardern-Jones[1], R. Kenen (joint first author)[2], E. Lynch[3], R. Doherty[4], R. Eeles[1,4].* 1) Dept Genetics, Royal Marsden NHS Trust, Sutton Surrey, United Kingdom; 2) The College of New Jersey, Department of Sociology and Anthropology, New Jersey, USA; 3) The Royal Melbourne Hospital, Melbourne, Australia; 4) The Institute of Cancer Research, Cotswold Rd, Surrey, UK.

The professional specialists who care for breast cancer patients with uninformative BRCA genetic test results, as well as their unaffected high risk female relatives - geneticists, genetic counsellor/nurses, oncologists, gynaecologists and breast surgeons, are an understudied group. We conducted a small qualitative study that investigated women who had developed breast cancer under the age of 45 and who had received the information that their BRCA1/2 genetic test results were inconclusive, thus no pathogenic mutation had been identified despite a significant family history of breast cancer. We interviewed 12 health professionals who were involved in the care of such women and their families. We conducted three focus groups with thirteen women - those affected with breast cancer who had undergone testing, as well as their unaffected sisters/daughter who had not had any testing. We asked them questions regarding their understanding and reactions to the inconclusive test result of their affected sister/mother. The majority of the women had a good grasp of the meaning of their own, or a sister's inconclusive result, but a few indicated some misunderstanding. A difficult issue for professionals caring for such families relates to prophylactic surgery, mainly for the unaffected women, but also for the affected women. Affected women have an increased risk of contralateral breast cancer as well as ovarian cancer, if ovarian cancer is present in the family. The unaffected women in this study were mostly concerned about breast cancer risk and options. The professionals were sensitive to the difficulties in explaining an inconclusive result to the cancer patient who has had the test. Some felt frustrated that technology had not as yet provided them with a better tool for prediction of risk. For the most part, the professionals believed that they should support the women in whatever management decisions they consider, provided these decisions were based on a complete and accurate understanding of the current limitations of the genetic test. The Hospital's Clinical Research Committee and Local Regional Ethics Committee approved the study.

### 1466/T/Poster Board #15

**A different key mutation among their family members in Familiar Neurofibromatosis Type1.** *Y. Jo[1], H. Kim[1], H. Song[2], K. Yoon[1].* 1) Dept. of Biochemistry and Mol.biology(BK21 project),School of Medicine, kyungHee univ.,; 2) Dept. of Orthopedics, Korea Univ.,Guro hospital, Seoul, Korea.

Neurofibromatosis Type1 (NF1) is a one of the most common autosomal dominantly inherited disorder, and NF1 (MIN162200) gene maps to chromosome 17q11.2. The main clinical features od the disease are café-au-lait spots, cutaneous neurofibromas, hamartomas of the iris (Lisch nodules), optic or chiasma glioma, and pseudoarthrosis. Almost everyone who inherits the NF1 gene has clinical features of the disease by the age of five years. The majority of sporadic mutations in neurofibromatosis type1 arise in paternally inherited alleles. The aim of this study was to investigate the pathogenic genes related to NF1 in 15 families with typical NF1 patients. We have analyzed 15 families, as 37 samples. RNA samples from these families' peripheral blood ware analyzed using RT-PCR and nucleotide sequencing with 12 primer sets including whole coding region. All patients had the premature termination codon involved premature formed protein. About 46% patients, 17 of 37, had multi site deletions or both deletion and insertion mutations. , 10 families' mutations had same pattern mutations but almost had multi site mutation have not identity. In these families, some progeny has more deletion site (2 cases) or larger deletion (2 cases) and others had fewer deletions (8 cases) than their proband. 1 family, members are 2 sons and mother, has not shown the inheritance, but brothers are same mutation site. 1 family, members are 2 sons and father, has not shown the irregular inheritance, one has spontaneous mutation, another has inheritance from the father and same mutation, spontaneous mutation, form his brother. Interestingly, many family members (10 of 15 families) are little difference mutation site, and their key mutations, as the shortest premature formed mutation, are different between progeny and parents. In this series of familiar studies, we reported the novel mutation and different key mutation in the family members. Some inheritance genetic mutation diseases are not from their parents or probands, because NF1 genes have very high rates (50%) of spontaneous mutation. These mutations caused abnormal transcript and premature termination of the mutation alleles. And we find many exon skipping appearance for splicing errors. Then we provide new information for pathogenesis of NF1 patients and assist in identifying the NF1 mutations in NF families.

### 1467/T/Poster Board #16

**Real World Experience with Cancer Genetic Counseling via Telephone.** *R. Sutphen[1,2], B. Davila[1], H. Shappell[2], T. Holtje[1], S. Vadaparampil[1], S. Friedman[1,4], M. Toscano[3], J. Armstrong[3].* 1) Dept Med Gen, H Lee Moffitt Cancer Ctr, Tampa, FL; 2) Informed Medical Decisions, Inc; 3) Aetna, Inc; 4) FORCE: Facing Our Risk of Cancer Empowered.

**Purpose:** One barrier to cancer genetic counseling and testing is the lack of access to genetic counselors. Recent studies suggest that telephone genetic counseling may be an effective delivery model, but published experience to date is largely confined to research in academic centers. We provided cancer genetic counseling via telephone, through a pilot project for employees of Aetna, Inc., a national insurer with approximately 35,000 employees nationwide. Knowledge transfer, behavioral intentions, and patient satisfaction were assessed by survey after genetic counseling.

**Methods:** All Aetna employees received email notification of availability of cancer genetic counseling via telephone as a covered benefit and were provided a link to a brief online hereditary cancer questionnaire. Individuals identified as having potential risk for inherited cancer based on the screening questionnaire were invited to schedule a telephonic genetic counseling session conducted by board-certified genetic counselors employed by Informed Medical Decisions, Inc., a national genetic services provider. After the session, respondents were asked to complete an online survey.

**Results:** 397 individuals completed the questionnaire. 39 proceeded with telephone genetic counseling, and 22 completed the follow-up survey. One third reported prior discussion about inherited cancer risk with their primary care provider (PCP). Twenty percent had an accurate perception of their personal cancer risk at baseline. After counseling, 94% reported understanding of their risk for cancer and 87% were aware of available risk-reduction strategies. Among high-risk respondents, 87% intended to engage in risk-management interventions. 93% reported high satisfaction with the service provided. 66% indicated they would not have pursued genetic counseling if it was not available by phone.

**Conclusion:** Results suggest telephone counseling is a viable option for increasing access to genetic experts. Telephone counseling increases knowledge of cancer risk, motivates intention to change relevant health-related behaviors, and elicits a high satisfaction level. Consequently, Aetna now offers telephone cancer genetic counseling to its 16 million members nationwide as a covered benefit.

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## 1468/T/Poster Board #17

**Integration of genetic counseling and testing into a Parkinson disease clinic: Assessment of patient knowledge, attitudes, and interest.** *D. Clay Falcone[1], E. McCarty Wood[1], P. Ahimaz[2], V. Van Deerlin[1].* 1) Center for Neurodegenerative Disease Research, University of Pennsylvania, Philadelphia, PA; 2) Genetic Counseling Program, Arcadia University, Glenside, PA.

Parkinson disease (PD) is a neurodegenerative condition characterized by tremor, rigidity, postural instability, and bradykinesia. The most common genetic contributor to both familial and sporadic late-onset PD is the leucine-rich repeat kinase 2 (*LRRK2*) gene. The discovery of *LRRK2* has significantly changed the landscape for PD genetics and clinical *LRRK2* genetic testing is available. In order to effectively integrate *LRRK2* genetic testing into clinical practice, an effective strategy tailored to the PD population must be developed. We assessed 126 individuals with Parkinson disease for baseline knowledge of basic genetics principles and PD genetics, interest and opinions regarding genetic counseling and testing options, as well as levels of concern regarding a possible familial risk. A structured patient interview was developed after a literature review of previously published tools in the field of adult-onset genetic conditions and was administered to patients seen at the Parkinson Disease and Movement Disorders Clinic at Pennsylvania Hospital, Philadelphia, PA. Knowledge responses were graded based on both the correct response and demonstration of concept understanding. Participants had an average knowledge score of 58% (range 32%-92%). For concept understanding, the majority of participants demonstrated understanding of Parkinson disease, gene functions and mutations, and risk factor genes, but did not demonstrate an understanding of Parkinson disease genetics, such as *LRRK2*, and genetic testing concepts. Only 11% of respondents had heard of *LRRK2*; 32% had heard of genetic testing for PD. While 71% of respondents thought it was likely they had inherited a PD gene, only 41% thought it was likely other family members would develop PD. The majority (64%) of respondents did not have any concerns about genetic testing and 88% believed genetic testing for PD would be useful. Fifty-seven percent of respondents were interested in obtaining genetic testing for PD and 51% were interested in a genetic counseling program for PD. These results indicate that there is a significant level of interest in genetics and genetic testing options within the PD patient population, but also highlight a considerable deficit in genetics knowledge as well as an over-estimation of gene carrier status. Genetic education and counseling tools to address these needs were developed to provide PD patients with the ability to make informed and knowledge genetic testing decisions.

## 1469/T/Poster Board #18

**Effectiveness of a visual media presentation as a decision aid tool for pregnant women considering early prenatal screening tests.** *S. Demsey[1], D. Rada[2].* 1) Dept. of Genetics, Kaiser Permanente Med Ctr, Bellflower, CA; 2) Dept. of Genetics, Kaiser Permanente Med Ctr, Fontana, CA.

Pregnant women are faced with multiple complex choices for prenatal screening and diagnosis. Providers of prenatal care have only limited time to discuss the options with their patients. For this reason, an alternative method for educating patients was considered. A narrated and animated PowerPoint presentation (video) was created to give prenatal patients complex information about options of early screening and testing for birth defects. In addition to factual information regarding timing, methods and accuracy of the screening options, the video posed questions intended to allow the viewers to consider their personal values regarding diagnostic testing and risk factors to aid in their decision process. METHODS: The subject population was composed of women over 18 years of age who presented for prenatal care in the first trimester to two different OB clinics at our institution. The women who participated in the study were English speaking between the ages of 20-44 years, half of whom were over 35 years of age. Subjects were given a questionnaire to assess knowledge before and after viewing the video. Data was analyzed with paired t-test. RESULTS: 16/44 (36%) of respondents answered all questions correctly or answered only one question incorrectly prior to the presentation, indicating a high baseline level of knowledge. The average score of correct answers before watching the media presentation was 77%; the average score of correct answers after watching the video was 85% which represents a significant improvement (p=0.02). Before watching the video, 98% (43/44) of subjects could correctly identify that there is an increased risk of chromosome anomalies in pregnancies of women over 35 years of age. However, even with this knowledge, 75% of the women over age 35 considered themselves "low risk" for chromosome defects prior to the educational video. 5/18 (28%) women over age 35 changed their perception of risk to "high risk" after the informational presentation. Questions regarding the timing and risks of diagnostic testing were answered incorrectly most often both before and after watching the video.-CONCLUSIONS: Participants stated the content, tone and organization of the presentation was "helpful, clear and thought provoking". Clients present for prenatal screening with predetermined values and assumptions. Additional studies could assess how these values affect client choices and learning about prenatal screening and diagnostic testing.

## 1470/T/Poster Board #19

**The telephone clinic model facilitates rapid BRCA genetic testing for patients with advanced cancer who are eligible for PARP-inhibitor clinical trials.** *K. Kohut[1,2], K. Myhill[1], S. Thomas[1], E. Bancroft[1,3], P. Fong[1,3], S. Kaye[1,3], J. de Bono[1,3], T. Yap[1,3], R. Eeles[1,3], S. Shanley[1,3].* 1) Clinical Academic Cancer Genetics Unit, The Royal Marsden NHS Foundation Trust, London, United Kingdom, SM2 5PT; 2) Barts and The London School of Medicine, London, United Kingdom, EC1M 6BQ; 3) Drug Development Unit, Institute of Cancer Research, Royal Marsden Hospital, Sutton, SM2 5PT.

Introduction: Genetic testing can have a vital impact on cancer patient management with respect to surgical decision-making and, more recently, targeted chemotherapy options. The telephone clinic model was initially a pilot study to triage all new GP referrals to Cancer Genetics with the aim of offering a more flexible and efficient service in a setting and time span to best suit patient needs. This model has been adapted for urgent clinical care of patients eligible for a new form of chemotherapy in the context of a clinical trial. The PARP inhibitors are a class of drugs that specifically target DNA repair-deficient tumours such as those caused by an inherited mutation in the BRCA1 or BRCA2 gene. In such trials, there is a need to provide genetic testing and advice quickly for patients with advanced cancer who may benefit from targeted treatment. Alternative methods of counseling and testing need to be explored in order to meet the changing needs of cancer patients. Methods: New referrals were reviewed by senior staff and appointments for a telephone clinic consultation were arranged where appropriate. Results: Eighteen patients were referred to the Cancer Genetics Unit for genetic assessment as part of eligibility evaluation for for a Phase I trial of PARP inhbitors in advanced cancer. Seventeen were eligible for BRCA mutation analysis and of these, six were found to have a mutation and one to have a variant of uncertain significance. Results were provided within three weeks of referral, and generally, result provision was by telephone. Conclusions: Rapid genetic testing of the BRCA1 and BRCA2 genes is facilitated by the use of a telephone clinic and fast turn-around time from the laboratory. Rapid entry into a PARP inhibitor clinical trial was made possible. Patients were relieved to have access to a fast test and appreciated not having to attend additional in-person appointments when dealing with an advanced cancer diagnosis.

## 1471/T/Poster Board #20

**An internet-based approach to enhance genetic data discovery in ALS.** *C.A. Brownstein, T.E. Vaughan, M.P. Massagli, P. Wicks, J. Heywood.* Research and Development, PatientsLikeMe, Cambridge, MA.

Purpose: Patients with familial ALS (FALS) have a strong need to understand their illness and the impact of genetics on their outcomes. To meet this need, a novel disease-focused social network (PatientsLikeMe.com) was used to investigate the variability in clinical phenotype and disease progression due to different ALS-causing mutations, with the aim of sharing this information with patients. Methods: Genetic data-capture capabilities were added to PatientsLikeMe.com, and all active members of the ALS community were asked to submit their genetic mutation, if known. A genetics search engine was launched on the site so patients could identify others with the same mutation. Clinical features of the mutation-carrying cases were summarized and compared with the published literature. Results: Fifty percent of Familial ALS (N=227) patients responded to a request to tell PatientsLikeMe any genetic information they had on their disease, including "no mutations found". Eighteen community members with 12 different mutations submitted their genetic mutations and self-report clinical outcome scores. The most frequently reported mutations were SOD1 A4V (N=4), SOD1 D90A (N=4), and the VAPB mutation P56S (N=3). Preliminary analysis indicate that D90A patients lost 0.22 points per month on the self-report Amyotrophic Lateral Sclerosis Functional Rating Scale, Revised (ALSFRS-R)(SE 0.06, CI95%: -0.40, -0.05), SOD1 A4V patients lost an average of 1.2 points per month (SE 0.5, CI95%: -2.5, 0), and VAPB P56S mutation patients had an average loss of 0.30 points per month (SE 0.16, CI95%: -0.82, 0). By comparison, Sporadic ALS patients with no genetic information (N=1727) had a mean loss of 0.68 points per month (SE 0.01, CI95%: -0.71, -0.66). Conclusions: Even with a small sample size, trends rapidly become visible in ALS progression rates due to different mutations. Our hope is that sharing this information with patients who want it will help them plan for and anticipate future disease progression. Using the internet to aggregate groups of patients by specific mutation criteria allows for the international sharing of predictive outcomes based on historical data. This presents the possibility of real time distribution of new information to improve clinical understanding and communication. Making patients the driver of this communication medium means that they become educated and engaged, and can more effectively contribute to research and their own clinical management.

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## 1472/T/Poster Board #21

**The psychosocial impact on healthcare professionals managing fetal abnormality.** *M.A. Menezes[1,2], M. Sahhar[2,3], J. Hodgson[1,2], S.A. Metcalfe[1,2].* 1) Genetics Education & Health Research, Murdoch Childrens Research Institute Parkville, Victoria, Australia; 2) Department of Paediatrics, University of Melbourne Parkville, Victoria, Australia; 3) Genetic Health Services Victoria, Royal Children's Hospital Parkville, Victoria, Australia.

Approximately 4% of pregnancies in Victoria, Australia each year are diagnosed with a fetal abnormality. Healthcare professionals working in prenatal settings are often involved in the diagnosis and/or counseling of patients at risk of fetal abnormality who are facing a decision as to whether or not to continue their pregnancy. This potentially raises professional, ethical, moral and legal issues for healthcare professionals working in this field, and could lead to feelings of stress and/or 'burnout'. This study aims to explore the experiences of healthcare professionals who work with fetal abnormality and to identify any support systems currently used and/or needed. A qualitative approach was used in this study. Over 40 semi-structured, in-depth interviews have been conducted with healthcare professionals who work with fetal abnormality including clinical geneticists, genetic counselors, obstetricians, maternal fetal medicine specialists, midwives etc. Interviews were audiotaped, transcribed verbatim and analyzed using thematic analysis. Participants identified a range of challenging situations they encounter in their work. Most participants believe that working with fetal anomaly has an impact on their daily lives, particularly when pregnant themselves. Pregnancy presented a unique challenge for healthcare professionals and many reported an increased risk perception resulting in their being worried about their own pregnancy fairly early on. Participants reported breaking bad news to be one of the most difficult areas of their work. They found the act of breaking bad news to be difficult and reported that unprepared patients complicated this process. Genetic healthcare professionals were more comfortable with breaking bad news and reported having more formal training in this area. Many participants discussed at length the emotional nature of working with patients in crisis. Working with fetal abnormality appears to have a substantial impact on healthcare professionals, both personally and for the workforce as a whole. This study identifies that additional training on breaking bad news would be beneficial to healthcare professionals working with fetal abnormality. The training received by genetic healthcare professionals could be used as a model for non-genetic trained healthcare professionals working with fetal abnormality and support could be put in place, particularly when healthcare professionals are pregnant themselves.

## 1473/T/Poster Board #22

**HUNTER: Development of a mnemonic screening tool for Hunter Syndrome using data derived from the Hunter Outcome Survey (HOS).** *G.M. Cohn[1], I. Morin[2], D.A.H. Whiteman[1] on behalf of the HOS Investigators.* 1) Shire, HGT Cambridge, MA; 2) Global Outcome Surveys, Shire HGT, Cambridge, MA.

**Introduction**: Hunter syndrome (mucopolysaccharidosis II, MPS II) is a rare, X-linked multisystem disorder that is caused by a deficiency in the activity of the lysosomal enzyme, iduronate-2-sulfatase (I2S) that leads to the lysosomal accumulation of dermatan sulfate and heparan sulfate in affected organs. Signs and symptoms of Hunter syndrome generally develop during the first decade of life, though age of onset and rate of clinical progression are variable. A recent publication of Hunter Outcome Survey data suggests that the median age of diagnosis of MPS II is 3.5 years and that a 1.4-year median lag exists between the initial onset of signs and symptoms and eventual diagnosis. As newborn screening for MPSII is lacking, this delay in diagnosis may reflect a decreased awareness of this rare condition among primary care providers as well as the challenge of diagnosing this complex disease of diverse signs and symptoms. **Objectives**: To address the issue of delayed diagnosis we have developed a screening tool, HUNTER: H (Hernia, Hearing) U (Unusual facies) N (Nasal obstruction) T (Tongue & Tonsils enlarged) E (Enlarged liver & spleen) R (Respiratory compromise, Range of motion decreased) that is a weighted mnemonic score (score 0-16) based on prevalence data of the most common signs and symptoms found at diagnosis and recorded in HOS. **Results**: This screening tool was initially applied to data derived from a random sample of 25 patients enrolled in HOS, and following modification, was applied to data from a second random sample of 25 HOS patients. The modified screening tool correctly identified over 95% of the MPS II patients at a score (six) unlikely to identify individuals unaffected by a mucopolysaccharidosis. The modified tool was then applied to data of a larger cohort of 237 HOS patients. Over 95% of patients were again correctly identified using a score of six. The median (P10-P90) score for patients $< 2$, 2 to 4, and $\geq 4$ years old at diagnosis was 12 (7-16), 13 (8-16), and 13 (7-16), respectively. **Conclusion**: The HUNTER mnemonic had a sensitivity of over 95% at a score unlikely to identify individuals unaffected by MPS. Validation in a clinical setting is required to determine its utility as a reliable clinical screen for MPS II. This approach highlights the potential benefit of utilizing patient registry data in the development of tools that may assist physicians in the diagnosis and management of rare genetic disorders.

## 1474/T/Poster Board #23

**Two new bioinformatics tools to help in distinguish neutral variations from pathogenic mutations.** *G. Collod-Beroud[1,2], F-O. Desmet[1,2], M. Lalande[1,2], M.Y. Frederic[1,2], D. Hamroun[3], M. Claustres[1,2,3], C. Beroud[1,2,3].* 1) INSERM, U827, Montpellier, F-34000 France; 2) Université MONTPELLIER1, UFR Médecine, Montpellier, F-34000 France; 3) CHU Montpellier, Hôpital Arnaud de Villeneuve, Laboratoire de Génétique Moléculaire, Montpellier, F-34000 France.

Approximately, half of gene lesions responsible for human inherited diseases are due to an amino acid substitution, showing that this mutational mechanism plays a large role in diseases. In another hand, if these mutations usually have a direct impact on proteins, an increasing proportion is now believed to impact mRNA splicing and to be responsible for genetic diseases. They mostly involved donor or acceptor splice sites but synonymous, non-synonymous or even nonsense mutations can also create and/or disrupt splice sites or auxiliary splicing sequences. Being able to distinguish neutral sequence variations from those responsible for the phenotype is of major interest in human genetics. Since in vitro validation of mutations is not always possible in diagnostic settings, indirect arguments must be accumulated to define if an exonic or intronic variation is causative. To further differentiate harmless variants from pathogenic nucleotide substitutions, we developed two new tools: 1) The **UMD-predictor® tool** provides a combinatorial approach that includes: localization at the protein level, conservation, biochemical properties of the mutant and wild type residues and the potential impact of the variation on mRNA (http://www.umd.be). 2) The **Human Splicing Finder (HSF) web resource** is dedicated to the prediction of mutation's impact on splicing signals and/or the identification of these motifs in any human sequence. It contains all available matrices for auxiliary sequences prediction, new Position Weight Matrices to evaluate the strength of 5' and 3' splice sites as well as branch point sequences (http://www.umd.be/HSF/ ). These tools have been extensively tested on a thousand of exonic and intronic mutations from more than 15 genes and have given very accurate predictions (99.4% positive predictive value, 95.4% sensitivity and 92.2% specificity for exonic mutations; accurate predictions of 93% of branch point inactivating mutations; accurate prediction of all pathogenic mutations affecting donor or acceptor splice sites including +3 and +4 positions; accurate identification of auxiliary splicing motifs when available). These two tools could therefore help to rapidly scan for pathogenic mutations both for clinical and research purposes. They could be used to filter the numerous variations produced by large scale sequencing research programs such as the new solid sequencing technologies.

## 1475/T/Poster Board #24

**The inbred Bedouin population of southern Israel: from disease gene identification to massive carrier screening.** *O.S. Birk[1,2].* 1) Genetics Inst, Soroka Med Ctr, Beer-Sheva, Israel; 2) Morris Kahn Laboratory of Human Genetics, National Institute for Biotechnology in the Negev, Ben Gurion University, Beer-sheva, Israel.

The Bedouin population of southern Israel (~170,000) is unique in its combination of extreme inbreeding within tribes (~60% first or second cousin marriages) and an average of 8-9 children per couple, with many males having two or three wives. As a consequence, there is an extremely high incidence of both common and novel autosomal recessive diseases, taking a dramatic toll on this community. We have embarked on a massive research initiative leading to the discovery of 10 novel disease genes over the past 5 years, as well as several dozen mutations in previously known disease genes. The research is done in-house using cutting edge technology, with a major part of the team designing our own software for SNP data analysis as well as for selection of candidate genes from within a disease-associated genomic locus. Some of the severe neurological diseases whose molecular basis we have recently deciphered include lethal congenital contractural syndrome (arthrogryposis) types II and III, mitochondrial complex 3 deficiency, infantile neuroaxonal dystrophy and the novel genomic-imprinting Birk Barel syndrome. Based on our findings, we initiated a government-financed free carrier testing operation, making use of the existing network of maternity clinics. A significant effort has been made also in educational programs from highschool through adulthood. The combination of the massive research initiative leading to the massive free carrier testing had a major impact, with a dramatic fall in infant mortality in this community from 17/1000 to 8/1000 over the past 3 years. As the mutations we find are often common to other Arab populations, we are now seeking collaborative projects to share our experience with others to the benefit of Arab communities elsewhere.

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## 1476/T/Poster Board #25

**Manchester Project to Eradicate Sickle Cell Disease.** *I.H. Ejebe[1], F. Gibson1[1], K. Mason[1], G. Serjeant[1, 2].* 1) Sickle Cell Trust Kingston, Jamaica; 2) Southern Regional Health Authority Ministry of Health.

Sickle cell disease (SCD) results from the inheritance of abnormal hemoglobin genes from both parents, and its frequency at birth is determined by the frequency of these genes in the population. In Jamaica, these abnormal genes are the sickle cell (HbS) trait in 10%, the HbC trait in 3.5% and the beta thalassaemia trait in 1.5%. Therefore 15% or 375,000 Jamaican are at risk of having a child with a type of SCD which occurs once in every 150 births. Can any of these births be prevented? In Bahrain, a programme of voluntary screening in place for the last 10 years, has halved the frequency of births with SS disease. The Manchester Project is a 5-year pilot project of school education and student screening coupled with newborn screening in the Manchester Parish of Jamaica. Gel electrophoresis and HPLC are used to detect abnormal hemoglobin. The newborn screening project began in August 2008 in three different Manchester hospitals and has screened over 2,700 babies (91% coverage) and detected 20 babies with sickle cell disease. A survey of 100 mothers at the Mandeville Hospital maternity ward revealed over 95% of the mothers felt that this free service was important for their child. The student-screening program couples sickle cell disease education with optional screening and genetic counseling. The program has screened 5th and 6th form students in 15 different secondary schools in the Manchester Parish. In the 2007-2009 school years 4,748 students have been screened (75% coverage) with the detection of 691 abnormal hemoglobin types. A survey of students that opted out of the test showed that fear of the pain of the needle was a significant reason opting out of testing.

## 1477/T/Poster Board #26

**Development of a Rapid Genetic Screening Panel for the Southern Californian Persian Jewish Community.** *C.A. Riley-Portuges[1], J.R. Lopategui[1], M.M. Kaback[3], D.L. Rimoin[2].* 1) Pathology, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 3) Department of Pediatrics, University of California, San Diego, San Diego, CA.

Introduction: Genetic screening for mutations common in the Ashkenazi Jewish population is widely accepted, but has not been developed for Persian Jews (PJ), despite their having a number of unique prevalent founder mutations. We developed SNP detection assays for 3 common PJ diseases with known founder mutations. : Hereditary Inclusion Body Myopathy (HIBM, mutation: M712T); Autoimmune Polyendocrinopathy Syndrome Type I (AIRE, mutation: Y85C); and Pseudocholinesterase deficiency (BCHE, mutation: D70G). These conditions are considered appropriate as potential candidates for community- based screening as each of these conditions is either avoidable (BCHE), treatable (AIRE) or preventable (HIBM). In Israel, carrier frequencies among PJ are reported as: 1:12 for HIBM; 1:50 for AIRE; and 1:9 for BCHE. PJ residing in the United States may have slightly different frequencies due to differences in migration patterns. To verify the presence of these mutations in the Southern California PJ community, and to evaluate the feasibility of targeted community-based screening, we genotyped a group of 86 asymptomatic persons of Middle Eastern Jewish descent referred for Tay Sachs carrier testing and 111 individuals previously electing HIBM carrier screening. Methods: 86 anonymous patients of Middle Eastern Jewish descent had genomic DNA isolated using Qiagen QIAamp kit. An additional 111 DNA specimens were provided by HIBM Research Group (HRG, Encino, CA), 11 of who were homozygous for HIBM. DNA was quantitated by spectrophotometry and normalized to 25 ng/μl. Each SNP was analyzed using a single primer pair and wild type and mutant allele specific probes in Quantitect Probe master mix (Qiagen, Valencia, CA). Results: All samples yielded interpretable genotypes for the 3 SNPs tested. Of 197 total samples tested, 18/197 were identified as heterozygotes (het) for HIBM; (2/86 and 16/111 in the 2 sample sets); 5/197 were het for AIRE (2/86 and 3/111 in the 2 groups); and 17/197 were mutation positive for BCHE (8/86 and 9/111 in the 2 groups). Conclusions: Our results demonstrate the feasibility of PJ carrier screening for HIBM, AIRE and BCHE in the Southern California PJ community. We are planning a large population survey, which will allow a more accurate determination of the heterozygote frequencies of these and other conditions in this population, without ascertainment bias, and where interventions are feasible.

## 1478/T/Poster Board #27

**Susceptibility mutations and aminoglycoside-induced deafness in high-risk newborns.** *E. Sartorato[1], P. Andrade[1], J. Bergmann[1], V. Moraes[1], M. Azevedo[3], M. Câmara[2].* 1) CBMEG, Universidade Estadual de Campinas, Campinas, SP, Brazil; 2) Departamento de Fonoaudiologa, Universidade de Fortaleza, UNIFOR, Fortaleza, CE, Brazil; 3) Departamento de Fonoaudiologia, Universidade Federal de São Paulo, SP, Brazil.

An early diagnosis has been a priority in the audiological practice. Identifying the hearing loss until 3 months old and make an intervention before 6 months old minimizes the impact of auditory loss in the health and communication development of these children. Nonsyndromic sensorioneural hearing loss (NSHL) affects 1 in 1000 births. The prevalence of profound bilateral deafness elevates from 1 per 1000 newborns to 20-40 per 1000 when neonatal intensive care unit (NICU) newborns are considered. Mutations in the mitochondrial DNA have also been found to be associated with NSHL and some of them with aminoglycoside-induced deafness. The aims of this work were to determine the etiology of the hearing loss in children admitted into Neonatal Intensive Care Unit (NICU) and compare with other factors, mainly ototoxic medication. The subjects were divided in four groups in order to elucidate the etiology of their hearing impairment and the bases of aminoglycoside ototoxicity. Group A - 25 newborns at risk with aminoglycoside-induced and sensorineural non-syndromic hearing impairment. Group B - 25 newborns at risk with aminoglycoside-induced and normal hearing. Group C - 25 full term newborns with sensorineural non-syndromic deafness and no history of ototoxy medication. Group D - 25 full term newborns with normal hearing and no history of ototoxy medication. All groups were screened for A7445G, G7444A, A1555G, C1494T, A827G, T961G and 961delT/insC mitochondrial mutations, del(GJB6-D13S1830) and del(GJB6-D13S1854) in the GJB6 gene, and mutations in the GJB2 gene. Our findings suggest that in these cases the deafness is not related to susceptibility mutations and ototoxy medication in NICU pre-term newborns.

## 1479/T/Poster Board #28

**Screening Hemoglobinopathies by High Performance Liquid Chromatography in a Filipino population.** *C.L.T. Silao[1,2], M.L.T. Naranjo[3], M.L.E. Leporgo[3], C.D. Padilla[1,2], E.dJ. Yuson[3].* 1) Institute of Human Genetics, National Institutes of Health Philippines, University of the Philippines Manila; 2) Department of Pediatrics, College of Medicine-Philippine General Hospital, University of the Philippines Manila; 3) Thalassemia Center of the Philippines, Dr. Fe Del Mundo Medical Center, Manila, Philippines.

Background: The hemoglobinopathies are a heterogeneous group of congenital hemolytic anemias, which include hemoglobin (Hb) variants, thalassemia and hereditary persistence of fetal hemoglobin. Thalassemias and hemoglobinopathies, a group of autosomal recessive inherited human disorders, are prevalent in many parts of the world. The automated cation-exchange High Performance Liquid Chromatography (HPLC) system is a sensitive and precise method for detecting thalassemia and abnormal Hbs. A pilot study using this system was conducted to determine the prevalence of hemoglobinopathies in the Philippines. Methods: Blood samples from two hundred eighty-five (285) randomly selected healthy individuals and individuals suspected to have thalassemia or a particular hemoglobinopathy, irrespective of age and gender, were collected during the local celebration of World Thalassemia Day in May 2008 and were subsequently screened for thalassemias and hemoglobinopathies using the HPLC system (VARIANT TM, BioRad). Results: Of the 285 samples, 35 (12.3%) were shown to have beta thalassemia with high A2, 19 (6.6%) had beta thalassemia with normal A2, and 14 (4.9%) had beta thalassemia with E interacting. Four (4) subjects were found to be heterozygous E and one (1) subject was found to be homozygous E. Interestingly, 3% of the samples yielded results suggestive of alpha thalassemia. Conclusion: A significant proportion of thalassemias among randomly selected Filipino subjects were noted. The HPLC method was found to essential for rapid screening of beta thalassemia and hemoglobinopathies. This is important for proper clinical management/counseling and for early and precise diagnosis.

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## 1480/T/Poster Board #29

**Combination of SMN2 copy number and NAIP deletion predicts disease severity of spinal muscular atrophy in Northern China.** *Y. Zhang, Y. Luo, L. Cao, S. Wang.* Medical Genomics, China Medical University, Shenyang, Liaoning, China.

OBJECTIVE: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by decreased levels of survival motor neuron protein (SMN). In the majority of cases, this decrease is due to absence of the SMN1 gene. The SMN2 gene is highly homologous to SMN1 and has been reported to be correlated with severity of the disease. The clinical presentation of SMA varies from severe to mild, with four clinical subtypes (type I, type II, type III and type IV) that are assigned according to age of onset and severity of the disease. In this study, we aim to investigate the potential association between the number of copies of SMN2 and the deletion in the NAIP gene with the clinical severity of SMA in patients of Northern Chinese origin. METHODS: Multiplex ligation-dependent probe amplification (MLPA) is a modern quantitative molecular method. Applied in SMA cases, it improves diagnostics by simultaneously identifying the number of copies of several target sequences in the SMN1 gene and in nearby genes. Forty-one probable SMA cases were examined by MLPA and those carrying deletions of the SMN1 gene were enrolled in the study. Altogether thirty-one SMA patients (3 of type I, 24 type II, 3 type III and 1 type IV) were selected. SMN2 and NAIP copy number were determined by MLPA. RESULTS: One type I patient carried one copy of SMN2, while the other two carried 1.5 copies. Among the type II SMA patients, 8 percent (2/24) of cases carried 1.5 copies of the gene, while 92 percent (22/24) carried 2 or 2.5 copies of SMN2. All the type III patients carried three copies and the asymptomatic type IV patient carried four copies. Deletion analysis of NAIP showed that two type I SMA patients had a homozygous deletion of this gene, while the other one had a heterozygous deletion. Only one out of 24 type II SMA cases carried a homozygous deletion, while all the other type II patients had a heterozygous deletion. All type III patients carried intact copies of the NAIP gene. CONCLUSION: There exists a close relationship between SMN2 copy number and SMA disease severity, suggesting that the determination of SMN2 copy number may be a good predictor of SMA disease type. Furthermore, NAIP gene deletion was found to be associated with SMA severity. In conclusion, combining the analysis of deletion of NAIP with the assessment of SMN2 copy number increases the value of this tool in predicting the severity of SMA.

## 1481/T/Poster Board #30

**Mutation analysis of ATP7B gene in Korean patients with Wilson disease.** *B.Y Kim[1,2], C.H. Lee[2], E.S. Park[2], J.S. Lee[2].* 1) BK 21 Project for Medcal Sci, Graduate Sch Yonsei Univ, Seoul, Korea; 2) Dept Clinical Genetics, Yonsei Univ Col Medicine, Seoul, Korea.

Wilson disease is an autosomal recessive disorder of copper transport that results in accumulation of copper primarily in the liver, the brain and the cornea. The incidence of the disease is between 1 in 35,000 and 1 in 100,000 live births, and caused by mutations in the ATP7B gene. The ATP7B gene encodes for a membrane copper transport protein. In this study, we examined the ATP7B gene in 61 Korean patients with Wilson disease. Genomic DNA was obtained from peripheral leukocytes and coding regions of the ATP7B gene were amplified using PCR followed by direct sequencing. We have identified 11 different mutations in 28 Wilson disease patients: Gln717Term, Arg778Leu, Ala874Val, Thr1029Ile, Gly1035Val, Leu1083-Phe, Val1216Met, Asn1270Ser, c.2304dupC, c.2695_2721del27bp, IVS13+5G>C. The Arg778Leu mutation is the most common mutation in Korean patients with Wilson disease. Eleven different mutations were detected in exon 8 (Gln717Term, c.2304dupC, Arg778Leu), exon11 (c.2695_2721del27bp, Ala874Val), exon14 (Thr1029Ile, Gly1035Val), exon15 (Leu1083Phe), exon17 (Val1216Met), exon18 (Asn1270Ser) and intron 13.

## 1482/T/Poster Board #31

**Bloodspot based genetic testing identifies a high prevalence of bi-allelic *GJB2* mutations from infants who fail newborn hearing screening.** *L.A. Schimmenti[1], B. Warman[1], A.M. Jurek[2], K.A. Daly[3], M.R. Schleiss[2], J.A. Ross[2], S.A. Berry[1], M. McCann[4].* 1) Department of Pediatrics, Institute of Human Genetics, University of MInnesota, Minneapolis, MN; 2) Department of Pediatrics, University of MInnesota, Minneapolis, MN; 3) Department of Otolaryngology, University of Minnesota, Minneapolis, MN; 4) Minnesota Department of Public Health, St. Paul, MN.

**Background:** Newborn Hearing Screening (NHS) is performed at the infants's bedside with automated devices that test functional hearing. Despite nearly universal screening, about half of all infants who fail NHS do not return for follow-up evaluation. To determine the prevalence of bi-allelic *GJB2* mutations in bloodspots from infants who fail and pass NHS, we performed a pilot study using anonymous residual newborn screening bloodspots. We tested the hypothesis that genetic screening would detect a greater prevalence of bi-allelic *GJB2* mutations in bloodspots from infants who fail than in a control group of bloodspots from infants who pass NHS. We also tested the hypothesis that we would identify bloodspots from infants with bi-allelic mutations in *GJB2* that escape detection by NHS. **Methods:** We performed a case-control study comparing the prevalence *GJB2* mutations from anonymized bloodspots. Over a 22-month period, we analyzed 1177 bloodspots from infants who failed NHS in both ears and 1177 contemporaneously selected control bloodspots from infants who passed NHS. Mutation analysis was performed by allele specific melt curve analysis for common mutations 35delG, 167delT, 235delC, and V37I. Bi-directional sequencing of *GJB2* exon 2 was performed on samples identified to have one mutant allele by melt curve analysis, an abnormal melt curve, or samples from ethnic groups where common mutations are not typically identified. This study was performed with the approval of the Institutional Review Boards of both the University of Minnesota and the Minnesota Department of Health. **Results:** The prevalence of bi-allelic *GJB2* mutations in a cohort of bloodspots from infants who failed NHS was 22 in 1177 while the prevalence of bi-allelic *GJB2* mutations from a control group of spots was 2 in 1177 ($p<0.0001$). **Conclusions:** There are a significant number of bloodspots from infants who fail NHS harboring bi-allelic *GJB2* mutations. This high prevalence emphasizes the need for follow-up of all infants who fail NHS as nearly 1 in 50 will have bi-allelic mutations in *GJB2*. Bi-allelic *GJB2* mutations were identified in bloodspots from infants who pass NHS. These results demonstrate that infants who pass NHS could still be at risk for hearing loss and that molecular screening approaches may be preferable to functional NHS for identification of genetic etiologies of hearing loss. *Funding: March of Dimes.*

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## 1483/T/Poster Board #32

**Molecular Results and Deaf Community Identification in GJB2-Associated Deafness: Relationship Between DNA and Culture.** *J.L. Deignan[1], P. Boudreault[2], E. Baldwin[3], M. Fox[4], Y. Kobayashi[2], J.J. Zhou[5], J.S. Sinsheimer[5], W.W. Grody[4,5,6], C.G.S. Palmer[3,5].* 1) Medical Genetics Institute, Cedars-Sinai, Los Angeles, CA; 2) Deaf Studies Department, CSU Northridge, Northridge, CA; 3) Department of Psychiatry and Biobehavioral Sciences, UCLA, Los Angeles, CA; 4) Department of Pediatrics, UCLA, Los Angeles, CA; 5) Department of Human Genetics, UCLA, Los Angeles, CA; 6) Department of Pathology, UCLA, Los Angeles, CA.

The most common genetic cause of nonsyndromic sensorineural deafness worldwide is sequence variation in the GJB2 gene, encoding the protein connexin 26. Around 100 protein truncating (T) and non-protein truncating (NT) GJB2 variants have been described, with truncating variants more likely to result in audiograms in the severe-profound range compared to non-truncating variants. The recent availability of genetic testing for deafness has caused some concern within the Deaf Community since deafness is not considered a disability but is one component that defines themselves, their families, and their culture. An affiliation with either the Hearing or Deaf Community is an important aspect of cultural identity, and many factors participate in shaping this affiliation. One aspect that has not been previously analyzed is how genetic variants for deafness relate to a deaf individual's affiliated community. In our cohort of 202 deaf and hard-of-hearing adults drawn from regions of California, we sequenced exons 1 and 2 of GJB2, tested for a common deletion in the GJB6 gene, and assessed cultural affiliation using questionnaires. A majority (55%) of our subjects reported an affiliation with the Deaf Community, 9% with the Hearing Community, and 34% with both communities; 85% self-identified as deaf, 10% as hard-of-hearing, and 5% as hearing impaired. The frequency of truncating alleles was 38%, with 35delG being seen in 30% of Caucasians (78% of our sample), 9% in Hispanics, and 0% in other ethnic groups, while each of the other allele frequencies fell below 8%. We identified two novel variants (delF141, -3177C>T), and the GJB6 deletion was rarely observed (0.5% of alleles). Biallelic GJB2 variants were found in 40% of our subjects; the most common genotype was 35delG homozygosity (18%). The genotypes of those with biallelic GJB2 variants were categorized based on protein truncation [T/T (87%), T/NT (9%), NT/NT (4%)], and a homozygous truncating genotype was found to be significantly correlated with both a self identification as deaf (FE $p=0.006$, $n=79$) and a preferential identification with the Deaf Community (FE $p<0.001$, $n=79$). This is the first study to empirically associate the functional nature of an individual's deafness-causing variants as a factor related to both self identification and community affiliation, which underscores the importance of fully examining the ethical and social issues raised by genetic testing for deafness.

## 1484/T/Poster Board #33

**Prenatal genetic counseling in fetuses with mosaic 45,X/isodicentric Y. Is this Turner syndrome?** *C.L. Blout[1], S.E. Hickey[1], B.D. Rink[1,2], J.F. Atkin[1,2].* 1) Dept Gen, Nationwide Children's Hosp, Columbus, OH; 2) The Ohio State University, Columbus, OH.

Isodicentric Y is a common structural abnormality of the Y chromosome. Individuals with this condition often have a 45,X (Turner syndrome) cell line in addition to their isodicentric Y due to the unstable nature of an isodicentric chromosome. Depending on the nature of the structural abnormality and the level of mosaicism individuals can present as normal males, normal females, or ambiguous genitalia with or without a Turner-like phenotype.

We present three families with isodicentric Y mosaicism, including monozygotic twins, who were prenatally ascertained with ultrasound abnormalities. Their prenatal diagnosis and postnatal outcomes illustrate the complexity of counseling patients with this condition.

Family 1: Baby prenatally diagnosed with hypoplastic left heart syndrome (HLHS). Amniocentesis revealed an abnormal karyotype ultimately confirmed to be mosaic 45,X/isodicentric Y. Clinical evaluation confirmed female gender with absence of other Turner stigmata. Family 2: Monochorionic, diamniotic twin gestation with both fetuses appearing female by ultrasound at 18 weeks gestation. Twin A presented with a cystic hygroma, heart defect, club feet and developed hydrops with intrauterine fetal demise around 28 weeks. Twin B had no known prenatal anomalies detected until the labia appeared edematous during a 33 week ultrasound. Postnatally the neonate demonstrated ambiguous genitalia with palpable but undescended gonads and hypospadias. Karyotype revealed mosaic 45,X/isodicentric Y. The parents identified Twin A as female and elected male gender assignment for twin B. Family 3: Baby prenatally diagnosed with HLHS, mitral valve stenosis, and ambiguous genitalia. Amniocentesis revealed an abnormal karyotype of mosaic 45,X/isodicentric Y. Neonatal exam confirmed male gender with bilateral cryptorchidism.

Our families represent the gender spectrum associated with this complex karyotype which makes prenatal genetic counseling difficult. Presentation may be consistent with clinical Turner syndrome, but gender assignment should not be made until the baby is evaluated after birth with the help of an experienced team of subspecialist physicians.

## 1485/T/Poster Board #34

**Array CGH characterizes abnormal prenatal karyotypes and identifies chromosome imbalances missed by conventional cytogenetics.** *J. Coppinger, B.A. Bejjani, R. Schultz, L.G. Shaffer, B.A. Torchia, A.N. Lamb.* Signature Genomic Lab, Spokane, WA.

Microarray-based CGH (aCGH) has become a routine test in the neonatal/postnatal setting for the characterization of suspected genetic disorders. The use of this technology in a prenatal setting, however, has been limited. We performed aCGH on 68 prenatal cases that had previous findings of either abnormal karyotype or abnormal FISH. Sample types included 54 cultured amniotic fluids (79.4%), nine cultured CVSs (13.2%), four direct amniotic fluids (5.9%), and one DNA specimen from cultured CVS (1.4%). aCGH characterized all 20 cases submitted with previous findings of aneuploidy, suspected deletions or additions, suspected variant 16p or unbalanced translocations detected by karyotyping and/or FISH. In addition, aCGH identified unexpected clinically significant imbalances in three of these cases. Nearly half of the specimens were submitted because of full or mosaic marker chromosomes (45.6%). Of these, aCGH identified and further characterized the marker chromosome in 7/13 non-mosaic cases, and in 7/18 mosaic cases. The marker chromosome cases not identified are interpreted as composed of heterochromatin, regions not represented on the array or undetectable due to the low level of mosaicism. Benign CNVs (7.4%) and results of unclear significance (2.9%) were found with equal frequencies on the BAC-based and oligo-based platforms. Our data demonstrate that array CGH is an important diagnostic tool to further characterize chromosome imbalances identified prenatally by karyotyping or FISH, and to identify clinically significant imbalances that conventional cytogenetics did not detect. In many circumstances, such as cases with non-mosaic marker chromosomes, a normal aCGH result can provide added reassurance that the abnormality is either balanced or composed of heterochromatic material. Pre-test genetic counseling is critical to prepare parents for the possibility of normal aCGH results, or for abnormal clinically significant results that may or may not be related to the chromosome abnormality detected by karyotyping or FISH, or for results of unclear clinical significance. In some cases pregnancy outcome may be difficult to predict even if aCGH further characterizes the chromosome abnormality.

## 1486/T/Poster Board #35

**A severe congenital brain disorder explained by microarray.** *R. Forbes[1], R. Leventer[3,2], G. McGillivray[1,2].* 1) Genetic Health Services, Royal Children's Hospital, Victoria, Australia; 2) Murdoch Children's Research Instiute, Royal Children's Hospital, Melbourne, Aust; 3) Dept Neurology, Royal Children's Hospital, Melbourne, Aust.

We describe the clinical phenotype and genetic counselling issues in a female patient with a del(5)(q31.2q31.3) microdeletion. She presented in the neonatal period after term delivery with marked central hypotonia, poor suck, abnormal movements, hiccough and an encephalopathy with altered level of consciousness. She has mild dysmorphic features, profound motor and intellectual disability, visual impairment with enlarged optic discs and dystonic movements and posturing. The initial MRI brain scan showed immature frontal gyration and abnormal white matter. Subsequent scans showed cystic lesions of the basal ganglia and internal capsule, delayed myelination and cerebral atrophy. Developmental assessment at two years was consistent with profound motor and intellectual disability, but not regression. Multiple metabolic investigations including mitochondrial biopsies were normal. SNP microarray (Affymetrix SNP 6.0) subsequently diagnosed a 3.36 Mb microdeletion of 5q containing 89 known genes. We discuss family issues and psychosocial impact of this diagnostic information.

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## 1487/T/Poster Board #36

**Novel and recurrent mutations in the *NF1* gene in 44 Korean patients with neurofibromatosis type 1.** *H. Kim[1,2], J.M. Ko[1,2], G.N. Baek[1], Y.C. Kim[3], L.M. Messiaen[4].* 1) Ctr Gen Disease, Ajou Univ Hosp, Suwon, Korea; 2) Dept of Medical Genetics, Ajou Univ Hosp, Suwon, Korea; 3) Dept of Dermatology, Ajou Univ Hosp, Suwon, Korea; 4) Dept of Medical Genetics, University of Alabama at Birmingham, Birmingham, Alabama, USA.

Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant disorders in humans. NF1 is caused by mutations in the *NF1* gene. Mutation detection is complex due to the large size of *NF1* gene, the presence of pseudogenes and the great variety of lesions. Numerous studies have looked at genotype-phenotype correlation, however, there was no obvious genotype-phenotype correlation has been found in NF1. In order to assess the *NF1* mutation spectrum and genotype-phenotype relationship in Korean NF1 patients, we screened 48 (16 for familial and 32 for sporadic cases) unrelated Korean patients for mutations in the *NF1* gene. The mean age of diagnosis was 22.5 yr (range; 2 - 45 yr) in familial cases and 14.7 yr (range 0.2-58 yr) in sporadic cases. Mutation analysis of the entire coding region and flanking splice sites was carried out, and included the use of a combination of FISH and MLPA. Thirty nine distinct *NF1* mutations were identified in 44 (91.7%) patients. Causative mutations were detected in all patients having positive family history, and we could not find any mutations in 4 of 32 patients without family history. The mutations included 22 single base substitutions (9 missense and 13 nonsense), 10 splice site mutations, 3 small deletions, 2 small insertions, and 2 large deletions. Twenty (51.3%) mutations have been previously reported in other studies and 19 (48.7%) mutations were novel. c.6792C>A was found in three patients, and c.2033_2034insC, c.4537C>T, c.5546G>A were identified in each two patients. These four recurrent mutations were already reported mutations. The mutations are evenly distributed across exon 3 through intron 47 of the *NF1* gene and no mutational hot spots were found. The mutation spectrum shown in our study was similar to those of other reports performed in different ethnic background. This analysis revealed a wide spectrum of *NF1* mutations in Korean patients. A genotype-phenotype correlation analysis suggests that there is no clear relationship between specific *NF1* mutations and clinical features of the disease. As technologies advance in molecular genetics, mutation detection rate will become higher. Considering that about 50% of detected mutations were novel, exhaustive mutation analysis of the *NF1* seems to be one of the important tools in early diagnosis and genetic counseling, especially in young NF1 patients without family history.

## 1488/T/Poster Board #37

**Identification of a putative region associated with primary microcephaly through SNP array analysis in a consanguineous family.** *J. Wiszniewska, S-H.L. Kang, R. Pace, C.M. Eng, A. Patel, C.A. Bacino.* Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Chromosomal Microarray Analysis (CMA) is a sensitive method for the detection of genomic gains and losses, and a widely available clinical tool to help establish a diagnosis. Recent implementation of SNP arrays in clinical diagnostics allows not only for the detection of copy number changes but also enables identification of copy neutral changes such as uniparental disomy, and absence of heterozygosity (AOH) that occur through identity by descent. It may also help elucidate rare genetic syndromes due to recessive conditions in consanguineous families. Here we describe two male siblings, from a consanguineous family who presented with identical phenotypes including congenital microcephaly. The two brothers were evaluated because of severe microcephaly and moderate to severe developmental delay. The family history reveals that both parents were consanguineous, first cousins once removed. The 8 yr old has a head circumference (FOC) of 45.5 cm (below the 3rd centile, 50th centile for 9 months). He can follow simple commands, but has few words and is not toilet trained. The 5yr old brother had an FOC at 16 months of age of 39.5 cm (less than 3rd centile, 50th centile for 2 1/2 months). He has moderate developmental delay. Extensive genetic and metabolic testing was undertaken and failed to identify an underlying genetic condition. Subsequently, both siblings were analyzed by a SNP array (Illumina 610Quad) in order to identify common regions of absence of heterozygosity. SNP array did not detect any abnormalities in copy number however continuous regions of copy neutral AOH were present on several chromosomes in both children and were consistent with parental consanguinity. Only two regions of AOH showed overlap in both siblings, approximately 14Mb on 9q12-q21.21 and approximately 15Mb on 19q12-q13.31. Interestingly, the region of AOH on chromosome19 includes a previously mapped primary microcephaly (MCPH2) locus. Primary microcephaly (MCPH) is a genetically heterogeneous autosomal recessive neurodevelopmental disorder that is linked to at least 7 loci of which four MCPH genes have been identified thus far. Given the AOH was found in both siblings, it is possible that both are homozygous for a mutation in yet unidentified MCPH2 gene mapping to this region. These results demonstrate that analysis by SNP arrays is a useful clinical tool to narrow the region of interest and can be potentially valuable for gene discovery.

## 1489/T/Poster Board #38

**Pena-Shokeir syndrome : uncommon ins/del mutation in RAPSN gene.** *V. Benoit[1], U. Ullmann[2], C. Verellen-Dumoulin[1], P. Hilbert[1].* 1) Human Genetic Center, Inst Pathology & Gen, Charleroi, Belgium; 2) Centrum Medische Genetica, Universitair Ziekenhuis, Brussel, Belgium.

Pena-Shokeir syndrome is a rare early lethal autosomal recessive disorder mainly characterized by neurogenic arthrogryposis, facial and brain anomalies, polyhydramnios and lung hypoplasia. This syndrome was first described to be a deformation sequence caused by a reduction of foetal movements in the uterus (FADS, foetal akinesia deformation sequence). These manifestations are usually severe, and death occurs during pregnancy, at birth or in the neonatal period of life. Mutations in the RAPSN gene are usually associated with congenital myasthenic syndrome but reports also underlined RAPSN mutations in Pena-Shokeir syndrome We reported on the identification of a new causing mutation in the RASPN gene for two Pena-Shokeir affected male foetuses from consanguineous parents (first cousins). The first foetus died in utero at 29 weeks of gestation. He had generalized oedema, overlapping fingers, bilateral pulmonary hypoplasia and bilateral pleural effusion. An amniocentesis was performed and the karyotype results were normal. DNA material was kept for additional tests. During a second pregnancy, male foetus presented at 23 weeks similar signs. This second gestation was terminated and the couple refused any sampling. Having a suspicious diagnosis of Pena-Shokeir syndrome, we collected DNA samples from the parents and we sequenced the RAPSN gene. We were able to identify an heterozygous c.149-153del ins24 (p.Val50fs) mutation for both samples. We then tested the DNA extracted from the amniocytes collected during the first pregnancy and found a homozygous c.149-153del ins24 mutation resulting in a truncated protein along with maternal DNA contamination. This strongly suggested that this Pena-Shokeir syndrome was the result of a very severe form of congenital myasthenic syndrome. The mutation we found is very uncommon and, to our knowledge, it is the first time that an ins/del mutation is described in the RAPSN gene. Moreover, our data clearly confirm that sequencing of the RAPSN gene must be considered in case of Pena-Shokeir syndrome.

## 1490/T/Poster Board #39

**A NOVEL QF-PCR BASED ASSAY FOR DETECTION OF MECP2 GENE DELETIONS AND DUPLICATIONS.** *S.P. Rivera, M. Peng, R. Owen, A. Buller, F. Quan, A. Rebuyon, W. Sun, C. Strom.* Molecular Genetics, Quest Diagnostics, Nichols Institute, San Juan Capistrano, CA.

PURPOSE Rett syndrome (RTT) is an X-linked dominant, progressive neurological disorder caused by mutations in the gene for methyl-CpG-binding protein 2 (MECP2). Deletions/duplications account for approximately 16% of classic RTT and 7% non-classical RTT female patients. Males with MECP2 deletion are rare but duplications have been found in males with severe mental retardation. Direct sequence analysis of the MECP2 gene has been offered at Quest Diagnostics Nichols Institute to aide the diagnosis of RTT. In order to offer a comprehensive MECP2 gene test for classic and non-classic RTT, and nonsyndromic X-linked mental retardation disorders as well as for the recently defined MECP2 duplication syndrome a MECP2 gene dosage test has been created. Methods: Fragments sampling the four exons and proximal promoter of the MECP2 gene, Amelogenin and two autosomal gene fragments are amplified in a single multiplex polymerase chain reaction (PCR) in the presence of fluorescently-labeled primers. Products of the amplification reactions are analyzed on an automated capillary DNA sequencer. Dosage analysis is carried out using SeqPilotTM software. Summary We have created a QF-PCR based dosage assay to detect MECP2 gene deletions/duplications. We have examined two hundred specimens submitted for RTT sequencing analysis with this assay and detected a partial deletion of exon 4 in a female Rett syndrome case with negative sequencing result. We have also confirmed a large full gene duplication in a case originally identified by CGH array in a male patient with behavior problem, speech and motor delay, and learning disability.

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## 1491/T/Poster Board #40

**DNA diagnostic testing for nephrogenic diabetes insipidus: novel mutations in the AVPR2 and AQP2 gene.** *M. Nagel, S. Nagorka, M. Brzeska.* Laboratory for Molecular Diagnostics, Center for Nephrology and Metabolic Disorders, Weisswasser, Germany.

Hereditary nephrogenic diabetes insipidus (NDI) is a chronic disease that occurs soon after birth or in childhood. Affected patients have incongruous polydipsia, polyuria, dehydration, vomiting, and hypernatraemia. The disease can result in brain damage followed by permanent mental retardation or retarded physical growth. Hereditary NDI is caused by mutations in the AVPR2 (X-linked) or AQP2 (autosomal recessive or dominant) genes. In this study, we performed direct sequencing of entire coding regions of the AVPR2 and AQP2 genes in 50 unrelated individuals referred to our laboratory with a clinical diagnosis of NDI. The patients were from different ethnic backgrounds. Overall, 70% of these patients were male and 36% of them were 1-5 years old. Since most NDI is caused by AVPR2 mutations, we started the analysis with AVPR2 sequencing. If no mutation found, AQP2 sequencing was performed. The goal was to identify the mutation in order to confirm the clinical diagnosis of NDI. Thirty one different mutations were identified with an overall detection rate of 62%. There were 23 mutations identified with the AVPR2 gene and 8 with the AQP2 gene. Notably, many mutations were novel (17 of the 23 AVPR2 mutations and 6 of the 8 AQP2 mutations). We found 9 novel hemizygous missense mutations: S54I, Y117H, I130N, P173T, Q174E, W200C, L211P, A216P, and C283Y, 2 novel hemizygous nonsense mutations: W208X and Q291X, 4 novel hemizygous deletions (c.823_834del, c.832_834del, c.415_447, c.783delC), 1 novel hemizygous insertion (c.573insCAG), and 1 novel splicing mutation (IVS1+3G>A) in the AVPR2 gene. Additionally, we identified 2 novel heterozygous missense mutations: L18R and T125R, 2 novel splicing mutations (IVS1+3G>A and IVS2+3A>G), and 2 novel heterozygous deletions (c.754_772del and c.727_736del) that affected the AQP2 gene. Missense mutations were the most common mutation identified in the AVPR2 gene and account for 56% of all identified mutations in this gene. 6 out of 8 mutations identified in AQP2 gene were involved in autosomal recessive NDI. In conclusion, this study is an update on mutations in the AVPR2 and AQP2 genes. All identified mutations confirm the clinical diagnosis of NDI. There are no differences in clinical symptoms in patients with X-linked and autosomal NDI, thus if no mutation are found in AVPR2 gene, the analysis should be followed by AQP2 sequencing.

## 1492/T/Poster Board #41

**Identification and prenatal diagnosis of a novel G→A splicing mutation at position +1 of intron 7 of PHEX gene in a large Han Chinese family affected with vitamin D resistant rickets.** *G.R. Qiu[1,2], J. Wang[1], J. Li-Ling[1,2,3], Y.Y. Zhao[1,2], C.L. Jin[1,2], K.L. Sun[2], C.X. Liu[1].* 1) Liaoning Center for Prenatal Diagnosis, Department of Gynecology and Obstetrics, Shengjing Hospital of China Medical University, Shenyang 110003, China; 2) Department of Medical Genetics, China Medical University, Shenyang 110001, China; 3) Sino-Dutch Biomedical and Information Engineering School, Northeastern University, Shenyang 110003, China.

Short stature comprises a group of heterogeneous disorders featuring retarded development and reduced adult height, among which X-linked hypophosphatemia has been characterized by growth retardation, rachitic and osteomalacic bone disease, hypophosphatemia, and renal defects in phosphate reabsorption and vitamin D metabolism. The causative gene of the latter, i.e., phosphate-regulating endopeptidase (PHEX) gene, has been mapped at Xp22.2-p22.1. Here we report on a large Chinese family having at least 11 members (3 males and 8 females) affected with the disease. Pedigree inspection was consistent with X-linked dominant inheritance. The proband was a healthy pregnant woman querying for prenatal testing. Medical history revealed that her husband and 10 of his relatives have features including short stature (adult males < 160 cm in height, adult females < 150 cm in height), chest wall deformity, scoliosis and curving of lower limbs, with males to be more pronounced affected. Blood and urine analysis indicated hypophosphatemia in conjunct with increased urine phosphate, whilst blood and urine calcium were both normal. At the request of the family and with the consent from the Ethics Committee of Shengjing Hospital, China Medical University, genetic testing and prenatal diagnosis were provided. With 29 pairs of primers designed, the 22 exons of the PHEX gene, in addition with all exon-intron junctions were screened with PCR and DNA sequencing. As revealed by the result, all patients from the family have carried a novel G→A splicing mutation at position +1 of intron 7 of the gene, with male and female patients being, respectively, hemizygous and heterozygous for the mutation. The same mutation was found in none of the unaffected members from the family and 100 non-related normal controls. Bioinformatics analysis has suggested above mutation has abolished the corresponding splicing site, whilst a potential splicing site at 517 bp downstream may have been activated (to be verified by experiment). Prenatal testing suggested that the fetus did not carry the same mutation, and a healthy boy was born at full term. Conclusion: a novel G→A splicing mutation at position +1 of intron 7 of the PHEX gene may be pathogenic in the reported family. Prenatal testing may also be extended to a 34-year relative of the family, who has also been affected and is planning for pregnancy.

## 1493/T/Poster Board #42

**Hispanic Male with Invasive Breast Cancer Is Negative for a Known Deleterious Family BRCA 1 Mutation : Complex Genetic Counseling Issues and Possible Explanations.** *J. Wetzel[1], S. Demsey[2].* 1) Genetics, Kaiser Permanente, Anaheim, CA; 2) Genetics, Kaiser Permanente, Bellflower, CA.

Breast cancer affects more than 10% of women during their lifetime. Approximately 1% of breast cancer occurs in males. This is a report of genetic counseling for a 49 year old male affected with invasive ductal carcinoma who is negative for a familial deleterious BRCA1 mutation. BRCA1 mutation K654X results in the premature truncation of the BRCA1 protein at amino acid position 654. This mutation was previously noted in his affected sister. Site-specific analysis with reflex to comprehensive BRCA analysis (including large rearrangements; "BART" analysis) was performed and failed to reveal any known mutation in BRCA1 or 2. Site-specific analysis was repeated on a second blood specimen and was again negative. Counseling issues addressed included: a Mexican American male with a "female cancer", cultural issues, reluctance to inform his family of his diagnosis, initial refusal to request family assistance regarding necessary medical records, personal concern regarding risk for other cancers, and concern for his daughter's risk to inherit a cancer susceptibility. Counseling was further complicated by an unexpected negative result and the implications of not identifying a mutation in a very high risk individual. The negative result caused the counselor to challenge assumptions about the testing process, the presence of a cancer susceptibility mutation in an affected male and family dynamics. It required the client to reconsider his desire for confidentiality versus divulging personal information in order to obtain family cooperation with the testing process. Medical information from multiple affected family members was considered necessary to interpret his results. The patient's negative results could indicate that: a) there are two BRCA mutations in the family and the patient carries a BRCA mutation that is not detectable with current technology, b) there are two mutations in the family and the patient developed cancer due to a mutation in a more rare cancer susceptibility gene, possibly one which has not yet been identified or one for which testing is not available, c) the patient's cancer is due to a somatic mutation. Counseling a male with breast cancer poses a much higher initial level of suspicion regarding a cancer susceptibility mutation. When there is a known deleterious BRCA family mutation, a negative result is usually a true negative. This case demonstrates the complexity of genetic counseling and test result interpretation.

## 1494/T/Poster Board #43

**The application of next generation sequencing technology for the diagnostics and study of mental retardation.** *J. Brezo[1], M. Domanus[2], D. Antonopoulos[2], S. Das[1].* 1) Human Genetics, University of Chicago, Chicago, IL; 2) Institute for Genomics and Systems Biology, Argonne National Laboratory, Argonne, IL.

Nonsyndromic mental retardation(MR) presents a formidable challenge for molecular diagnostics because of the high number of implicated genes and low throughput of traditional Sanger sequencing. Next generation sequencing (NGS) is a promising new technology capable of simultaneous sequencing of hundreds of potential gene candidates in patients in whom clinical diagnosis is difficult. This capability can be used in sequencing entire pathways rather than a small panel of established genes. We are evaluating the potential of NGS for the analysis of patients with non-syndromic MR. To this end, we have compiled a panel of 100 genes implicated in MR that will be sequenced and analyzed on the Roche Genome Sequencer FLX. An important aspect for the use of this technology is the selection of a robust high-performance amplification method for downstream NGS. We are in the process of evaluating and comparing two amplification methods, array-based capture by hybridization (Nimblegen's 385K array) and microdroplet-based amplification by PCR (Raindance's RDT 1000). We selected five samples with known genotypes based on Sanger sequencing that have been amplified using both methods. An approximate coverage of 96.8% was obtained using the sequence capture array platform and approximately 96.9% coverage was obtained using the Raindance platform. The Roche Genome Sequencer FLX will be used to compare the two amplification methods, investigate the performance of multiple identification tags for multiplexing, and assess the depth of coverage that yields the best-read accuracy. Using the Genome Sequencer, we will further analyze a series of twenty patients collected through our diagnostic laboratory with a suspicion of Rett syndrome that were negative for testing for the MECP2, CDKL5 and FOXG1 genes. These samples will be re-sequenced for additional pathway genes that have been empirically, evolutionarily, or theoretically linked to these three established Rett syndrome genes. Additional pathway genes have been selected using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) interaction database and have been included in our panel of MR genes. This study will provide useful information on the comparison and performance of currently available amplification/enrichment platforms for NGS and the application of NGS to the study of MR genes. The applicability of this technology to the diagnostics of MR will be evaluated and our results presented.

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## 1495/T/Poster Board #44

**Williams-Beuren syndrome microdeletion diagnosis: the use of MLPA.** *R.S. Honjo[1], R.L. Dutra[1], C.C. Rebelo[1], I.M. Furquim[1], D.R. Bertola[1], L.M.J. Albano[1], P.C. Pieri[1], J.F.M. Araújo[2], C.A. Kim[1].* 1) Unidade de Genética, Instituto da Crianca, Universidade de São Paulo, São Paulo, SP, Brazil; 2) Departamento de Genética e Biologia Evolutiva - Instituto de Biociências - Universidade de São Paulo, São Paulo, SP, Brazil.

Introduction: Williams-Beuren syndrome (WBS) is MR/MCA syndrome caused by microdeletion of 1.5 to 1.8Mb in 7q11.23 region. Patients usually present with typical facies, congenital heart disease (particularly supravalvar aortic stenosis), mental retardation, arterial hypertension and peculiar hypersociable behaviour. Most cases are sporadic and the gold-standard method for diagnosis is FISH. Objectives: to evaluate MLPA (Multiple Ligation-dependent Probe Amplification) as a reliable alternative method for the diagnosis of WBS. Material and Methods: 23 patients with clinical suspicion of WBS were tested for the presence of 7q11.23 microdeletion using MLPA kit SALSA P029 (MRC-Holland). Results: Twenty-three patients with clinical suspicion of WBS were tested by MLPA and 21 were positive (91%). Among them, eight had also FISH with positive results and nine had a positive analysis using polymorphic genetic markers. One patient had MLPA negative for WBS but FISH and polymorphic markers confirmed the clinical diagnosis. Conclusion: MLPA was able to confirm the diagnosis of WBS in 91% of the patients. It failed to diagnose one patient, who has FISH and polymorphic markers positive for WBS microdeletion. In general, MLPA seems to be a reliable and cost-effective alternative to FISH for WBS diagnosis.

## 1496/T/Poster Board #45

**Molecular Diagnosis of Wolman/CESD Disease: Mutation Detection and Genotype-Phenotype Correlations.** *Y. Yang[1], P.H. Fernandes[1], I. Miloslavskaya[1], P. Orchard[2], S.J. Schwarzenberg[2], M. Grimley[3], W.E. O'Brien[1], C.M. Eng[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, University of Minnesota, Minneapolis, MN; 3) Texas Transplant Institute, San Antonio, TX.

Lysosomal acid lipase (LAL, gene: *LIPA*) deficiencies associated with two allelic autosomal recessive disorders: Wolman disease, which is characterized by complete absence of LAL activity, and the milder cholesteryl ester storage disease (CESD) which has residual LAL activity. Until recently, biochemical testing for LAL activity was the only method to confirm Wolman/CESD suspected by clinical criteria. We have recently developed LIPA gene sequencing for confirmatory and carrier testing for Wolman/CESD.

Since offering clinical DNA sequencing of the *LIPA* gene, we have analyzed five Wolman/CESD patients by sequencing the entire coding region of the gene. All the patients had deficient LAL activity by clinical biochemical analysis. A 27 day old patient with calcified adrenal glands was found to be homozygous for the p.G66V mutation. The p.G66V mutation in the heterozygous state was previously reported in a CESD patient as well as a Wolman patient, in trans with heterozygous c.894G>A and p.G5R mutations respectively. A 39 year old patient with microvesicular steatosis was heterozygous for p.G266X and c.894G>A mutations; the same genotype was previously reported in a CESD patient. The third patient was 4 months old and heterozygous for p.G266X and a novel c.822+1G>A mutation. A 6 year old patient with deficient activity had only one mutant allele of c.894G>A detected. The second unidentified mutation may be outside our standard region of sequence analysis or may be a large deletion or duplication. Finally, a four-month-old with calcified adrenal glands, elevated triglycerides and hepatosplenomegaly had two unclassified variants, c.894G>C and p.D345N. While in silico predictions showed conflicting results on the deleterious effects of the p.D345N unclassified variant, the c.894G>C unclassified variant, which affects the last nucleotide of exon 8, is predicted to cause exon 8 skipping at mRNA level, the same effect as the common c.894G>A mutation. The availability of clinical molecular testing and the identification of acid lipase sequence variants may help determine genotype-phenotype relationships in Wolman/CESD patients and provide information for enhanced genetic counseling.

## 1497/T/Poster Board #46

**Diagnostic method validation : High resolution melting (HRM) analysis for mutation screening of the ACVRL1, Endoglin and CFTR genes using the 7500- Fast system.** *S. PATRI, D. HAYE, A.M. DELVAUX, D. HAYE, B. GILBERT-DUSSARDIER, A. KITZIS.* SERVICE DE GENETIQUE, CHU LA MILETRIE, POITIERS, France.

We describe here optimized conditions for HRM analysis using the 7500 Fast Real Time PCR System (Applied Biosystem). We tested 3 genes which were routinely studied in our laboratory : ACVRL1, ENG and CFTR. The aim of this work was to perform all the studies with a unique condition (reaction mixture and cycling profile). Genomic DNA was extracted from whole blood using Magtration system 12GC instrument (PSS Co.Ltd) according to the manufacturer's instructions and diluted to 10 ng/μl. Genomic DNA samples from patients with previously characterized genetic variants and from normal control were used to determine the sensitivity of this analysis. Primers were designed to amplified entire exons and their flanking regions. Amplicon length was kept relatively short (< 400 bp) to improve genotype discrimination. Some larger exons were covered by 2 or 3 overlapping amplicons. Amplifications were performed in a final volume of 20 μl containing 30 ng of genomic DNA, 0.3 μM of each primer and 10 μl of Master Mix 2X (Applied Biosystem) containing the SYTO-9 dye (Invitrogen). The cycling profile was 10 min denaturation at 95°C, followed by 40 cycles of denaturation at 95°C for 15 sec and annealing/extend at 59°C for 1 min. High resolution melting was carried out after PCR denaturation at 95°C and renaturation at 50°C for 1 min followed by a slow (rate 1%) denaturation from 50°C to 95°C. This method does not require processing, reagent addition or separation after PCR. Amplification and melting curve analysis are performed in the same tube or plate which avoid the risk of contamination. All the known nucleotide variations were detected with the HRM analysis, point mutations as well as deletions. In complement we studied several patients and we detected some abnormal HRM profile. Each PCR product with abnormal profile was directly sequenced after ExoSAP (USB) treatment. The sensitivity was excellent with a very few number of false positive. For the polymorphism c.314-35A>G in the intron 3 of the ACVRL1 gene, we were able to distinguish the 3 genotypes (homozygote AA or GG and heterozygote AG). For all the samples, heterozygotes were easely discriminated from wild type, however mutations within the same exon were not necessary distinguishable one from the other. On a unique plate and with the same mixture reaction (except primers) and the same cycling profile, it was possible to genotype 5 patients for 12 amplicons or 9 patients for 8 amplicons.

## 1498/T/Poster Board #47

**Novel Neurofibromatosis 1 mutation in a patient with neurocutaneous and optic nerve lesions.** *F. Bolduc.* Dept Pediatrics, University of Alberta, Edmonton, Canada.

NF1 is the most common autosomal dominant disorder. Some of the major features are cutaneous, cerebral and neoplastic manifestations. Several mutations in neurofibromin have been described so far, affecting various regions of the protein. Neurofibromin contains a Ras-GAP domain involved in the regulation of the Ras-GTPase activity and CRAL-TRIO domain. We identified a 2 years old right-handed male with 9 cafe-au-lait macules and macrocephaly. He had normal psychomotor development. His MRI of the brain revealed the presence of a gadolinium-enhancing region in the right optic nerve, suggestive of optic nerve glioma. His father, as well as multiple members of his family, had clinical diagnosis of NF1. Multiplex ligation-dependent probe amplification (MLPA) analysis detected no whole-exon deletion or duplication. Sequencing of the NF1 gene revealed a point mutation in exon 8 resulting in a heterozygous L247P alteration that has not been previously reported. Based on alignment analysis, PolyPhen preditcs this to be damaging with a score of 2.110. Our case expands the range of mutation associated with NF1 and underline the need for sequence analysis in cases where MLPA may be negative.

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## 1499/T/Poster Board #48

**A Comparison of 23andMe and Navigenics Predictions for Five Individuals.** *P.C. Ng[1], S.S. Murray[2], S. Levy[1], J.C. Venter[1].* 1) Genomic Medicine, J. Craig Venter Institute, San Diego, CA; 2) Scripps Genomic Medicine, Scripps Health and The Scripps Research Institute, San Diego, CA.

Direct-to-consumer (DTC) companies such as 23andMe, Navigenics, and deCODE provide disease risk predictions to customers who have their DNA genotyped. We compare the predictions between two DTC companies, 23andMe and Navigenics, for five individuals. We find that on average, approximately a third of the predictions do not match between the two companies. The primary reason for differences in disease prediction is determined by whether the companies used the same markers with moderate to high odds ratio (OR). This indicates that markers with high OR should only be included if there is strong evidence to suggest that they are real, and that these markers have been verified in replication studies. While consensus among which markers to include will cause prediction agreement between the DTC companies, the markers that are currently used may not have good predictability because their effect size is still small. This may be improved by sequencing in the future, when rare variants with large effects might be identified.

## 1500/T/Poster Board #49

**Analysis of large genomic rearrangements of BRCA1 genes in Korean breast cancer patients.** *D.Y. Cho[1], S.Y. Choi[1], S.Y. Moon[1], S.J. Hwang[1], E.J. Park[1], H.M. Cheon[1], B.H. Son[2,3], B.K. Koo[2,3], S.H. Ahn[2,3].* 1) Clinical Research Institute, LabGenomics, Seoul, Korea; 2) Dept of Surgery, Asan Medical Center, Seoul, Korea; 3) Dept of Surgery, College of Medicine, University of Ulsan, Seoul, Korea.

Background : The BRCA1 and BRCA2 genes are associated with inherited susceptibility to breast and ovarian cancer. Most of disease-causing mutations in BRCA1/BRCA2 are point mutations and small insertion/deletions, but recently increasing number of large genomic rearrangements in BRCA genes have been reported in different populations with various prevalence. However, little is known about the prevalence and types of genomic rearrangements of BRCA genes in the Korean population. In this study, we have analyzed for the presence of BRCA1 large genomic rearrangements in Korean breast cancer patients. Methods : Multiplex ligation-dependent probe amplification (MLPA) was used to screen BRCA1 large genomic rearrangements in 249 Korean breast cancer patients at a priori risk of BRCA1/2 mutations due to known risk factors. The patients have been comprehensively analyzed for germline mutation in the entire regions of the BRCA1 and BRCA2 genes, using a combination of fluorescent-conformation sensitive capillary electrophoresis (F-CSCE) and direct sequencing, and were found negative. Positive MLPA result was confirmed and located by long-range PCR and sequencing. Results : We identified one large deletion in BRCA1, deleting exon 13-15, in one patient with family history of breast cancer. Breakpoints of this deletion are novel. Conclusion : Our results suggest that the large genomic rearrangements in BRCA1 gene are not a major cause for increased breast cancer susceptibility in Korean population.

## 1501/T/Poster Board #50

**Awareness and Utilization of *BRCA1/2* Testing and the US Preventive Services Task Force Guidelines among US Primary Care Physicians, 2007.** *C. Bellcross[1], K. Kolor[1], K. Goddard[2], R. Coates[1], M. Khoury[1].* 1) Office of Public Health Genomics, Centers for Disease Control and Prevention, Atlanta, GA; 2) The Center for Health Research, Kaiser Permanente, Portland, OR.

**Purpose:** While commercial testing for mutations in the breast/ovarian cancer susceptibility genes *BRCA1* and *BRCA2* (*BRCA*) has been available since 1996, there remains limited information regarding application in primary care. The United States Preventive Services Task Force (USPSTF) and other organizations have published guidelines regarding *BRCA* counseling and/or testing. However, there is a paucity of data regarding adherence to these recommendations in clinical practice. In addition, recent direct-to-consumer (DTC) advertising efforts may influence both awareness and utilization of *BRCA* testing. **Methods:** We assessed awareness and use of *BRCA* testing among physicians in the 2007 DocStyles national survey (1500 respondents: 515 family practitioners, 485 internists, 250 pediatricians, 250 obstetricians/gynecologists). **Results:** Overall, 87% of physicians were aware of *BRCA* testing, while 25% reported having ordered testing for at least one patient in the last year. Obstetricians/gynecologists were the most likely to be aware of (100%) and to have ordered (62%) testing, while pediatricians were the least likely (53% aware, 1.2% ordered). In multivariate logistic regression analyses, predictors of having ordered testing included physician specialty, higher patient socioeconomic status, and more than 10 years of practice. Physicians were asked to select indications for *BRCA* testing from seven different clinical scenarios representing increased (4) or low risk (3) situations. Of the increased risk scenarios, three were specifically identified by the USPSTF as considerations for genetic counseling referral. Among ordering physicians, 45% selected at least one low risk scenario as an indication for *BRCA* testing. In contrast, 19% chose none of the low risk, but all four increased risk scenarios. The “all correct” response rate rose to 31% when the analysis excluded the one increased risk scenario that was consistent with, but not directly addressed by, the USPSTF guidelines. **Conclusions:** While the substantial majority of primary care physicians are aware of *BRCA* testing and many have ordered it, a minority appear to consistently recognize the indications for counseling and testing set forth in the USPSTF guidelines. As the USPSTF concluded that potential harms outweigh benefits for women at low risk, these results suggest the need for improved provider awareness of existing guidelines, particularly in this era of increased *BRCA* DTC marketing and testing.

## 1502/T/Poster Board #51

**Utilization of genetic counselors to disclose clinically relevant genetic information obtained through research.** *L.L. Cheng[1], L. Le Marchand[2], N.M. Lindor[3], T.L. Burnett[2].* 1) Comprehensive Genetics Center, The Queen’s Medical Center, Honolulu, HI USA; 2) Epidemiology Program, University of Hawaii Cancer Research Center, Honolulu, HI USA; 3) Medical Genetics, Mayo Clinic, Rochester, MN USA.

The return of results generated in research laboratories has generally been discouraged; however, there are circumstances in which available medical management is of sufficient recognized value that the risk-benefit ratio may lean toward diagnosis disclosure. The Colon Cancer Family Registry (CCFR) is an international consortium dedicated to interdisciplinary studies in the genetics and genetic epidemiology of colorectal cancer. The six registries including the University of Hawaii Cancer Research Center (UHCRC) recruited population-based families at various risk for the disease and collected family history, epidemiological and clinical data, screening behavior, tumor blocks and blood samples from probands and their relatives. Samples were tested for DNA mismatch repair deficiency. When a deleterious germline mutation was identified in a proband, samples from consented relatives were also tested. Given the potential impact of these research test results on the subjects’ medical management, the CCFR recognized an ethical obligation to disclose these results. Statement of Purpose: To describe the collaboration between UHCRC researchers and clinical genetic counselors to develop a protocol for disclosure of test results. Methods: 1) Subjects whose blood was tested for deleterious mutations in MLH1, MSH2 and MSH6 were provided the option to receive test results; 2) Willing subjects were scheduled for an appointment with a genetic counselor; 3) Informed consent was obtained prior to results disclosure; 4) Confirmation of test results in a clinical laboratory was recommended; 5) Follow-up genetic counseling in a clinical setting was offered to all subjects; 6) A written summary of the counseling session was provided to the subject and optionally to their physician; and 7) Participants were encouraged to contact at-risk family members to share their genetic test results, inform relatives of the opportunity for testing through participation in the CCFR or community services, and options for genetic counseling. Summary: The protocol for subject notification of test results was approved by the University of Hawaii Committee on Human Subjects and The Queen’s Medical Center Research and Institutional Review Committee. This protocol is expected to increase enrollment of relatives into the Hawaii CCFR, identify new carriers of deleterious mutations and improve the medical management of participants. To date, 19 Hawaii CCFR participants have been counseled.

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## 1503/T/Poster Board #52

**Predictive Testing for Inflammatory Bowel Diseases: The Views of Unaffected Siblings.** *A.M. Mindlin[1], C. Shuman[1], T. Walters[2], D. Chitayat[1], C. Popalis[2], S. Reif[3], K. Frost[2], M. Wright[2], A. Griffiths[2].* 1) Clinical & Metabolic Genetics, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Gastroenterology, Hepatology, and Nutrition, Hospital for Sick Children, Toronto, Ontario, Canada; 3) Pediartric GI unit, Dana Children's Hospital, Tel Aviv, Israel.

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory conditions of the colon and small intestine with an incidence of around 10 cases per 100,000 in the United States. Both CD and UC exhibit a multifactorial mode of inheritance with well recognized genetic, immunological, and environmental contributions to disease susceptibility. Although genetic testing for IBD is not currently utilized to predict disease, research in the genetic etiology of CD and UC suggests that such testing will likely become available. First degree relatives and especially siblings are at the highest risk of developing IBD and thus they are the ones that could potentially benefit the most by understanding their individualized risk. The objective of this exploratory study was to investigate factors considered important in adolescents' and young adults' decisions to participate in predictive testing for IBD. Unaffected individuals aged 13 to 25 years with at least one sibling with IBD were invited to participate. Recruitment took place through the IBD clinics at The Hospital for Sick Children in Toronto, through online sources including Facebook and the Crohn's & Colitis Foundation of Canada, and through the Dana Children's Hospital in Tel Aviv. Participants were asked to complete a 15 minute online questionnaire developed from constructs of the Health Belief Model. Data analysis was performed on 130 questionnaires of which 70% of respondents were female and 30% were male. 88% of participants expressed interest in predictive testing for IBD and this did not significantly differ by gender or age of participant. This is consistent with previous studies looking at patients with IBD and their interest in genetic testing for themselves and for their children. Perception of severity of ulcerative colitis correlated with interest in testing. The top two reasons cited in favor of testing included: to watch for early symptoms of the disease and to help science. However, 45% of the individuals felt that a positive test result would make them feel anxious with females being significantly more likely to worry about developing IBD than males. The results of this study provide insight into the perceptions of adolescents and young adults regarding potential genetic testing for IBD; this data is important to consider in the development and implementation of testing for common, complex disorders.

## 1504/T/Poster Board #53

**Comparing emotions and cancer screening behavior between family members who do or don't receive genetics services for Lynch syndrome.** *D. Hadley, S. Ashida, A. Giroux, H. Devlin, C. McBride, L. Koehly.* Social & Behavioral Research Branch, National Human Genome Research Institute / NIH, Bethesda, MD.

Purpose: Uptake of genetics services in families with inherited, adult onset diseases with preventive strategies range from 23 to 45%. This leaves a majority of at risk family members without the benefits of genetics education, counseling and risk assessment. Little is known about the psychological wellbeing and preventive health practices of those not receiving genetics services. In families with Lynch syndrome, relatives at risk to inherit mismatch repair mutations but not pursuing molecular testing are recommended to follow cancer-screening guidelines for mutation carriers. The aim of this study was to compare mood and use of colonoscopy between at risk family members receiving and not receiving genetic services in families with Lynch syndrome. Methods: One hundred sixty-three (163) family members (20 families) at risk to inherit a deleterious mutation completed questionnaires assessing the variables of interest prior to their receipt of genetics counseling and the offer of genetic testing. Family members at risk of inheriting the identified mutation, but not receiving genetic services (GS) (n= 49), were recruited through relatives receiving GS to complete a questionnaire assessing the variables of interest. Linear and logistic regression models were used to test for differences in 1) the number of depressive symptoms reported and 2) colonoscopy use between family members who went on to pursue GS as compared to those who did not, respectively. Analyses adjusted for the within family clustering and relevant covariates. Results: Mean scores for the number of depressive symptoms reported was significantly higher for at risk family members not receiving GS than for at risk family members presenting for GS. Colonoscopy use by relatives who did not receive GS was significantly lower than relatives receiving GS and identified as mutation carriers. Conclusions: Research to identify effective clinical and family based interventions that promotes cancer screening in at risk relatives not receiving genetic services is needed to facilitate prevention and early diagnosis of colon cancer. Higher numbers of depressive symptoms in family members not receiving genetics services may play a role in limiting uptake. Genetics counseling should include discussions that help family members who receive genetics services encourage those who don't to undergo cancer screening and offer support.

## 1505/T/Poster Board #54

**Support for patients with multiple endocrine neoplasia type 1 and their spouses: what do they want?** *S. Maruyama, A. Sakurai, Y. Fukushima.* Department of Medical Genetics, Shinshu University, Matsumoto, Japan.

Multiple endocrine neoplasia type 1 (MEN1) is a relatively rare autosomal dominantly inherited disorder characterized by hyperplastic and neoplastic disorder of endocrine organs such as parathyroid, anterior pituitary and gastroenteropancreatic endocrine tissues. Less frequent manifestations include adrenal cortex adenoma, thymic/bronchial neuroendocrine tumor and cutaneous tumors including facial angiofibroma, collagenoma and lipoma. Most subjects with MEN1 carry a heterozygous germline mutation in the MEN1 gene which localizes to chromosome 11q13 and encodes 615 amino-acid nuclear protein menin. Patients with MEN1 need to have various medical managements including periodic biochemical- and imaging studies, medication for various symptoms and surgery which usually need to have more than once. Genetic testing of patients and family members can cause them significant psychological distress. To know what they have felt when they were diagnosed as having MEN1 and what kind of supports they needed, we carried out a written questionnaire-based survey for affected patients and their spouses. Twenty-nine patients and 22 their spouses completed questionnaires and those were analyzed. Age of respondents varied from twenties to seventies, and ratio of males and females were nearly equal. More than three fourths and more than half of patients, respectively, were diagnosed based on results of genetic testing and family history. At the time of diagnosis, with surprise and uneasiness, many respondents concerned "inheritance of the disease to their children" as well as their own future health problems. As time passes after the diagnosis, majority of patients reported a relief from anxiety, but some reported further increase of anxiety, probably related to aggravation of the diseases and concern about genetic testing for their children. At the time of diagnosis, medical information was on the top of the list they wanted, but as time passed, they asked more social- and physical support. In addition to an appropriate medical management, sustained assistance which meets variety of needs of patients and family members is necessary to achieve better quality of life for them, and establishment of an appropriate administration system is desired for that purpose.

## 1506/T/Poster Board #55

**Recurrence risk of severe osteogenesis imperfecta (OI): parsing the risk due to parental mosaicism for dominant inheritance and recessive mutations.** *S. Pyott, U. Schwarze, M. Pepin, P. Byers.* University of Washington, Seattle, WA.

About 95% of individuals with lethal forms of OI have dominant mutations in type I collagen genes and the remainder have recessive mutations in two known (CRTAP and LEPRE1) and additional genes that remain to be identified. Measured recurrence risk in families with unaffected parents range from 2% to about 7% and depends on the methods of ascertainment of the sample. Recurrence risks are at the lower end with samples comprised by only those with one affected. The risk of recurrence after a second infant is substantially increased and is likely the result of a mixture of recessive inheritance and parental mosaicism, but the contribution of the two is unknown. To determine the relative contributions of the two mechanisms, we identified 45 families in which two or more infants with lethal and severe forms of OI were born to unaffected parents (a total of 146 children with 102 affected). We sequenced genomic DNA or cDNA from cultured cells, or from DNA provided from affected infants, to identify mutations in COL1A1, COL1A2, CRTAP, LEPRE1, PPIB, and SERPINH1 until we identified causative alterations. In 56% of these families (66 children with 52 affected) we found dominant mutations in the type I collagen genes, COL1A1 (16) and COL1A2 (9). In 27% of the families (43 children with 29 affected) we found homozygosity or compound heterozygosity for mutations in CRTAP (6), LEPRE1 (5), or PPIB (1). We failed to find mutations in the remaining 18% of the families (8) (37 children with 21 affected). Accounting for ascertainment by two affecteds, the overall recurrence risk was 21%; in families with recessive mutations it was 26% (5 of 19); in families with parental mosaicism for dominant mutations it was 13% (2 of 16), and in families with no mutation identified it was 24% (5 of 21). This last group is consistent with additional genes in which recessive mutations account for recurrence in these families. The rate of recurrence in mosaic families depends on the proportion of primordial germ cells that contain the mutant allele. The recurrence risk (13%) is compatible with 1 in 4 cells allocated to the germ line containing the mutant allele and argues for a relatively small pool of cells destined for the germline. These data support the contention that mutation identification is important in the assessment of recurrence risk and provides the basis for prenatal and preimplantation diagnosis when requested by the family. Supported by grants from USPHS and OI Foundation.

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## 1507/T/Poster Board #56

**Incorporating locus heterogeneity into recurrence risk estimates in Bardet-Biedl syndrome.** *J.C. Sapp[1], D. Nishimura[2], J.J. Johnston[1], E. Stone[4], E. Heon[3], V. Sheffield[2], L.G. Biesecker[1].* 1) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA; 2) University of Iowa Hospitals and Clinics, Department of Pediatrics, Iowa City, IA, USA; 3) Hospital for Sick Children, Department of Ophthalmology and Vision Sciences, Toronto, ON, Canada; 4) University of Iowa Hospitals and Clinics, Department of Ophthalmology, Iowa City, IA, USA.

Bardet-Biedl syndrome (BBS) is a pleiotropic multiple anomaly syndrome inherited in an autosomal recessive pattern. Prior to genetic analysis, this disorder was assumed to be a unitary clinical and genetic entity. Recurrence risk calculations based on these assumptions estimate the risk for an offspring of a sibling of an affected individual who reproduces with an unrelated person to be approximately 1/950. It is now known that this disorder has remarkable locus heterogeneity, with causative mutations identified in as many as 14 genes. While the risk for a subsequent pregnancy for parents of an affected child is 1/4 regardless of which gene is mutated in that family, the risks for other relatives can vary substantially because of this locus heterogeneity. Clinical practitioners are increasingly called on to evaluate, molecularly diagnose, and counsel individuals and families affected by BBS, and risks to unaffected siblings and the affected probands themselves are among families' primary concerns. Thus, we set out estimates that can be used for recurrence risk counseling based on locus heterogeneity and a large cohort of families with mutations in BBS genes. To estimate these risks, we counted the relative proportion of families with mutations at each of 10 loci (BBS1-7, 9, 10, & 12) from a cohort of 182 families with one or more members who have two mutant alleles in any BBS gene. We estimated the carrier rate for mutations at each locus using Hardy-Weinberg principles and an aggregate population frequency of 1/100,000 for the phenotype. Taking into account locus heterogeneity, the risks for an offspring of the sibling of an affected individual actually range from ~1/1,500 to ~ 1/13,000, compared to ~1/950. As there is substantial phenotypic variation in BBS, locus-specific risk calculations may also be relevant for affected patients themselves. For the two common loci (BBS1 and BBS10) the magnitude of the difference between locus-specific and aggregate estimates is modest, yet for rare loci this difference is large and likely to be clinically relevant. Variation of recurrence risks using a locus-specific model is likely to have implications for genetic counseling about prenatal testing and other reproductive options.

## 1508/T/Poster Board #57

**Early Experience with a Prognostic Test for Adolescent Idiopathic Scoliosis.** *L. Nelson, M. Singleton, R. Chettier, K. Ward.* Axial Biotech, Inc, Salt Lake City, UT.

Introduction: We recently developed and validated a prognostic test for prediction of curve progression in adolescent idiopathic scoliosis (AIS) (Submitted). AIS affects 2-3 % of the pediatric population and 10% of patients will require bracing or fusion surgery. Although mandated in 26 states, school screening, to provide early identification of AIS patients, is controversial since 90% of AIS patients have non-progressive disease. Our test was designed to address problems in school screening such as cost, patient and parent anxiety, serial X-ray exposure and missed school and work. We report our initial experience with this test. Methods: The initial indications for testing includes female Caucasians age 9-13 that had been identified as having mild AIS. AIS is diagnosed using the Cobb method (radiographic measurement of the angle of intersection from the start to the end of the curve; angle of at least 10 degrees). Angles greater than 25 degrees are typically treated. The AIS-PT uses a panel of 53 DNA markers and current Cobb angle to assign a risk progression score (1-200). Risk scores correlate with the patient's risk of progressing to a severe curve (progression to a curve >40° in a skeletally immature individual or progression to a curve >50° in an adult). Low risk patients (<1% risk of progression to a surgical curve) are those with scores ≤ 40 and high risk patients are those with scores between 181-200. A follow-up survey after testing assessed how the test is used in clinical practice. Results: 316 patients with initial Cobb angles <20 degrees were tested during the first 5 months of use. 49% had "low-risk" scores, 50% had "intermediate risk", and 1% had a "high-risk" In the subset of patients with Cobb angles of 10-14 degrees there were 146 patients. 72% of them had low-risk scores, 28% had intermediate scores and 0% had high scores. Surveys were returned on approximately a third of the patients. Conclusions: The first patients tested appear to represent a population with a more severe prognosis (older patients with more severe Cobb angles) compared to typical mild patients. In research and validation populations from similar practices approximately 68% of patients were identified as low risk. Surgeons report that the AIS-PT helps in making management decisions especially in low and high-risk patients.

## 1509/T/Poster Board #58

**Collection and use of family history information in the primary care setting.** *S.E. Hahn[1,4], K.L. Powell[2], S.H. Blanton[1,4], A.H. Buchanan[3], C.A. Christianson[2], V.C. Henrich[2], A. Agbaje[5], J.M. Vance[1,4], M.A. Pericak-Vance[1,4].* 1) Miami Inst for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) The Center for Biotechnology, Genomics, and Health Research, The University of North Carolina at Greensboro, Greensboro, NC; 3) Cancer Center, Duke University, Durham, NC, United States; 4) Dr. John T. Macdonald Foundation Department of Human Genetics, Miller School of Medicine, University of Miami, Miami, FL; 5) Moses Cone Health System, Greensboro, NC.

Healthcare infrastructure does not support appropriate disease-related risk assessment. Family history (FH) is our most powerful tool to identify patients at risk for complex disorders, but it is underused. One goal of The Genomedical Connection is to determine the feasibility of using a computer-based self-administered family history and genetic risk assessment tool to facilitate the practice of genomic medicine in primary care. To determine the effectiveness of the tool we plan to measure primary care providers' (PCPs) practices regarding the collection of family history, genetic risk assessment and management strategies at baseline and subsequent intervals. Here we report the results of the baseline chart review conducted in two pilot practices. A modified version of the chart checklist developed by the Michigan Department of Community Health was used. A list of patients age 18 or older seen for a new patient- or a well-visit in the 6 months preceding 11/1/2007 was generated. 264 patient charts were randomly pulled and analyzed; at least 23 for each of 11 clinicians (except one in transition). 50% were for female patients. 98% included FH information (none in pedigree form). Parents were most frequently reported in FH (91%), followed by siblings (69%), children (20%), grandparents (8%); aunts/uncles (7%); and cousins (0.5%). Age of onset for diseases in relatives was rarely reported ("never" 90% of time). When relevant (e.g. cousins), maternal vs. paternal side of the family was missing 31% of the time. No patients had been referred to a genetic specialist. Two patients had genetic testing - one for hemochromatosis (not by PCP) and one for factor V. Five were referred to a non-genetics specialist based on family history - three to a cardiologist; one to an oncologist; and one to a gastroenterologist. Eleven (4%) were referred for cancer surveillance (e.g., colonoscopy at early age, breast MRI) and 2 (1%) were referred for EKG due to FH. While PCPs collect FH information, they are frequently missing critical pieces that would aid risk assessment, such as 2nd and 3rd degree relatives, age of onset, and side of family. Also, only a very small proportion of patients had recommendations based on FH. By comparing baseline results with the data collected at subsequent points in this study we will be able to assess the effectiveness of the model and secondary effects on overall physician practice.

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## 1510/T/Poster Board #59

**Family HealthLink: A Unique, Online, Automated Risk Assessment Tool for Cancer and Coronary Heart Disease.** *A. Sturm[1], C. Craven[1], P. Coss[2], B. Bennett[2], S. Nottingham[2], K. Sweet[1].* 1) Dept Internal Med/Div Human Gen, Ohio State Univ, Columbus, OH; 2) Chronic Disease & Behavioral Epidemiology, Center for Public Health Statistics and Informatics, ODH, Columbus, OH.

Even with Human Genome Project advances and related efforts, family history (FH) remains the "gold standard" for assessment of the interaction of familial and environmental risk factors. This is especially so for common diseases such as cancer and coronary heart disease (CHD), the top two causes of death in the developed world. We previously demonstrated the rate of FH collection (69%) and assessment of high risk cancer status (14%) was inadequate at a Comprehensive Cancer Center. In 2008, we added FH questions to the Ohio Behavioral Risk Factor Surveillance System survey, n= 6,079. While 86% surveyed indicated their physician or health care professional (HCP) asked them about FH, 36% did not receive adequate risk assessment and 38% did not receive recommendations based on FH. Importantly, most (95%) were willing to spend at least 15 minutes completing FH, either on their own or at their HCP's office, and most were very likely (66%) or somewhat likely (30%) to make prevention and lifestyle changes based on increased risk. To address this need, we developed an automated tool known as Family HealthLink (FHL), https://familyhealthlink.osumc.edu, which is unique in providing assessment for all known hereditary cancer syndromes and CHD. The FHL consists of branched-point decision-making screens that guide the user through the program, mimicking face-to-face FH collection and distinguishing this interactive media from a paper-based FH questionnaire. The user enters demographic information, personal cancer and CHD history including age at diagnosis, and the cancer and CHD history of first- and second-degree relatives, in both the maternal and paternal lineages. This takes the average user less than 15 minutes. The individual's familial risk (average, moderate, or high) for cancer and CHD is then computed based on published algorithms. A qualitative risk assessment with recommendations for prevention is provided in PDF format. Since October 2008, the FHL public access website has had 722 users (590 females, 132 males). 57% of users were assessed as high risk, most for one criterion, although 67 (9.3%) were at high risk for both cancer and CHD; 28% of users had moderate risk for at least one disease. Regarding CHD risk specifically, 35% were at high risk and 14% were at moderate risk. Local marketing initiatives on the importance of FH for cancer and CHD risk correspond with tool usage, and higher risk individuals may be seeking such services on the Internet.

## 1511/T/Poster Board #60

**Combining genotypes and family history for prediction of complex disease.** *E. Ziv[1], S. Rosset[2].* 1) Dept Medicine, Dept of Epidemiology and Biostatistics, Institute for Human Genetics, Helen Diller Family Comprehensive Cancer Center, Univ California, San Francisco, San Francisco, CA; 2) Department of Statistics, Tel Aviv University, Tel Aviv, Israel.

As the number of associations discovered by genome wide association studies increases, the results from these studies are often considered in the context of predictive tests. Such predictive testing can be used to risk stratify individuals and consider preventive interventions. However, genome wide association studies often identify only a fraction of the causative variants which account for only a fraction of the heritability. Thus, family history will continue to be a useful predictor of complex disease in the near future. In addition, family history may continue to remain an important factor in individual risk assessment since it may also capture shared environmental factors. Therefore, properly combining information from genotypes and family history is important to maximize the utility of individualized risk prediction. We demonstrate how to combine information from genotypes and sibling relative risk for a complex trait to estimate individual risk. In particular, we demonstrate how to partition sibling relative risk into the proportion that has been accounted for by the genotypes measured and the proportion that remains unaccounted for, or the residual sibling relative risk. Combining information from the genotypes and the residual sibling relative risk gives the optimal risk assessment from the available data. We illustrate our approach with recent results from genome wide association studies of breast and prostate cancer.

## 1512/T/Poster Board #61

**A computational approach to predict the pathogenic effect of missense mutations in genetic diagnostics of hypertrophic cardiomyopathy (HCM).** *D.M. Jordan[1,2], B.H. Funke[3], R.C. Green[4,5], S. Sunyaev[2].* 1) Program in Biophysics, Harvard University, Cambridge, MA; 2) Division of Genetics, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 3) Laboratory for Molecular Medicine, Harvard-Partners Center for Genetics and Genomics, Cambridge, MA; 4) Department of Genetics, Harvard Medical School, Boston, MA; 5) Departments of Neurology, Genetics, and Epidemiology, Boston University Schools of Medicine and Public Health, Boston, MA.

Many mutations identified by sequencing in clinical diagnostic labs are not clearly correlated with the disease phenotype. These mutations are considered variants of unknown significance (VUS) and are difficult to interpret clinically. A reliable *in silico* method to assess these variants would be a valuable tool to assist in their classification. However, current methods are considered too unreliable for clinical use, and have not been validated to the standard required by physicians.

Using the PolyPhen software, we have designed a new tool to identify missense variants that cause hypertrophic cardiomyopathy (HCM), a genetic heart disease that affects approximately 1/500 individuals in the general population and is thought to be the most common cause of sudden death in the young (including trained athletes). Our goals were to improve on the native performance of PolyPhen, and to assign a confidence to our predictions, which is required for clinical use. HCM is caused mainly by variants affecting contractile proteins of the cardiac sarcomere. We focused on ten of these genes (ACTC, LAMP2, MYBPC3, MYH7, MYL2, MYL3, PRKAG2, TNNI3, TNNT2, and TPM1). This enabled us to improve performance by incorporating new statistics that rely on specific prior knowledge of the phylogenies, structures, and functions of these genes. The small number of patient sequences to be analyzed in a clinical setting also allowed us to use more computationally expensive algorithms.

We assigned confidence to our predictions based on a set of 106 missense variants that had been previously classified using a variety of traditional genetics criteria, relying heavily on frequency in controls and segregation with disease in families. We used a jackknife validation method to identify which variants we could classify confidently, and chose classification thresholds that allow us to claim a confidence of ~90%. Out of 137 missense variants classified as VUS from the same data set, we were able to classify ~50% of them at this level.

In addition to producing a tool that may assist in classification of VUSs for HCM, this methodology is also widely applicable to other genetic diseases. This work suggests new avenues for the clinical application of computational genetic diagnostics.

## 1513/T/Poster Board #62

**ABO Genotyping by Capillary Electrophoresis.** *A.A Pradhan[1], S.R. Berosik[1], A. Chhibber[1], C.J. Davidson[1], Y. Doi[4], R.N. Fish[1], S.C. Hung[1], B.F. Johnson[1], M. Kondo[3], J. Lee[1], R.A. Padilla[1], D. Rodriguez[1], A.C. Felton[1], Y. Yamamoto[5], M. Yamazaki[2], L.K. Joe[1].* 1) Life Technologies Corporation, Foster City CA USA; 2) Hitachi High Technologies, Naka, Ibaraki, Japan; 3) Life Technologies, Tokyo, Japan; 4) Criminal Investigation Laboratory, Okayama Prefectural Police Headquarters, 1-3-2 Tonda-Cho, Okayama City, Okayama 700-0816, Japan; 5) Department of Legal Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences.

Identification of specific combinations of SNPs in the ABO locus on chromosome 9 can be used to determine ABO blood type, and this method is often used in identification of suspects, victims, or missing persons in criminal investigations. ABO blood-group identification by genotyping can also be a valuable tool in medical applications where routine serological typing is either not feasible or inconclusive, or where greater detail about precise allele composition is required. However, standard methods of ABO genotyping are either too time consuming (e.g. PCR-restriction fragment length polymorphism (PCR-RFLP)) or too complex (e.g. single-stranded conformational polymorphism (SSCP)) for routine laboratory use in forensic or clinical settings. Furthermore, sensitivity is an important consideration, with ABO typing in forensic cases often relying on very small quantities of genomic DNA. A simple and rapid procedure for a multiplex single-base primer extension reaction for ABO typing using six SNP sites within the ABO gene has been described in the literature. We present a method for capillary electrophoresis of such multiplex primer extension reactions. This method allows for sensitivity capable of accurate genotyping down to 0.05 ng of genomic DNA and improved consistency across instruments and laboratories. In addition, we demonstrate a robust and rapid secondary analysis workflow that minimizes the need for manual data interpretation of each sample.

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## 1514/T/Poster Board #63

**Human Stem Cell Research; the State of the Art: Scientific and Ethical Challenges.** *A.I. Al-Aqeel[1,2], A.A. Alaiya[2], S. Coskun[3], C.N. Adra[2].* 1) Deptartement of Pediatrics, Riyadh Military Hospital, PO Box 7897-W951, Riyadh 11159, Kingdom of Saudi Arabia; 2) Stem Cell Therapy Program, Research Centre, King Faisal Specialist Hospital and Research Centre, MBC-03, P.O. Box 3354, Riyadh 11211, Kingdom of Saudi Arabia; 3) Assisted Reproductive Technology, King Faisal Specialist Hospital and Research Center, P.O. Box 3354 MBC 10, Riyadh, 11211, Kingdom of Saudi Arabia.

Stem cells are unspecialized cells able to divide and produce copies of themselves and having the potential to differentiate, i.e. to produce other cell types in the body. Because of the latter ability, the scientists investigate their possible use in regenerative medicine. Especially embryonic stem cells (ESCs) have huge therapeutic potential because they can give rise to every cell type in the body (pluripotentency) as compared to stem cells from certain adult tissues which can only differentiate into a limited range of cell types. For this reason scientists stress the importance of embryonic stem cell research. However, this research raises sensitive ethical and religious arguments, which are balanced against possible great benefit of such research for the patients suffering from so far incurable diseases. Serious questions remain about safety. The main technical problems are the control of cell differentiation in vitro and in vivo, the correct targeting of the desired tissue and the control of proliferation. Only when the processes of proliferation and differentiation are fully understood can stem-cell therapy become a viable option for treatment. The integration of stem cells into the target tissue is not yet fully understood. Research must also find efficient methods for controlling differentiation into tissue cells of the liver, nervous system, bones or heart and prevent uncontrolled proliferation after insertion. The difficulties of culturing these cells to maintain their pluripotencyas can be done for mouse ESCs has made the manipulation of hESCs an even more highly skilled art and one that few scientists have yet mastered. A major limitation on the field of hESC research is, therefore, a shortage of human research skill that can only be overcome slowly. Recent advances in the field of cloning and stem cell research has raised many complex questions. It is rare that a field of science causes debate and challenge not only among scientists but also among ethicists, religious scholars, governments and politicians. There is no consensus on the morality of human cloning, even within specific religious traditions. In countries in which religion has a strong influence on political decision making, the moral status of the human embryo is at the center of the debate. We will discuss our experience; how Islamic teachings make this very promising research and therapeutic technique, and modality of treatment permissible; and the Islamic perspectives about reproductive/therapeutic cloning. In conclusion, it is still unclear which human stem cellswhether embryonic or adultwill be developed and for which conditions. Qualities of the ideal stem cell in a clinical setting are expected to be extensive and far reaching. The ability for stem cells to be expanded in culture without genetic and epigenetic abnormalities, their ability to form functional cell types in vitro and in vivo, and their immuno-compatibility with the patient need to be studied. Given this, the focus of research community, should be on developing human research capacity in both ASCs and ESCs. Each type of research will take time to mature. The ethical debate will need to produce acceptable policy and regulatory compromises so that the regulatory burden can be reduced and investors' risk aversion can be overcome. If these things happen, the major remaining barrier to realizing the medical benefits of stem-cell research might be the lack of skilled scientists in the field.

## 1515/T/Poster Board #64

**When the Findings are Not Certain: Dissemination and Disclosure Options for Ecogenetics Research.** *K.M. Beima-Sofie[1], J. Hofmann[4], C. Jansen[5], R. Cunningham[3], M. Negrete[6], P. Palmandez[6], M. Keifer[3], K. Fryer-Edwards[1,2].* 1) Institute for Public Health Genetics, University of Washington, Seattle, WA; 2) Bioethics and Humanities, Univeristy of Washington, Seattle, WA; 3) Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA; 4) Department of Epidemiology, University of Washington, Seattle WA; 5) School of Public Health, University of Washington, Seattle, WA; 6) Pacific Northwest Agricultural Safety and Health Center, Yakima, WA.

Increasingly, we need to understand how ambiguous research findings impact the translation and distribution of research results. Incomplete understanding of direct correlations between genetic polymorphisms and disease risk make it difficult to translate research results into clinical and public health practices. In addition, the ethical, legal and societal implications arising from the disclosure of uncertain or unclear genetic information are not well understood. Because of these uncertainties, current guidelines advise against returning results to individuals unless they are valid, reliable, and have clinical utility. These recommendations create a dilemma for researchers who believe results should be returned to study subjects regardless of whether they meet these criteria. We build on an existing study at the University of Washington Pacific Northwest Agricultural Safety and Health Center that provided pesticide handlers with individual results regarding their paraoxonase (PON1) status and information on cholinesterase (ChE) inhibition and personal protective behaviors. Our project utilized various outreach methods to disseminate information about genetic susceptibility, environmental exposures, and individual genetic test results. Study subjects received information by letter, phone call, and/or informational video. Preliminary interviews show that workers who received individual genetic results by letter did not understand genetics or what their results meant. However, because of previous education efforts, participants were aware of ChE monitoring programs and were more interested in obtaining individual ChE levels than genetic susceptibility information. Current projects are evaluating the effectiveness of video risk communication strategies. The goal of this poster is to review guidelines for returning results, to demonstrate where genetics creates ambiguity, and to share successful outreach methods with other researchers in order to ethically and effectively communicate research results to study participants. We point to education and research practices that preserve trust and ensure that study participants understand the results they receive and are motivated to engage in healthy behaviors. By better understanding how one particular cohort of study participants reacts to results with ambiguous implications, we hope to contribute to emerging best practices for future research and outreach projects in ecogenetics and environmental health.

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## 1516/T/Poster Board #65

**Bioethics and socioethics: The paradoxes associated with non-invasive prenatal diagnosis performed on fetal cells obtained from maternal blood.** *C. Bouffard, A. Ducos, K. Krabchi, R. Drouin.* Dept Ped, Fac Med Hlth Sci, Univ Sherbrooke, Sherbrooke, PQ, Canada.

Pregnant women may one day be able, merely by providing a blood specimen, to obtain non-invasive prenatal diagnoses (NIPDs) on fetal cells circulating in their blood. This form of prenatal diagnosis is performed during the first trimester and makes it possible to determine, without recourse to such invasive methods as amniocentesis or chorionic villi sampling, whether a fetus presents with chromosomal or genetic anomalies. Testing on fetal cells from the mother's bloodstream is deemed non-invasive and non-threatening to the pregnancy. It thus offers undeniable bioethical benefits. Nevertheless, it presents significant socioethical problems. Our paper deals with the ethical paradoxes raised by NIPD. Method: Comparative discourse analysis of the scientific and bioethical literature on NIPD. Results: On one hand, NIPD would allow for solving numerous bioethical problems raised by prenatal diagnosis. Its non-invasive nature; its freedom from risks to the pregnancy; the earliness of the diagnosis, allowing more time for decision-making or for earlier and thus less distressing recourse to abortion; the overall lessening of parental anxiety in connection with prenatal diagnostic testing; greater equity among pregnant women in terms of access to such testing; reliable results; low cost: these are among the benefits that NIPD would bring. On the other hand, the technique gives rise to some fundamental socioethical concerns. For example, in democratic countries, NIPD could allow, under the banner of liberal individualist values, for recourse to genetic testing (and consequent decisions about interrupting a pregnancy or not) based on individual or non-medical social criteria (family balancing, paternity, social sex selection). Conclusion: We can easily imagine that any diagnostic method that reveals the fetus's state of health without threatening either the mother's health or the pregnancy would be well received by most women and couples at risk of transmitting a genetic disorder. The introduction of NIPD into clinical service delivery brings with it the obligation to develop empirical knowledge about: 1) our societies' response to intellectual handicaps and to individuals with trisomy 21; 2) social pressures exerted both by and on pregnant women; 3) support structures for parents who continue a pregnancy when the fetus is known to present with a disorder; 4) the impact of informed consent and informed choice; and 5) the effects of direct-to-consumer tests.

## 1517/T/Poster Board #66

**Consent given from the desire to please versus the exercise of free will: The involvement of adolescents with neurofibromatosis in social science research, viewed from an ethical standpoint.** *C. Legendre, R. Drouin, C. Bouffard.* Dept Ped, Fac Med Hlth Sci, Univ de Sherbrooke, Sherbrooke, PQ, Canada.

Although the individual's free will in relation to consent is a key concern in the ethics of genetics research, several factors continue to represent obstacles to the genuine exercise of free will. Despite the existence of numerous statements of principle, laws, and guidelines designed to ensure that all are in a position to give free and informed consent, the influence exerted by those in a position of authority still plays a preponderant role in the dynamics of consent. In Canada, children may refuse to take part in a study protocol. Minors aged fourteen and over also have the right to refuse to take part in a non-medical study without their parents' permission. These measures do not, however, protect adolescents with genetic disorders, who may wish to consent, from their parents' perhaps well-meaning influence. Our research on neurofibromatosis (NF) shows that parental influence is an especially significant factor in the case of adolescents with that disorder. While they may be able to act independently as regards consent, certain aspects of NF make them particularly vulnerable to the influence of those closest to them. Methodology: Ethnographic methodology (analysis of the literature on research ethics; interviews and participant observations with members of Canadian NF associations). Results: Since participants in social science studies are in effect subject to little or no physical risks, parents' desire to see their children's lives improve often leads to the over-riding of their adolescents' right to exercise free will in granting consent When it comes to NF, this problem is exacerbated by the scarcity of psychosocial research and the importance of such studies for the intellectual and psychosocial development of children with the condition. In these circumstances, we must be concerned about the nature of the children's consent. Added to this is the known fact that children with NF are inclined to want to please and tend to have a more highly developed capacity for empathy than usual. Conclusion: Ethically speaking, it is important to take into account that, even if adolescents are unlikely to suffer parental sanctions for refusing to take part in a study, the parent-child relationship is hierarchical, with parents in a position of authority. This we must wonder about the real benefits of the right to free and informed consent when consent may be subject o the desire to please.

## 1518/T/Poster Board #67

**Direct-to-consumer genetic testing: An overview of regulatory aspects in Europe.** *P. Borry, H.C. Howard.* Biomedical Ethics and Law, Katholieke Universiteit Leuven, Leuven, Belgium.

There has been a recent rise in companies offering genetic testing services directly-to-consumers and bypassing the traditional face-to-face consultation with a health care professional from the established health care system. The presence of these services, mostly concentrated in North America, but also present in Europe and Asia, have generated a lot of debate regarding their potential benefits and harms. Supporters of direct-to-consumer genetic testing point to, among others, increased access and empowerment for consumers, and enhanced confidentiality of results as potential benefits. Meanwhile, detractors have many concerns, including, but not limited to, issues regarding tests' analytical and clinical validity, as well as clinical utility; issues related to the quality of pre- and post-test counselling; and the lack of an adequate legal framework. Through a systematic review of the literature, we present a summary of important existing European legal and ethical documents framing the sale of genetic tests sold directly to consumers. Among others, we will discuss the European IVD Directive, the Additional Protocol to the Convention on Human Rights and Biomedicine concerning Genetic testing for Health Purposes, as well as specific national initiatives to regulate these services.

## 1519/T/Poster Board #68

**The Emerging Language of Genetic Biobanking: A Contest for Transparency, Control, and Power.** *J.M. Conley[1], A.M. Davis[2], J. Cadigan[2].* 1) University of North Carolina School of Law, Chapel HIll, NC; 2) Department of Social Medicine, University of North Carolina, Chapel Hill, NC.

The Emerging Language of Genetic Biobanking: A Contest for Transparency, Control, and Power John M. Conley University of North Carolina School of Law CB#3380, Chapel Hill, NC 27599 USA This paper will report early results from a sociolinguistic study of the discourse taking place in and around the creation and maintenance of research-focused DNA repositories, or biobanks. Health researchers around the world are accelerating their efforts to develop large-scale repositories of human DNA, with an ultimate goal of deciphering the relationship between genetic variation and health outcomes. Research biobanks are being created at every level, including comprehensive, state-sponsored projects like Biobank UK and the samples being collected by numerous university-based projects in the United States and elsewhere. The latter depend on enrolling large numbers of volunteer subjects, a reality that has already provoked a vigorous ethical and legal debate (sometimes waged in court) over such issues as informed consent, the relationship between researcher and subject, and log-term control over samples and the genetic information derived from them. Strikingly, the various participants have yet to arrive at a taken-for-granted vocabulary. In each instance, the variants that are in play convey strong messagesoften with legal implications--about transparency, control and power. For example, is the subject (itself a heavily laden term) a "donor," a "participant," a "patient," or something else? Is the contribution of a sample a "gift," a "donation," a "transfer," a "loan," or even a "license"? Is the researcher a "fiduciary," a "trustee," a "custodian," or an "owner"? Even the ubiquitous "biobanking" assumes many conclusions. This paper is based on the qualitative discourse analysis of lengthy, semi-structured interviews of both research subjects and researchers. This method has previously been used to study various aspects of science and medicine, as well as such domains as law and business. The analysis reveals that the two groups (subjects and researchers) often have strikingly different ways of speaking about themselves and their relationship. Moreover, the subjects frequently voice their concerns and fears about biobanking in a vocabulary and idiom that has yet to be represented in the "official" discourses of law and medical ethics. The authors argue that these "native" concerns deserve more serious attention in law and policy debates.

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## 1520/T/Poster Board #69

**Attitudes toward human research protections in genetic research among ASHG members: Results from the Genetics Research Review and Issues Project (GRRIP).** *K.L. Edwards[1], A.A. Lemke[2], H. Starks[1], G.L. Wiesner[3], "GRRIP" Team.* 1) University of Washington, Seattle, WA; 2) Northwestern University, Chicago, IL; 3) Case Western Reserve University, Cleveland, OH.

**Background:** Protocols submitted to Institutional Review Boards (IRB) for genetic research are increasing in number and complexity. A collaboration between the Univ. of Washington Center for Genomics and Healthcare Equality and Case Western Reserve Univ. Center for Genetics Research Ethics and Law, has sought to assess experiences with and attitudes toward review of genetic research. GRRIP was conducted in two phases; 1) qualitative interviews of researchers and IRB members, and 2) national surveys of the ASHG and Public Responsibility in Medicine and Research (PRIM&R) memberships. The results from the ASHG survey are presented here, while results from PRIM&R are reported separately. **Methods and Results:** An anonymous, web-based survey was sent to the ASHG membership in April 2009. The survey included questions on experience with the genetic research application and review process, views toward specific genetic research issues, and basic demographic information. To date, 360 /4,908 individuals have responded to the online survey. The majority of respondents are female (54%), U.S. based researchers (82%), and involved in research as their primary activity. Most respondents (48%) reported 15 years or more experience in conducting human genetic research studies. Issues related to consent of subjects has caused considerable discussion with their IRB (51%), followed by procedures related to protecting personal information of research participants (35%), and return of results to participants (31%). When asked about consequences of IRB review, 54% of participants indicated that an excessive delay of a project had occurred, and 27% were dissuaded from pursuing similar projects in the future. On the positive side, 25% reported improved participant protections, 23% help in planning for future research, and 12% reported identification of potential harms to subjects that were not addressed. Participants reported they would be equally or slightly less likely to experience these consequences in nongenetic studies and generally felt that review of research protocols should not be different for genetic studies. **Significance:** Findings from this study will be used to inform a consensus process aimed at proposing guidelines regarding human subjects protection in genetic research.

## 1521/T/Poster Board #70

**Association for Molecular Pathology v. USPTO: A Controversy Involving Gene Patents, Intellectual Property Law and the U.S.Constitution.** *D.L. Greenfield[1,2,3,4,5].* 1) Center for Society and Genetics, UCLA, Los Angeles, CA; 2) American Bar Association, Sub-Committee on Reproductive & Genetic Technologies (Family Law Section) 2001-2009; 3) California State Bar; 4) Los Angeles County Bar Association Bioethics Committee; 5) Center for Policy on Emerging Technologies: http:www.c-pet.org.

The recently filed case, Association for Molecular Pathology v. U.S. Patent and Trademark Office, does not simply concern a narrow issue, a rarified intersection of intricate patent law and complex science. Rather, the ACLU suit on behalf of plaintiffs including leading groups and members of the scientific and medical research communities as well as individuals affected by breast and ovarian cancer, against defendants USPTO and Myriad Genetics, has broad and historically significant implications for human genetics. The important knowledge and discoveries of the 20th Century Human Genome project nonetheless created 21st Century social, ethical, political and legal dilemmas: controversies have arisen where tensions between biotechnological achievement and civil liberties exist. One of which is whether or not human DNA and its embodied information should be subject to intellectual property regimes, specifically, patent rights; and if allowed, are Constitutional guarantees of the First Amendment, specifically freedom of speech and expression, infringed? A discussion of the legal history and societal effects of gene patenting that brought about the unique convergence of the ACLU and the plaintiffs will introduce the issue. Following which, an argument will support the conclusion that these patents are contra to the law which specifies and defines the subject matter for which patents may be obtained, as well as free speech guarantees. The specific requirements for statutory subject matter, including exceptions to what is generally considered patentable, and definitions of allowable categories will be presented, concluding that patents on human DNA and DNA sequences, are, as both products and laws of nature, exceptions to what is statutory subject matter, and exclusions to the enumerated categories: as explained and articulated in case law, human DNA is not a process; machine; manufacture; or composition of matter. When these genes are nevertheless privatized, free speech rights are implicated. Individuals, including patients, biomedical researchers, scientists and physicians cannot use their own genes to communicate something about themselves to others and are denied access to both their own information as well as the information of the human genome: freedom of thought is restricted and academic freedom is denied. In conclusion, it will be suggested that new paradigms should govern the use and control of human DNA.

## 1522/T/Poster Board #71

**Ethical implications of research on the genetic aspects of autism spectrum disorders: A qualitative study of parental opinions in Japan.** *J. Higashijima[1], K. Kato[1,2,3].* 1) Dept Biostudies, Kyoto Univ, Kyoto City, Kyoto, Japan; 2) Institute for Research in Humanities, Kyoto Univ, Kyoto City, Kyoto, Japan; 3) Institute for Integrated Cell-Material Sciences, Kyoto Univ, Kyoto City, Kyoto, Japan.

Genetic research on autism spectrum disorders (ASD) is being given much attention these days. From a historical perspective, such as eugenics and social stigma, as well as emerging practical issues, such as privacy, informed consent, and data sharing, it is necessary to consider the ethical and social aspects of ASD's genetic research (Journal of Genetic Counseling, 15, 41-50; American Journal of Medical Genetics, Part C, 142C, 52-57). In this process, a consideration of opinions of ASD patients and parents is essential. The aims of the current research are to clarify and examine Japanese parental attitudes and knowledge regarding genetic research on ASD. Participants were recruited through a nonprofit autism agency in Japan. The data were collected through a survey of parents with at least one child diagnosed with ASD. Approval was obtained from the Medical Ethics Committee of Kyoto University, Japan, and data were gathered using a semistructured interview format developed for this study on the basis of a comprehensive literature review and on the results of earlier interview studies. Questions in the survey consisted of 4 format types: "fill in the blank," "Likert response," "nominal," and "open-ended." Also, they were divided into 4 parts: (i) Demographics, (ii) Parental knowledge of gene mutation, (iii) Parental knowledge of ASD research, and (iv) Parental attitudes toward ASD research, focusing especially on genetics. Answers and comments to open-ended questions were analyzed by content analysis in relation to quantitative information, though we conducted no quantitative analyses. About 20 surveys were completed, and our results have revealed many important issues to consider and discuss for researchers, medical personnel, and genetic counselors. For example, although many parents favored a medical diagnosis at birth, some respondents considered the prediagnosis child-rearing period necessary to gradually prepare their minds for a medical diagnosis of ASD. Some respondents expressed serious concerns over research focusing on genetic differences between ASD patients and ordinary people, though many strongly supported research on the genetic mechanism of ASD. However, respondents showed negative attitudes toward research on the causes of ASD, which concerned prenatal genetic testing and abortions, and some considered direct scientific or therapeutic benefits to the subject unimportant.

## 1523/T/Poster Board #72

**Ethical issues surrounding the research activities of companies selling direct-to-consumer genetic tests.** *H.C. Howard, P. Borry.* Biomedical Ethics and Law, Katholieke Universiteit Leuven, Leuven, Belgium.

There has been a recent rise in companies offering genetic testing services directly-to-consumers and bypassing the traditional face-to-face consultation with a health care professional from the established health care system. The presence of these services, mostly concentrated in North America, but also present in Europe and Asia, have generated a lot of debate regarding their potential benefits and harms. Supporters of direct-to-consumer (DTC) genetic testing point to, among others, increased access and empowerment for consumers, and enhanced confidentiality of results as benefits. Meanwhile, detractors have many concerns, including, but not limited to, issues regarding tests' analytical and clinical validity, as well as clinical utility; issues related to the quality of pre- and post-test counselling; and the lack of an adequate legal framework. Moreover, there is evidence suggesting that consumers may be confused as to the worth and ultimate meaning of such test results for their health. Some companies are also inviting and/or recruiting consumers who choose to purchase testing to also take part in genetic research being conducted by the company. With this specific aspect in mind, we performed a content analysis of the websites of five companies offering DTC genetic testing. We described how companies present their research activities to consumers as well as their policies regarding the storing of biological samples and data. We then discussed issues surrounding informed consent including consumer understanding, company transparency, and the right to withdraw. Finally, we also address this recruitment method and what it means to be a consumer of services versus being a research subject.

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## 1524/T/Poster Board #73

**Public attitudes toward genetic testing for children in Japan: A comparative study between nationwide opinion surveys in 2005 and 2008.** *I. Ishiyama[1], K. Muto[2], A. Tamakoshi[3], T. Maeda[4], A. Nagai[5], Z. Yamagata[5].* 1) Teikyo-Gakuen Junior College, Hokuto, Yamanashi, Japan; 2) The University of Tokyo, Tokyo, Japan; 3) Aichi Medical University, Nagakute, Japan; 4) The Institute of Statistical Mathematics, Tokyo, Japan; 5) University of Yamanashi, Yamanashi, Japan.

**Purpose:** Rapid developments in genetic technologies increase the ability to identify the asymptomatic children susceptible to disease. These developments confront parents and their children with ethical and legal issues. The purpose of this study was to assess public attitudes toward genetic testing for children, to examine the attitude change of Japanese general public by comparing two nationwide opinion surveys conducted in 2005 and 2008, and to explore the factors related to attitudes toward genetic testing for children. **Methods:** The participants were comprised of 4,000 people (age, 20-69) in 2005 and 3,000 people in 2008, selected from the Japanese general population by using the two-step stratified random sampling method. They were queried about the following topics in a mail survey: (1) attitudes toward genetic testing for disease susceptibilities of children on common diseases, (2) images of the genomic studies related to medicine, (3) awareness of the benefits and risks of genomic studies related to medicine, (4) level of genomic literacy, (5) demographic and socioeconomic background, and (6) knowledge and attitudes toward science in general. We examined the attitude change using $\chi^2$test and identified the factors related to the attitudes toward genetic testing using logistic regression models. **Results:** The response rate was 54.35% (2,171/4,000) in 2005, and 53.8% (1,613/3,000) in 2008. The genetic testing for disease susceptibilities of children on common diseases was favored by 55.5% people in 2005, and 66.2% people in 2008. A difference in the attitudes was observed ($p<0.001$). The negative image of 'scary' decreased ($p<0.01$), and awareness of the risks about 'discrimination' and 'unexpected harmful effect' decreased ($p<0.05$) in 2008. Multivariate analysis of 2008 survey revealed that positive attitudes toward genetic testing for children related to positive images of the genomic studies (the highest quartile of score versus the lowest: adjusted odds ratio (OR), 3.69; 95% confidence interval (CI), 2.57-5.30) and having cognition of 'scientific development has more advantages than disadvantages' (adjusted OR, 1.62; 95% CI, 1.26-2.09). By contrast, respondents of 40-49-year-old group (adjusted OR, 0.64; 95% CI, 0.41-0.99), and having a child (or children) actually (0.65; 0.46-0.91) showed negative relations. At the same time, increases of the genomic literacy score did not relate to positive attitude toward genetic testing for children.

## 1525/T/Poster Board #74

**Direct-to-consumer personal genomic services: An exploratory case study of product claims, disclaimers, and performance.** *R. Sterling[1], S. Adams[2], H. McLeod[3], J. Evans[4].* 1) Center for Genomics and Society, University of North Carolina, Chapel Hill, NC; 2) School of Medicine, University of North Carolina, Chapel Hill, NC; 3) School of Pharmacy, University of North Carolina, Chapel Hill, NC; 4) Department of Genetics, University of North Carolina, Chapel Hill, NC.

Advances in genomic research have enabled the commercialization of whole genome profiling or personal genomic services (PGS). Direct-to-consumer (DTC) marketing and sale of PGS has raised concerns among clinicians, researchers, and policy makers. In 2008, the Secretary's Advisory Committee on Genetics, Health and Society reported 29 gaps in the oversight of genetic services and New York and California issued a provisional moratorium on the DTC sale of PGS, fueling debates about whether restrictions that limit consumer choice are warranted to avoid potential harm to consumers. As policy makers work to improve oversight of genetic services, the continued DTC sale of PGS warrants examination of advertising. Although federal regulations do not specifically address advertising for genetic services, various laws and recommendations set forth basic requirements and a normative standard. In this study, we purchased two PGS kits from two companies (23andMe and deCODE) and examined: 1) whether websites provided information recommended by experts, 2) website claims and disclaimers, and adherence to Federal Trade Commission (FTC) requirements for product advertising, and 3) whether claims were supported by product performance. In our content analysis of websites, we found both PGS companies presented information on topics repeatedly recommended by experts, such as lab certifications, test limitations, and privacy. However, they did not meet FTC requirements that disclaimers be presented in close proximity to claims. FTC also requires that disclaimers qualify and not contradict claims; however, we found several examples of disclaimers that contradicted claims, particularly in regard to medical relevance. Finally, our independent genotyping of submitted specimens confirmed the accuracy of genetic profiles reported by both companies, but reported risk profiles showed significant variability. Of the 14 conditions profiled by both companies, risks for five conditions were reported in opposite directions for the same specimen. The current lack of a "gold standard" to guide the interpretation of genotypic profiles is a substantial limitation to the utility of PGS and poses a major challenge to advertisers charged with both increasing PGS sales and avoiding deceptive advertising. We encourage PGS companies in the US and abroad to adhere to FTC requirements regarding product claims and disclaimers to enable informed decision-making by consumers.

## 1526/T/Poster Board #75

**Resurrecting the Dead?: Research on Archived Samples (The ENGAGE Scenario).** *A.M. Tassé[1], I. Budin Ljøsne[2], B.M. Knoppers[3].* 1) Faculté de droit, Université de Montréal, Montréal, Québec, Canada; 2) Norwegian Institute of Public Health, Oslo, Norway; 3) Faculty of Medicine, Department of Human Genetics, McGill University, Montréal, Québec, Canada.

Human biological material has always been collected for different purposes: medical care, biopsies, pathology, research, rare disease archives, tumor banks, etc. These resources are highly coveted for research purposes that often differ from the initial objectives of the collection. Access and use of such archived samples and data raise legal and ethical issues distinct from those of prospective studies, especially when the samples come from deceased individuals. The secondary use of biological samples and data from deceased individuals is increasingly important for epidemiological and international research initiatives such as the European Network for Genetic and Genomic Epidemiology (ENGAGE). An analysis of the consent forms of 50 ENGAGE cohorts reveals that most did not anticipate the use of samples and data after the death of the participant/individual. Moreover, this secondary use is regulated quite differently between countries, especially when the biological material has been collected for clinical diagnostic purposes. A comparative analysis of the legal frameworks governing the secondary use of biological data from deceased individuals demonstrates that contradictory approaches can affect research objectives. In the USA, research is permissible on samples from deceased individuals whereas in Canada free and informed consent must be expressed in a prior directive provided at the time of the collection, or by an authorized third party. Within Europe, there is an equally broad range of possible options. But in the context of international collaborative research, distinctions in legal and ethical frameworks create hurdles which hinder (if not block) research. Perhaps a re-interpretation of ethical principles specific to this context can provide direction.

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## 1527/T/Poster Board #76

**Enabling Responsible Public Genomics.** *D.B. Vorhaus[1], J.M. Conley[2], D.J. Clark[1].* 1) Genomics and Genetics Practice Group, Robinson, Bradshaw & Hinson, P.A., Charlotte, NC; 2) School of Law, University of North Carolina, Chapel Hill, NC.

As understanding of the complex relationship between genes and traits advances, researchers require increasingly rich datasets that combine genomic, phenotypic and environmental data from increasingly large numbers of individuals. A dramatic decline in the cost of genomic sequencing has made such datasets economically feasible. The generation of individuals' genomic sequence data inescapably produces clinically actionable information even as the linkage of that data with personal and phenotypic data simultaneously precludes traditional privacy promises; features not contemplated by the established ethical, legal and social framework governing human genomic research. The researcher's duty with respect to clinically significant data, the boundary between genomic research and the practice of medicine and the challenges to privacy presented by the return of genomic dataall these issues must be addressed if the next generation of genomic research is to be pursued in a responsible fashion.

Responsible public genomics combines identifiable data contributed by an informed and fully consenting cohort of information altruists with a public domain database model, resulting in rich datasets that maximize research potential and seed a genomic commons that is freely and globally accessible, enabling ethically, legally, and socially responsible genomic research in full view of the public. This Article examines the risks and benefits of the public genomics model in the context of one of the most ambitious genetic research project currently under waythe Personal Genome Projectand (i) demonstrates that large-scale genomic projects are both inevitable and desirable, (ii) evaluates the risks and challenges presented by public genomics research, and (iii) assesses the current legal and regulatory regimes that govern public genomics research and determines that even as they fail to adequately protect research subjects they simultaneously restrict beneficial and responsible scientific inquiry. The proposed solution is a modified normative and legal framework that embraces and enables a future of responsible public genomics.

## 1528/T/Poster Board #77

**Using Genetics to Overturn The Legacy of Slavery? The Hope and the Hype of Popular Representations of Personal Genomics, U.S. African Americans and Genetic Ancestry Testing.** *E. Clay.* Zygon Center, Lutheran School of Theology at Chicago, Chicago, IL.

The hope that advances in genomic research would discredit the biological legitimacy of 'race' as an operative category in research design and practice has been tempered by misleading hype in popular media that uncritically promotes social categories of race as biologically distinct; hype initiated in part by sensationalized genetic narratives and misrepresentations or omissions within researcher statements. In recent years, media coverage of genetic genealogy, ancestry-testing estimations, and approximated ancestral origins for U.S. born African Americans or Black British persons has increased. Described in several films as a means to overturn the legacy of slavery and reclaim black identity, ancestry tests are framed as a means to achieve personal empowerment and reconcile ancestral origins. These claims go far beyond what can be supported by currently available scientific methods. Expanded media attention to personal genomics, the marketing of direct-to-consumer (DTC) genetic testing and dramatic media portrayals of race and ancestry pose social, ethical, educational, and policy challenges. This paper provides an overview of representations of genomics, race and ancestry. Examples of hype in film, radio shows, and news print media from 2003 to 2009 are examined to investigate the role that messages of personal empowerment, images of travel to homelands and genetalk of newly discovered genetic 'kin' play in the representation of African American consumption of personal genomics. Many representations inaccurately depict human genetic diversity and seldom question the utility, direction, or methods of ancestry tests Media storylines not only make grandiose claims concerning ancestry tests, they also map older social categories of race onto less than 1% of human genetic variation. Following thematic plots of reversal of fortune or a quest for heritage, popular media portrays test-takers as pioneers, geneticists as keepers of secret knowledge, and genetic tests as having the ability to predict the past and the future well being of persons with certainty. The ethics of manufactured demand for a product of limited utility and validity should be scrutinized. Serious information asymmetries between testing companies and consumers call for increased regulation of ancestry claims and commercial genetic testing. Lastly, the legal implications of using ancestry tests to grant U.S. African Americans dual citizenship in African countries are considered.

## 1529/T/Poster Board #78

**Understanding the impact of long qt syndrome in a BC First Nations community.** *L. Huisman, R. McCormick, L. Arbour.* The University of British Columbia 3800 Finnerty Road Victoria B.C. V8P 5C2.

**Background:** There is a disproportionately high rate of hereditary Long QT Syndrome (LQTS) in Northern British Columbia First Nations people, partly due to a novel missense mutation in KCNQ1 (V205M). The effect has been previously described (Arbour et al 2008) predisposing those affected to syncope, arrhythmia and sudden death. A community based participatory research approach has enabled over 200 community members to take part, identifying more than 30 carriers of the mutation. Although a great deal of previous research has been carried out on the biological aspects of LQTS, there has been little study into the impact of living with a mutation that predisposes to sudden death, and no previous studies have provided cultural insights into the issues a remote First Nations community might face. **Objectives:** 1) From a cultural context explore what facilitates and hinders resiliency and coping for individuals and families 2) To explore issues of barriers to effective physical and psychological management, 3) understand the multigenerational impact of LQTS. **Methods:** Participants were invited to partake in their choice of 1) One to one interviews 2) photovoice and 3) talking circles. Interviews and talking circles were recorded, transcribed, and analyzed qualitatively using the Systematic Text Condensation method. **Results:** Consistent with the core study, men were reluctant to participate, however ten women shared their personal experiences of living with LQTS. Five of the women had known mutations and 5 had unknown genetic status. Most had affected children. In general, learning about a LQTS diagnosis was perceived as traumatic, with gradual acceptance that lead to coping. The main factors that facilitate resiliency and coping are: 1) positive family relationships, 2) spiritual faith, and 3) knowledge about LQTS. The main factors that hinder resiliency and coping were: 1) a poor understanding of the biological or clinical aspects of LQTS, 2) conflicting medical advice, especially about necessary physical restrictions, 3) LQTS not being taken seriously by both social contacts and health care providers, and 4) concern for the wellness of the next generation. **Conclusion:** Learning to live with LQTS is an ongoing process, requiring balance and interconnectedness between all aspects of wellbeing. These issues warrant further exploration. Recommendations to enhance genetic counseling within FN communities will be presented to reflect the Medicine Wheel concept.

## 1530/T/Poster Board #79

**Genetic health attitudes and reproductive intention in college students: Implications for public health genomics.** *H.H. Honoré[1,2], L-S. Chen[3], P. Goodson[3], D.M. Latini[1,2].* 1) Michael E DeBakey VA Med Ctr, Houston VA HSR&D Center of Excellence, Houston, TX; 2) Baylor College of Medicine, Houston, TX; 3) Department of Health & Kinesiology, Texas A&M University, College Station, TX.

Purpose: Although the relationship between certain disease-specific risk perceptions and sexual/reproductive intentions has been previously studied, fewer studies have explored the relationship between individual perceptions of genetic/genomic risk and these intentions. As college students are of reproductive age, this mixed-methods study examined the relationship between genetic risk perceptions and reproductive decision-making among college students. Method: This study consisted of qualitative (8 focus groups; n=86) and quantitative (survey; n=2,576) phases. Participants were primarily young, female college students. We asked participants how genetic/genomic risk might affect their reproductive decision-making. Responses were assessed using content analysis. Later, we developed hypothetical genomic-related scenarios (i.e., individuals with unknown genotype, asymptomatic carrier status, symptomatic carrier status, genetic/genomic-related physical disabilities, genetic/genomic-related mental disorders, and any genetic/genomic disorder). We used descriptive analyses to examine reproductive intentions under each scenario. Results: Focus group participants expressed overall negative attitudes related to childbearing with individuals having personal/familial histories of mental and physical health issues. These issues were considered "genetic" and stigmatized. Survey respondents were more likely to have childbearing intentions towards unknown genotype (41.7%), asymptomatic (26.2%) and symptomatic (18.4%) carriers, or any genetic disorder (17.1%) than those perceived to have physical disabilities (13.4%) or mental disorders (8.4%). Conclusion: Our mixed data show similar patterns: negative attitudes and lower reproductive intentions towards individuals with genetic/genomic disorders. With increased genetic/genomic test availability, caution must be taken to avoid genetic/genomic stigma and discrimination. Additional research is necessary to determine psychosocial factors influencing the perceived genetic risk-intentions relationship and develop psycho-educational and stigma-reducing interventions.

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## 1531/T/Poster Board #80

**Genomic Medicine: The Tip of the Iceberg.** *A. Pai.* Healthcare Practice , IBM Global Business Services, Toronto, ON, Canada.

Recent data on genomics from a number of publications and Web sites demonstrate that the role of genomics in healthcare represents the "tip of the iceberg". As genomics technologies (e.g. genomic testing) improve in sensitivity and decrease in cost, genomic medicine will reach a tipping point and become an integral part of health care delivery and a key part of a citizen's electronic health record. Healthcare stakeholders thus have a remarkable opportunity at their hands; one that promises to increase in uptake as such technologies mature. Healthcare and wellness decisions will routinely include a genomic component and change its approach to care delivery from retrospective, interventional care to prospective and preventative care which is highly personalized and pre-emptive. More specifically, in the case of chronic diseases such as diabetes, mental health disorders and cancer, such data will lead to significant pre-emptive measures to prevent the onset of disease, years in advance of symptoms appearing. Genomic data will thus influence chronic disease and prevention management strategies. Key enablers in this regard include genomic literacy, privacy and security and the role of e-health (and corresponding information technology). The impact of genomic medicine will also not occur in isolation, but will instead converge with advances in information technology (e.g. the Internet and electronic health records), communication (e.g. social networking), and other innovative areas of medicine (e.g. clinical research and regenerative medicine). Instead of debating whether genomics is really impacting healthcare or not, it will be prudent for healthcare leaders to grapple with how quickly the game will change as a result of genomics. Given the importance of success of enablers such as genomic literacy, privacy and security of genomic information and the key role that e-health and information technology will play in this regard, strong policy measures that support these enablers will be crucial for the successful incorporation of genomic medicine in healthcare.

## 1532/T/Poster Board #81

**Population Prevalence of Stratified Familial Cancer Risk and Common Hereditary Cancer Syndromes: The 2005 California Health Interview Survey.** *M. Scheuner[1,2,3], T.S. McNeel[4], A. Freedman[5].* 1) RAND Corp, Santa Monica, CA; 2) VA Greater Los Angeles Healthcare System, Los Angeles, CA; 3) Department of Health Services, UCLA School of Public Health, Los Angeles, CA; 4) Information Management Services, Inc., Silver Spring, MD; 5) National Cancer Institute, Bethesda, MD.

Background: Family history helps guide cancer screening, prevention, and referral for genetic services. Using data from the 2005 California Health Interview Survey, we estimated the population prevalence of increased familial risk for breast, ovarian, endometrial, prostate and colorectal cancers and hereditary cancer syndromes that include these cancers. Methods: For each respondent, a weak, moderate, or strong familial risk was assigned for each cancer type using rules that consider age at diagnosis, number of affected 1st and 2nd degree relatives, their relationship to each other, and occurrence of related cancers. Guidelines were applied to identify individuals with family histories suggestive of hereditary breast-ovarian cancer (HBOC) and hereditary nonpolyposis colon cancer (HNPCC). Results: Of 33,187 respondents between the ages of 18 and 64 years, 2% reported a personal history of breast, ovarian, endometrial, prostate or colorectal cancer. Among respondents without a personal history of these cancers, women had higher prevalence of both moderate and strong familial risk for each cancer, except prostate cancer. Whites had the highest prevalence, and Asians and Latinos had the lowest prevalence of moderate and strong familial risk for each cancer. Of all familial cancer, familial breast cancer was most prevalent (5% of family histories were strong and 7% moderate risk). Among women without history of breast or ovarian cancer, 3% met criteria for HBOC; 89% had a strong and 11% a moderate familial risk for breast or ovarian cancer. Of women who did not meet HBOC criteria, only 4% had a strong and 9% a moderate familial risk. Among individuals without history of colorectal, endometrial or ovarian cancer, 0.3% met criteria for HNPCC; 99% had a strong and 1% a moderate familial risk for these cancers. Of those who did not meet HNPCC criteria, only 3% had a strong and 7% a moderate familial risk. Conclusions: Based on the first large state survey of familial cancer history, we provide population-based prevalence estimates for moderate and strong familial risk for five common cancers and family histories consistent with HBOC and HNPCC. Among respondents without a personal history of cancer, 3% of the population had a family history indicative of HBOC and only 0.3% for HNPCC. Such estimates will be helpful for planning and evaluation of genetic services and prevention programs, and for assessment of cancer surveillance and prevention strategies.

## 1533/T/Poster Board #82

**A Policy to Develop Public Trust; the Discourses of Accountability and Transparency in Biobank Japan.** *S. Iwae.* Institute for Research in Humanities, Kyoto University, Japan.

It has been known that a human biobank project potentially generates a number of complex ethical and social issues. So, the necessity of establishing adequate governance framework has been emphasized in biobank researches. In particular, it can be said that the core of the governance policy is fostering public trust, which also consists of two significant components: accountability and transparency. In this research, I explored how those two factors were actually interpreted in the governance framework of Biobank Japan by interviews with several people involved in Biobank Japan project. Eventually, it seemed that solid and consistent governance policies did not exist in Biobank Japan, because people, who were involved, had many different views toward how accountability and transparency should be in the project. Such condition implied that those various views and activities in Biobank Japan were not well-organized and guided to build up the public trust. Finally, some future policy suggestions on how a biobank project may develop public trust will be discussed in this presentation.

## 1534/T/Poster Board #83

**If You Build It ... Will They Come?: Hurdles to Biobank Access.** *B. Knoppers, S. Wallace, S. Pathmasiri.* Dept CRDP, Univ de Montreal, Montreal, PQ, Canada.

In the last decade, public investment and participation in large, population genomics research studies has been constant. When such studies are harmonized, they can increase statistical power as well as serve as a resource for validation, replication or comparison (controls). The time to access such infrastructures has come. But how realistic are the possibilities for access and use by researchers? The identification of the core access elements across 21 major population genomics biobanks reveals both convergence and divergence. Not surprisingly, approaches to intellectual property vary greatly (if addressed at all!). But a myriad of other procedures might also hinder access and so thwart the "research -resource" mission of such biobanks. They include lack of clarity and transparency as to: i) the process of evaluation; ii) local requirements; iii) costs of access, and iv) necessary prior levels of ethics or security approvals. Providing an analysis of core elements and proposing a model for international sample and data access may foster their actual use for research.

## 1535/T/Poster Board #84

**Ethics in the Age of Direct-to-Consumer Personal Genomics: Evaluating Traditional Approaches towards Protecting the Public.** *S. Lee.* Dept Biomedical Ethics, Stanford Univ, Palo Alto, CA.

The rapid commercialization of cutting edge technologies often evokes challenging social and ethical questions. Over the past several years, increasingly efficient high throughput bioinformatics technology and ubiquitous internet use among the public have laid the foundation for academic and commercial efforts that provide personal genetic information directly to consumers and research participants. These developments have prompted a debate over the clinical utility of these services, focusing on questions over what information should be returned to individuals, what criteria should be used in making these decisions and who should be ultimately responsible for delivering results. As yet, there has been little empirical work that examines how the public understands, interprets and applies personal genomic information. This paper discusses initial findings from a qualitative pilot study of the attitudes, expectations and actions of consumers who have purchased personal genetic information through the internet. This paper evaluates traditional approaches to ethical issues concerning genetic testing that build on assumptions that genetic information is unique and discusses these in the context of attitudes among study participants. Building on emerging data from ongoing research of public attitudes and uses of personal genomic information, this paper addresses whether a paradigm shift is occurring in how genetic information is understood and applied by the public and discusses key social, ethical and policy issues related to this growing field.

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## 1536/T/Poster Board #85

**Un-enrolled Family Member Information Collected in NIH Sponsored Family Genetic Studies - Privacy Risk?** *M.T. Quinn Griffin[1], G.L Wiesner[2], M. Flatt[3], S. Lewis[2].* 1) Frances Payne Bolton School of Nursing, Case Western Reserve University, Cleveland, OH; 2) Department of Genetics, School of Medicine, Case Western Reserve University, cleveland, OH; 3) Department of Bioethics, School of Medicine, Case Western Reserve University, Cleveland, OH.

Purpose: Family genetic studies collect information on enrolled and un-enrolled family members. While privacy concerns have been raised over the collection and use of family based data, it is not clear what type of information is gathered and how it is used by researchers. Un-enrolled family members may be at particular risk for breeches of privacy as they did not provide informed consent for the collection of their information. The purpose of this study was to describe the collection of un-enrolled family member data in family genetic studies. Methods: 211 abstracts of eligible studies were identified through the online "CRISP" (Computer Retrieval of Information on Scientific Projects) database of projects funded by NIH. An email with a link to an online survey was sent to 175 unique investigators. The survey questions addressed the recruitment of family members, collection of demographic and health data, and use of pedigree information. 17 investigators did not match the inclusion criteria or had incorrect email addresses. The response rate was 30.4% (n=52). Most of respondents held Doctoral degrees (55.8%; n=29) and were experienced. Results: Almost one-half of the respondents (48.1%; n=25) asked participants asked to provide demographic or health information about un-enrolled family members. Five (10%) collected identifiers such as address while 7 (13%) recorded telephone numbers. Of these, 22 (88%) indicated that they drew a pedigree based on the information. The majority annotated the pedigree with health and personal/demographic information. Seventeen respondents (41.5%) included the pedigree in publications and 13 of these (81.2%) modified it before publication. There was no consistent method for gathering the family member's contact information from the proband, Eleven (21%) asked the proband for family members contact information before the family member expressed interest in being contacted. Conclusions: Varying amounts of data are collected from enrolled family members about their un-enrolled relatives. These data are used in pedigrees, many that are modified and published. This poses a risk of identification of these un-enrolled family members and the potential of a breach of privacy. This suggests a need for guidelines on specific protections for un-enrolled family members that may be useful to both researchers and to committees involved in the review of human subjects' research. Supported by CGREAL and NIH grant P50-HG003390.

## 1537/T/Poster Board #86

**Protecting Privacy, Trust, and the Future of Individualized Medicine: An analysis of genomic research practices and policies that fail to account for DNA as a unique identifier, and recommendations on how to fix them...** *J.T. Wu, B. Koenig.* Program in Professionalism and Bioethics, Mayo Clinic, Rochester, MN.

The growth of bioinformatics and genetic technologies has greatly increased the availability and accessibility of individual DNA data. This creates profound challenges to conventional notions of genetic privacy protection. Current policies regulating the collection and use of DNA data rely on administrative "anonymization" or "de-identification," and individual control of genetic information to protect privacy. As genomic and bioinformatics technologies have developed, current regulatory policy has fallen behind. Current policy cannot, and does not account for most of the privacy concerns that arise with the development of large-scale genetic biobanks and genome wide association studies, such as breaches in data security, re-identification of individual DNA data, and lapses in informed consent. This paper contains policy recommendations that aim to improve regulatory policy dealing with the collection, use, and dissemination of DNA data. A unique feature of our project was the structured integration of empirical and normative analyses. First, we conducted a review of conceptual literature relevant to genetic privacy and biobanking. This paper will provide an account of the social, scientific and legislative evolution of individualized medicine research. Second, we completed a collection of descriptive data from interviews with key informants. We conducted 15 semi-structured interviews with key stake holders in the fields of privacy, forensics, data safety, personalized medicine research, and government. Based on the qualitative analysis of our interviews, we will discuss the major themes addressed by the interviewees. Finally, we organized a multidisciplinary advisory board meeting where expert consultants came together to discuss the implications of the previous steps and outlined concrete policy recommendations based on prior steps. Here we present the major finding from each step in the above process.

## 1538/T/Poster Board #87

**A Framework for the Delivery of Genetic Testing: A Foundation for Translational Genomics Research.** *T. Bentley[1,2], S. Olmsted[3], E. Eiseman[1], S. Wooding[4], B. Kim[1,5], M.T. Scheuner[1,2,6,7].* 1) RAND Corporation, Santa Monica, CA; 2) UCLA Center for Health Policy Research, Los Angeles, CA; 3) RAND Corporation, Pittsburgh, PA; 4) RAND Europe, Cambridge, UK; 5) Department of Hematology-Oncology, David Geffen School of Medicine at UCLA, Los Angeles, CA; 6) VA Greater Los Angeles Healthcare System, Los Angeles, CA; 7) Department of Health Services, UCLA School of Public Health, Los Angeles, CA.

Purpose: With increasing availability of molecular genetic tests, strategies are needed to increase access to quality genetic testing services. We have created a model for the delivery of genetic tests to understand how best to organize and strategically plan for the delivery of these services. Methods: We adopted an existing structure-process-outcome model of provider behavior to characterize the factors that might influence the delivery of genetic tests used for medical decision-making. These factors reside in four key domains: external environment, organizational structure and processes, and provider and patient characteristics. We defined the different provider types that would be involved for various genetic testing indications, outlined the steps involved in delivering genetic tests, and detailed the process and provider assumptions. We convened an advisory board to validate our approach and refine our framework. Results: Our resulting seven-step framework represents the logical and temporal sequence of the delivery of genetic testing services. The steps include: (1) selection of the individual for testing, including assessment of pre-test probabilities and post-test implications; (2) selection of the test; (3) pre-test communication, including informed consent if necessary; (4) test uptake decision; (5) [if testing chosen] ordering of test; (6) results interpretation and disclosure; and (7) provision of medical recommendations. In the absence of testing, medical recommendations follow pre-test communication. The frequencies of actions taken at each step represent the outcomes of interest, which are influenced by use of clinical decision support, genetic counselors, and clinician referrals. The framework serves as a basis upon which to model specific genetic tests for the indications of susceptibility, diagnostic, prognostic, and pharmacogenetic testing. Types of providers include primary care clinicians, geneticists, other specialists, and direct-to-consumer marketing firms. Conclusions: We have developed a framework for the delivery of genetic tests that can be used in future translational genomics research including modeling studies, implementation research, clinical trials, and comparative effectiveness studies. By identifying processes and factors that influence the delivery of these services by different types of providers, applications of this framework will guide future development, planning and evaluation of genetic testing services.

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## 1539/T/Poster Board #88

**Informing evidence-based policy for expanded newborn screening: Engaging the general public about the provision and governance of newborn screening.** *Y. Bombard[1], F.A. Miller[1], J.C. Carroll[2], D. Avard[3], R.Z. Hayeems[1], J. Allanson[4], J. Bytautas[1], P. Chakraborty[4], Y. Giguere[5], J. Little[6], K. Senecal[7], B. Wilson[6].* 1) Faculty Medicine, Department of Health Policy, Management and Evaluation, University of Toronto, Canada; 2) Department of Family and Community Medicine, Mount Sinai Hospital, University of Toronto, Canada; 3) Centre for Genomics and Policy, Department of Human Genetics, McGill University, Montreal, Canada; 4) Department of Genetics, Children's Hospital of Eastern Ontario, and Department of Pediatrics, University of Ottawa, Canada; 5) Department of Medical Biology, Centre Hospitalier Universitaire de Quebec (CHUQ), University of Laval, Canada; 6) Department of Epidemiology and Community Medicine, University of Ottawa, Canada; 7) Centre de recherche en droit public, University of Montréal, Canada.

**Purpose:** Continued debate about expanded newborn screening (NBS) has led to calls for stakeholder engagement to inform policy. Research reports consumers' attitudes towards NBS, but little reflects the perspectives of the general public - important stakeholders of public health initiatives. **Methods:** We conducted a public engagement study using deliberative focus groups and pre-and post- questionnaires to assess the public's values and attitudes toward NBS. Participants were recruited through community agencies and advertisements to ensure socio-economic, ethnic, age and family life diversity. **Results:** Five focus groups were conducted (n=36). The majority supported expanded NBS that identifies serious disorders with highly effective treatment (36/36:100%), where treatment exists to reduce severity (34/36:94%) or where no treatment exists (26/36:74%). Participants reasoned that expanded NBS provides information, which even in the absence of treatment can help families plan for the future and help research. Potential harms, such as learning false positive or carrier results, were often minimized, but some respondents identified risks from stigma and unwanted knowledge. On consent, participants preferred that parents be *strongly encouraged* to have their baby screened (27/36:77%); they differentiated between highly treatable conditions where screening should be *required* (27/36:75%) and untreatable conditions where parents should be *able to choose* whether to have their baby screened (21/36:58%), but generally endorsed the need to ensure that parents were informed. The majority supported storing NBS samples for quality control (35/36:97%), diagnosis (35/36:97%) and anonymous research purposes (33/36:92%), but were ambivalent towards law enforcement or unspecified purposes (15/36:42% for both). Most preferred that parents should be s*trongly encouraged* to have their baby's sample stored (28/36:77%). The impact of deliberation on attitudes will be discussed. **Conclusions:** Most participants anticipated benefits from early disease identification through NBS, and expected few if any harms. Respondents anticipated that parents could make effective use of NBS results, and expected them to be able to make decisions about the scope of screening after being informed, ideally in the prenatal period. These findings should be considered in developing policy in this controversial domain.

## 1540/T/Poster Board #89

**Direct-to-Consumer Genetic Testing: Recommendations from the Secretary's Advisory Committee on Genetics, Health, and Society.** *S. Au, Secretary's Advisory Committee on Genetics, Health, and Society.* Genetics Program, Hawaii Dept Health, Honolulu, HI.

Advances in genetic and genomic technologies have allowed increasing amounts of genetic information to be provided to patients and consumers with declining costs. At the same time, there has been a shift toward consumer-driven health care and patient empowerment. Capitalizing on these technological and social developments, commercial entities offering genetic services directly to consumers have emerged. The unprecedented speed at which genetic technologies have been translated into commercial products, however, has raised some concerns. The Secretary's Advisory Committee on Genetics, Health, and Society (SACGHS) has developed a paper that outlines issues for consumers to consider in making an informed decision about using the services of companies that offer direct-to-consumer (DTC) genetic testing and the information they receive through testing. SACGHS was established to assist the Department of Health and Human Services (HHS) and, if requested, other Federal agencies in exploring issues raised by the development and application of genetic and genomic technologies, and the Committee makes recommendations to the HHS Secretary on how to address such issues. This presentation will describe areas of concern raised by DTC genetic testing, particularly potential issues that may arise when a consumer's personal health provider is not involved in health decisions or if government regulations do not apply to entities providing DTC testing. It will also highlight recommendations from prior SACGHS reports that address concerns about genetic testing regardless of the route in which tests are ordered (i.e., DTC or through a consumer's personal health care provider) and identify issues not adequately addressed by prior SACGHS recommendations. The scope of the paper is limited to DTC genetic testing that provides risk assessment or diagnosis of disease or health conditions, and information about drug response or other phenotypic traits. It excludes forensic analyses and paternity and ancestry testing.

## 1541/T/Poster Board #90

**The HumGen Spanish Portal Human Genetics Database of Law and Policies: A tool for the policy-making process in Latin America.** *P.F. Oliva-Sanchez[1], A. Arellano[1], C. Lara[1], R. Isasi[2], B. Knoppers[2], G. Jimenez-Sanchez[1].* 1) National Institute of Genomic Medicine, Mexico City, Distrito Federal, Mexico; 2) Research Center in Public Law (CRDP) at the University of Montreal.

Spanish is the second most-spoken language in the world, after Mandarin Chinese. Researchers, policy-makers, and the general public need accurate information to develop policy and legal regimes to inspire fruitful contributions to these debates. Due to the lack of this information in Latin America (LA), The National Institute of Genomic Medicine of Mexico (INMEGEN) and the Research Center in Public Law (CRDP) at the University of Montreal is participating in The HumGen International Project, developing it's Spanish portal. This project is an existing database of laws and policies in Human Genetics. The database is available in English and French to. HumGen includes Spanish-language sources and reach a Spanish-speaking audience. The portal is a research database of international, regional, and national instruments concerning aspects of human genetics. In the selection criteria we searching and included, documents that aim to set standards, either binding (such as laws and regulations) or non-binding (such as policies and recommendations of professional associations). We based the searching process in a list of keywords that includes important areas in like intellectual property or discrimination. The process of search and selection of documents is done by scientists to be part of the INMEGEN Team. The documents are sent to Canada Team, those who administer the portal.The Updated of the database HumGen began in August 2008. We found 252 new documents but only 46 documents in Spanish, were selected and added to the 64 documents that were available in the portal. From the documents found, 35% are laws and 65% are policies. By type of coverage for 22% of documents are national, 22% are international and 54% are regional. Documents were found belonging to 18 countries, of which 12 have Spanish as the official language. Regarding the spread of this portal, INMEGEN has conducted various academic events in institutions like the Senate of Mexico. Currently is organizing a presentation of the portal in the Pan American Health Organization. The Internet is the leading media for democratizing and disseminating knowledge and connecting providers of information with users. This portal serves for the dissemination of relevant information to policy-making in human genetics over Internet, aimed to researchers and policy-makers in the Hispanic countries, who are adapting in their cultures, new laws and policies based in a international regulatory framework.

## 1542/T/Poster Board #91

**Genome-wide genotyping linked to electronic medical records: demographics, ancestry, and other factors influencing consent in a large pediatric cohort.** *J.H. Flory[1], R. Chiavacci[1], K. Annaiah[1], W. Glaberson[1], H. Zhang[1], K. Wang[1], C. Kim[1], R. Grundmeier[2], S. Ostapenko[2], A. Thomas[1], K. Harden[1], A. Hill[1], S. Wildrick[1], C. Johnson-Honesty[1], K. Fain[1], S. Harrison[1], C. Drummond[1], D.E. Surrey[1], S.F. Grant[1], H. Hakonarson[1].* 1) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Center for Biomedical Informatics, Children's Hospital of Philadelphia, Philadelphia, PA.

Collection of genome-wide data is important for research and expected to become relevant to clinical practice. For the first 21,000 children recruited by the Center for Applied Genomics (CAG) at Children's Hospital of Philadelphia (CHOP), genome-wide data have been linked to their electronic medical record (EMR) for research. Data from CAG's enrollment, data security, and analytic activities can help inform debates over how genetic data can safely be used in research and ultimately clinical care.

80-95% of parents were willing to enroll their children for genome-wide genotyping at CAG from 2006-2009. This rate varied depending on enrollment site and the identity of the person obtaining informed consent. About 15% of parents only consented on the condition that there be no persistent link between the genetic data and their child's EMR or further contact with investigators. Consent to both further contact and persistent linkage to EMRs rose with time (80% in 2007 versus 90% in 2008, $P < 0.0001$). Participants' health had little effect on consent.

CAG was able to enroll a primarily African-American and Caucasian population roughly reflecting the demographics of CHOP's patient population. Multidimensional scaling to study participants' ancestry showed that self-reported race was accurate but that the African-American population in particular was highly admixed. After multivariable adjustment Caucasians were more willing than African Americans (OR=1.44, 95% CI 1.31-1.59) to consent to keeping data identifiable and Asians were less willing (OR = 0.68, 95% CI 0.54-0.85)). A history of visits to CHOP for routine care was also associated with consent to keep data identifiable (OR = 1.3, 95% CI 1.0-1.7, P=0.03).

The results show a substantial majority of Americans from diverse backgrounds in the Philadelphia area trust a large health care institution to collect their genetic information, and most are willing to have persistent linkage between that information and their identity. Several factors extrinsic to participantsnotably the year of recruitment and the identity of the recruiter who approached themhad a significant effect on willingness to give consent.

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## 1543/T/Poster Board #92

**Developing an Evidence Review Process for Newborn Screening Decision-Making.** *R. Howell[1], M. Lloyd-Puryear[2].* 1) Prof & Chair Emerit, Dept Ped, Univ Miami Sch Med, Miami, FL; 2) Maternal and Child Health Bureau Health Resources and Services Administration 5600 Fishers Lane Rockville, MD 20857.

When to test? What test to order? When to treat? How to treat? Who to treat? What guides our decisions that affect patient care and the publics' health in the provision of genetic testing and services? The development and use of systematic evidence reviews, professional guidance, and expert opinion will be considered as components of the decision making process in deciding what newborn screening and genetic services should be offered, to whom, and for what purpose within State newborn screening (NBS) programs. In the US, over 4 million infants a year receive a series of NBS tests. Standard criteria for synthesizing evidence are widely accepted for the evaluation of screening tests and treatments for common conditions. However, criteria need to be adjusted for evaluating screening for rare conditions, such as those identified by NBS. The screening tests, methods of diagnosis, and treatments for many conditions that NBS identifies have not undergone the large-scale and randomized trials that are the standard for other domains. The **Advisory Committee on Heritable Disorders in Newborns and Children** was created by Congress and began its work in 2004. The Committee's charge is to advise and guide the Secretary of the federal Department of Health and Human Services regarding the most appropriate application of childhood and universal newborn screening tests, technologies, policies, guidelines and programs for effectively reducing morbidity and mortality in newborns and children having or at risk for heritable disorders. The Committee also provides advice on a specific grant program delineated in its authorizing legislation. This presentation will outline the Committee's decision and recommendation process and its implications for screening newborns and children. We will also address other relevant Committee activities.

## 1544/T/Poster Board #93

**Transforming Research Practices: Achieving Benefits through Accountability.** *K. Fryer-Edwards[1], S. Goering[2], S. Holland[3], G. Geller[4], R. Sharp[5], M. Yarborough[6].* 1) Bioethics and Humanities, University of Washington, Seattle, WA; 2) Philosophy, University of Washington, Seattle, WA; 3) Religious Studies and Bioethics, University of Puget Sound, Tacoma, WA; 4) Berman Institute of Bioethics, Johns Hopkins University, Baltimore, MD; 5) Department of Bioethics, The Cleveland Clinic, Cleveland, Ohio; 6) Center for Bioethics and Humanities University of Colorado Denver, Aurora, CO.

The promise of biomedical research depends, quite pragmatically, on the translation of basic scientific findings into therapeutic applications that improve health. The translational imperative is particularly strong for areas of research like human genetics that carry a substantial expectation of benefit, and have involved significant investment. Yet some studies suggest that only 5% of "highly promising" basic genomic science findings ever result in applications suitable for clinical use, and only 1% are used according to clinical guidelines. This state of affairs suggests significant problems of translation. In the United States, the National Institutes of Health have recognized the importance of providing incentives for researchers to attend to translational obstacles through the creation of Clinical and Translational Science Awards (CTSAs). Such awards, given to interdisciplinary teams of researchers, are designed to speed the movement of new knowledge gained through bench science to the bedside, into regular clinical practice, and ultimately to improve community health. Yet while efficiency of translation is no doubt important, more fundamental questions remain: who benefits, and is justice served? Our work has focused on examining how standard research practices can inadvertently create harms, and how transformed practices might be possible. The usual practices of open data sharing, working with anonymized samples, prioritizing discovery-based science and publications in peer-reviewed journals, have done much to advance the research enterprise while not necessarily staying in touch with public needs and interests. In 2008, we met with community-health leaders in Colorado to explore issues related to translational research, and biobanking with genetic material more specifically. We heard clear messages regarding the need for the research institution to build relationships and develop two-way lines of communication, as well as have transparent systems of accountability. For example, participants discussed: developing mechanism for shared authority; traceable samples so participants can learn who has their samples, for what purpose; appreciating need for specific opt-out or withdrawal. This abstract reports on the work from two research groups: Testing Justice (funded by Greenwall Foundation and NHGRI through the Center for Genomics and Healthcare Equality) and TIES, or Trust, Integrity, and Ethics in Science (funded in part by NHGRI).

## 1545/T/Poster Board #94

**Age-related differences in attitudes toward participation in genomic research.** *S.M. Fullerton[1], S.B. Trinidad[1], J. Bares[1], G.P. Jarvik[2], E.B. Larson[3], W. Burke[1,2,3].* 1) Bioethics & Humanities, Univ Washington, Seattle, WA; 2) Medical Genetics, Univ Washington, Seattle, WA; 3) Center for Health Studies, Group Health Cooperative, Seattle, WA.

Members of the public are increasingly being called upon to participate in a range of large-scale genomic research projects designed to advance our understanding of genetic contributions to health and disease. However, relatively little is known about prospective participants' perceptions of the benefits and risks associated with genomic research, or how these perceptions might vary according to key demographic characteristics, such as age at time of recruitment. To explore the effect of age on attitudes toward genomic research participation, we compared responses of participants in 6 focus groups with members of a non-profit health maintenance organization (HMO), Group Health Cooperative in Seattle, Washington. The study, conducted as part of a Group Health-University of Washington eMERGE (electronic Medical Records and Genomics) project, was designed to elicit the viewpoints of consumer stakeholders regarding a number of ethical issues surrounding involvement in genome-wide association studies (GWAS) including data-sharing, privacy, secondary research uses, and informed consent. Two focus groups were held with each of three categories of HMO members not currently participating in Group Health-sponsored research: (1) those aged 18-34 (average age 27), (2) those aged 35-49 (average age 42), and (3) those aged 50 and above (average age 66). In these discussions, older participants (age 50+) were more likely to express altruistic motives for research participation and trust in Group Health as both health-care provider and research institution, whereas younger participants (age 18-50) were more likely to suggest that they would not be comfortable with broad terms of consent and would expect on-going engagement with the research team. Contrary to expectation, the major age-correlated difference was in views regarding risks to genetic privacy, with younger participants unexpectedly citing many more concerns about the security of genetic and electronic medical record data than older participants. Concerns included unauthorized access to computerized data, fears of identity theft, worries about insurance discrimination, and distrust of the government. Despite these misgivings, younger focus group participants reported a similar degree of willingness to participate in GWAS and related forms of genomic research.

## 1546/T/Poster Board #95

**Genome Diner: A strategy to increase community-researcher engagement.** *J.M. O'Daniel[1], T.M. Livingston[2], L. Boles[2], M.K. Rosanbalm[3], S.B. Haga[1].* 1) Institute for Genome Sciences & Policy, Duke Univ, Durham, NC; 2) Museum of Life and Science, Durham, NC; 3) Center for Child & Family Policy, Duke Univ, Durham, NC.

Large population studies often required in genome research could greatly benefit from a public who understands and values the potential benefits of the research and who trusts the researchers themselves. Researchers, however, may have difficulty communicating to diverse, lay audiences about the importance and relevance of their work. Further, the interests and unique perspectives of the public may not always align with what researchers wish to communicate. Thus, it is critical for researchers and the public to gain a better awareness of each other's perspectives. To address this need, we developed and piloted a unique strategy which bridges the concepts of formal (public school) and informal (Museum of Life and Science) science learning, with the experiential context of family learning and public communication training for university researchers. The resulting Genome Diner program engaged student/parent groups in thoughtful dialogue about scientific and social issues side-by-side with university researchers. The small group discussions were facilitated by moderators and a fun "menu" of genome discussion topics and questions to encourage participation by all. A total of 98 student/parent participants (47 students; 51 adult parents/guardians) and 16 genome science researchers took part in one of four Genome Diner sessions conducted at a local middle school. Using a series of pre-, post- and follow-up surveys, we assessed the impact of the program on: Community a) knowledge, perceptions and interest in genome science research and b) confidence in ability to seek and interpret genome science information; and Researcher a) perceptions about public understanding and attitudes, b) opinion of public input and c) confidence and willingness to participate in public science programs. Analysis reveals that participation in Genome Diner had significantly positive effects on perceptions and attitudes for both researcher and community participants. The outcomes of this project suggest that Genome Diner is a successful approach to building awareness and positive perceptions between community members and researchers.

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## 1547/T/Poster Board #96

**Community response to mental health intervention on the basis of genetic susceptibility in a large national population.** *A. Wilde[1, 3], B. Meiser[2], P.B. Mitchell[1,3,6], D. Hadzi-Pavlovic[1,3], P.R. Schofield[4,5,6].* 1) School of Psychiatry, University of New South Wales, Sydney, Australia; 2) Prince of Wales Clinical School, University of New South Wales, Sydney, Australia; 3) Black Dog Institute, Sydney, Australia; 4) Prince of Wales Medical Research Institute, Sydney, Australia; 5) School of Medical Sciences, University of New South Wales, Sydney, Australia; 6) Brain Sciences UNSW, Sydney, Australia.

Purpose. Despite an apparent high demand for predictive genetic testing, few data describe anticipated health behavior as a consequence of such testing. Using the example of the replicated association between a functional polymorphism in the serotonin transporter gene promoter and exposure to stressful life events resulting in an increased likelihood of major depression, this study aimed to evaluate anticipated community behavioral response to predictive genetic testing for depression risk. Methods. A quantitative survey of a large Australian nationally representative population sample of 1046 participants was ascertained through random digit dialing. Attitudes were assessed via structured interviews. Results. There was strong support for health-protective behaviors in the event of being identified with an increased genetic risk for major depression. Intention to start therapies (78%) was significantly and positively associated with higher self-estimation of risk for depression (p=0.001); endorsement of gene-environment interaction as a causal mechanism of mental illness (p=0.017); and endorsment of 'stressful life events' as a causal attribution for mental illness (p=<0.001). Intention to modify potential life stressors (84%) was significantly and positively associated with personal history of mental illness (p=0.032); endorsement of gene-environment interaction as a causal mechanism of mental illness (p= 0.005); and endorsment of 'abuse' as a causal attribution for mental illness (p<0.001). 'Do nothing' due to self perception of not being at risk (24%) was significantly and positively associated with being male (p=0.029), being older (p=0.001) and having no post school education (p=0.003) and significantly and negatively associated with endorsement of gene-environment interaction as a causal mechanism of mental illness (p=0.023). Conclusion. The study has identified strong community receptivity to behavioral mental health intervention in association with predictive genetic testing. In particular, we have identified target groups most likely to be motivated to engage in such interventions as those with a high risk of depression who endorse that mental illness may develop from both genetic and modifiable environmental risk factors. Our results suggest genetic risk information has potential value as an early intervention and prevention tool.

## 1548/T/Poster Board #97

**Public attitudes and knowledge regarding genetic research and participation in the establishment of a biobank in Portugal.** *M. Barreto, B. Nunes.* Department of Epidemiology, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal.

Cohort studies investigating genes, environment, and lifestyle require large samples of population. To recruit and retain participants, it is important to understand the factors that influence individual's motivation to participate. In this context, the aim of this study was to evaluate the Portuguese population's basic knowledge about gene-environment interactions in disease development, and their attitude regarding participation in a genetic research project, as well as the factors that would motivate people to participate. A Computer Assisted Telephone Interview (CATI) survey of Portuguese adults (≥18 years old) was conducted in January 2009. The questionnaire, consisting on 13 questions, was applied to a member of each of the 870 households comprising our random sample. Overall, 753 interviews were completed (87% response rate). To describe the estimates of population knowledge and participation in a genetic research study, the sample was weighted by region and sex. Most respondents (87.3%) believe that genetic research significantly contributes to disease prevention and 64.4% have good or very good knowledge regarding the interaction between genes and the environment in disease development. Regarding their willingness to participate in a genetic research study that required individuals to donate blood, 64.1% of respondents would be willing to participate and 16.7% would like to contribute to the study, but can't due to advanced age or due to the presence of chronic diseases. Only 13.5% of respondents would not participate. Refusal was mainly associated with having to donate blood (35.7%). To examine factors related to participation, an ordinal logistic regression model was used. Individual characteristics that potency participation were: belonging to the age group between 25-44 years old, having a higher education, supporting biomedical research and having very good genetics knowledge. The great majority of the respondents willing to participate (88.8%), would not mind to have their samples stored for large periods of time in a biobank to be used in other studies in the future. Overall, the Portuguese population believes that genetic research has an important role in disease prevention, has reasonable knowledge regarding gene-environment interaction in the development of certain disorders and is willing to participate in a study to investigate this type of interactions.

## 1549/T/Poster Board #98

**Validation of the Brazilian Database on Orofacial Clefts.** *V.L. Gil-da-Silva-Lopes[1], P.A. Mossey[2], I.L. Monlleó[3,4].* 1) Department of Medical Genetics, State University of Campinas, ampinas, SP, Brazil, Campinas, São Paulo, Brazil; 2) University of Dundee, UK; 3) Universidade de Ciências da Saúde de Alagoas, Alagoas, Brazil; 4) Universidade Federal de Alagoas, Alagoas, Brazil.

Introduction: In 1998 the National Health Ministry of Brazil created the Reference Network for Craniofacial Treatment (Rede de Referência no Tratamento de Deformidades Craniofaciais - RRTDCF). The first evaluation of the RRTDCF was held in 2004 by our team. Regional disparities, structural and functional constraints, and lack of coordination were problems identified. To address these problems we proposed a long-term project, starting with the Brazilian Database on Orofacial Clefts (BDOC). Aim: To discuss the validation phase of the BDOC. Methods: BDOC was designed according to the Who's recommendations. The proposal was promoted at four meetings and clinical geneticists were invited to test the case record form and manual for case registration, and to assess its feasibility. Results: Seven clinical geneticists entrusted with craniofacial care engaged in a six months test. Three of them are members of multidisciplinary teams of the RRTDCF while four are from genetic centres (two in the Northeast and Southeast, and three in the South of Brazil). After six months of collection, 126 record forms and 10 assessment questionnaires were received. Average time spent to complete the record form was 15 minutes. Reliability was assessed. Problems identified were need for revision of wording and spacing. Conclusion: Record form and manual have been assessed as useful and reliable tools and reduced time spent to complete would be advantageous. Overall, however, the BDOC seems to be a useful and feasible strategy to help address the challenge of orofacial clefts in Brazil. CAPES, CNPq, FAPEAL and FAPESP.

## 1550/T/Poster Board #99

**Developing a "Social Marketing Plan" for appropriate genomic medicine -meeting the needs of a cultural and linguistic minority group: The Deaf community.** *Y. Kobayashi[1], P. Boudreault[1], E. Baldwin[2], M. Fox[3], L. Dutton[2], L. Tullis[2], W.W. Grody[3,4,5], C.G.S. Palmer[2,4].* 1) Deaf Studies Department, CSU Northridge, Northridge, CA; 2) Department of Psychiatry and Biobehavioral Science, UCLA, Los Angeles, CA; 3) Department of Pediatrics, UCLA, Los Angeles, CA; 4) Department of Human Genetics, UCLA, Los Angeles, CA; 5) Department of Pathology, UCLA, Los Angeles, CA.

In addition to existing clinical genetic services, (e.g., prenatal, pediatric) an increasing number of genetic services devoted to common adult conditions, such as heart disease and diabetes, are anticipated; with personalized genomic medicine it is expected that everyone may benefit from genetic services in their lifetime. The integration of genomic medicine into public health to optimize the impact of this scientific knowledge will likely promote a broader understanding and appreciation of individual and population variability. Hence, this expansion in genomic medicine will likely affect public policy, healthcare and social service delivery. Historically, federal, state, and local programs have provided genetic services and use of information in genomic medicine to specific target groups; however, use of general genetic services is very low among members of the Deaf community, a linguistic and cultural minority group. Several researchers have addressed the economic, political, social, moral, cultural, educational, and ethical issues faced by members of the Deaf community and these issues seemingly interact with their use of genetic services, and use of genetic information. We describe a social marketing plan, one useful tool for health promotion, to increase use of genetics services in this group. Instead of using a top-down approach, social marketing focuses on the needs of the targeted audience, which in this case is a collective group that uses networking systems. As a community-based public health campaign with media components, social marketing strives to create a plan to best approach the target group that would ensure participation in the public health activity as well as integrating primary care for both individual and community. This marketing technique includes strategic planning, culturally and linguistically sensitive message and materials development, pretesting, implementation, and program evaluation. Design of the social marketing plan for this cultural and linguistic minority group is based on experience with a current research project examining impacts of genetic testing on deaf adults. Additional research is needed to determine if tailoring a culturally competent social marketing plan for members of the Deaf community increases the group's awareness of genetics services, increases effective use of genetic information, promotes health, and improves their overall quality of life.

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## 1551/T/Poster Board #100

**Using family history in primary care for predicting risk of common, complex diseases in individual patients.** *B. Wilson, Q. Hasanaj, J. Little, S. Hawken.* Epidemiology & Community Med, Univ Ottawa, Ottawa, ON, Canada.

**Background** Chronic diseases account for a large proportion of deaths, premature loss of life, and health costs. As well as known risk factors such tobacco and nutrition, unknown factors contribute substantially to risk. Family history (FH) may offer a clue to shared disease susceptibilities arising from genomic susceptibility, shared environments, and common behaviors (and do not require an understanding of molecular etiology). Including FH in risk assessment algorithms for common, complex diseases may permit better prediction of individual disease risk. FH, in isolation, is not likely to be highly predictive for complex disease risk, so it is important to examine its performance in conjunction with risk factors whose measurement is considered standard of care. **Objectives** 1. To identify how FH is incorporated into disease specific guidelines which are currently in use in North America. 2. To compare these recommendations with evidence on the predictive ability of FH information in order to identify gaps or errors. 3. To formally assess the incremental improvement in individual risk prediction which is gained by incorporating FH with other forms of clinical information recommended in the guidelines. **Methods** We performed an environmental scan of chronic disease prevention guidelines currently disseminated in North America, Europe, and Australia, using standard systematic review methods. Relevant data were extracted pertaining to the condition of interest, the specified FH items, how they are factored into recommendations, and quality of guideline development process. We compared the results with published systematic reviews of FH items as predictors of future disease and rated the original guidelines according to level of consistency with evidence. Finally, we used data from meta-analyses and secondary analyses of large datasets to calculate receiver-operator curves for 'predictive tests' based on different combinations of established risk factors and FH information for several exemplar conditions (including coronary heart disease, diabetes and cancers). **Preliminary Results** No consistency was found across guidelines for how FH was incorporated in risk assessment. A well-developed evidence base exists for FH as a risk factor (relative risk), but not for its use in individual discrimination of risk (sensitivity and specificity). Modeling of combinations of specific FH items with established risk factors is underway.

## 1552/T/Poster Board #101

**Preparing for the Next Generation of Biorepository Research: Points to Consider.** *G.F. Guzauskas[1], K. Fryer-Edwards[1,2], S.M. Fullerton[1,2], N. Anderson[3].* 1) Public Health Genetics, University of Washington, Seattle, WA; 2) Department of Bioethics and Humanities, University of Washington School of Medicine, Seattle, WA; 3) Division of Biomedical Health Informatics, University of Washington School of Medicine, Seattle, WA.

Biorepositories are invaluable resources for genetic epidemiology and genomics and are anticipated to be a key component of translational research. Researchers use samples and data stored in biorepositories to study the complex relationship of genetic and environmental factors, and wide sharing of such resources is increasingly seen as the best way to advance discovery science and its translation to clinical benefit. However, many new challenges accompany this anticipated paradigm shift. In this poster, we address these ethical and practical considerations, including issues of consent, de-identification, and data sharing. Traditional biorepositories ("first-generation") are single-institution, small-scale operations that typically use anonymized or de-identified biospecimens for a range of downstream research generally exempt from human subjects protections. In such settings, research oversight is maintained by biorepositories' investigators who control data uses according to terms defined by the initial informed consent agreement. With the advent of data sharing among biorepositories many others, not just those with whom the participants entered into direct agreement, will use the data, complicating oversight and governance. The traditional means of protection, data de-identification, is also less reliable in the face of large amounts of inherently identifying genomic data being generated by research. In response to these concerns, we propose that first-generation biorepository models must give way to a new set of ethical and practice guidelines better suited to current forms of biorepository-based data sharing ("second generation"). In such settings, we argue, the treatment of research as exempt from human subjects oversight is not tenable, nor is it feasible to broaden the terms of informed consent to permit all conceivable secondary research uses. New systems of governance must be devised which permit on-going review of research uses, based in the interests of biorepository donors, and can be dynamic and responsive to the continually evolving state of the science and technology. Rather than focus exclusively on privacy concerns, we examine systems that permit identified tracking of participants for greater accountability as well as improved follow up to expand research data and scope as needed. Such policy recommendations are guided by expert opinion, consensus development meetings, and policy analysis of existing international guidance.

## 1553/T/Poster Board #102

**Capacity Building for the Transfer of Genetic Knowledge into Practice and Prevention: Health Care Needs Assessment for Medical Genetic Services in Middle- and Low-Income Nations.** *I. Nippert[1], U. Kristoffersson[2], J. Schmidtke[3], A. Kent[4], A. Christianson[5], R. Raouf[6], C. Barreiro[7].* 1) Women's Hlth Res, Univ Muenster, Muenster, Germany; 2) Dept Clin Gen, Univ Hosp Lund, Lund, Sweden; 3) Inst Hum Gen, MHH, Hannover, Germany; 4) GIG, London, UK; 5) Div Hum Gen, Univ Witwatersrand, Johannesburg, South Africa; 6) Ain Shams University, Cairo, Egypt; 7) Hospital de Pediatria SAMIC, Buenos Aires, Argentina.

*Background:* CAPABILITY (http://www.capabilitynet.eu) is a 3-year model project developed by the European Network of Excellence: Genetic Testing in Europe - Network for test development, harmonization, validation and standardization of services (EuroGentest) (http://www.eurogentest.org) and by leading experts from emerging economies: Argentina, Egypt and South Africa, the latter being currently engaged in major development projects to integrate genetic services in primary care and prevention in their countries.

*CAPABILITY Objectives:*

identify priorities for capacity building for genetic services by a systematic health care needs assessment (HCNA) and

validate the HCNA approach by means of demonstration projects.

*Methods:* CAPABILITY has formalised a HCNA for medical genetic services that middle- and low-income nations can utilise for implementing genetic services. Key elements of the HNCA are the development of strategic aims; an evaluation of existing services and the environment in which they function; a review of epidemiology, effective interventions, opinions of consumers and professionals, available resources and possible contraints. Analysis of these elements enables the production of a HCNA. The approach is currently assessed by demonstrations projects in Argentina, Egypt and South Africa.

*In Conclusion:* A systematic HCNA will assist nations with emerging economies to marshal and allocate their limited resources when developing medical genetic services.

*Funded by:* EC Contract no.: FP6-037275.

## 1554/T/Poster Board #103

**The National Ophthalmic Disease Genotyping and Phenotyping Network: eyeGENE™ - Progress in Three Years.** *X. Wang[1], D. Blain[1,2], K. Goetz[1,3], V. NDifor[1,3], M. Reeves[1,3], S. Vitez[1,3], D. Scheim[4], C. Antolik[1], S. Tumminia[5].* 1) OGFVB/NEI, NIH, Bethesda, MD; 2) MedStar Research institute, Hyattsville, MD; 3) BioSearch/Team Placement Service Incorporated, Rockville, MD; 4) Private Systems Specialist, Blacksburg, VA; 5) OD/NEI, NIH, Bethesda, MD.

Genetic mutations are associated with many ocular diseases including glaucoma, cataracts, strabismus, corneal dystrophies, and many forms of retinal degenerative disease. As a result, gene-based therapies are actively being pursued to ameliorate genetic eye diseases that once were considered untreatable. However, molecular diagnostic testing for these diseases is not widely available to patients. The National Ophthalmic Disease Genotyping and Phenotyping Network (eyeGENE™) was created three years ago by the National Eye Institute (NEI), part of the National Institutes of Health, in partnership with research laboratories, ophthalmic clinics and CLIA-certified molecular diagnostic laboratories in the United States. The major goals of this network are to 1) broaden patient accessibility to diagnostic genetic testing, 2) facilitate research into the genetic causes of ophthalmic disease by establishing a genotype/phenotype database and a DNA repository, and 3) provide a de-identified genotype/phenotype-linked sample resource for ophthalmic disease researchers. This Network currently includes the eyeGENE™ Coordinating Center, more than 110 ophthalmic clinical organizations nationwide, and 12 clinical diagnostic laboratories. The Coordinating Center, based at NEI, manages a centralized repository for blood/DNA and a genotype/phenotype database. The Coordinating Center also manages the referral of clinical genetic testing for patients submitted from participating clinics to a Network CLIA-certified laboratory and reports the test results back to submitting physicians. To date, Network laboratories offer testing for mutations in more than 65 genes. Mutations in these genes correlate with 32 different clinical diagnoses. At the time of this submission, more than 1000 patient samples have been collected in the repository. Preliminary data analysis indicates that 40% of participants are in the Retinitis Pigmentosa or allied retinal degeneration category, 24% have a diagnosis of Stargardt Disease, and 7% are in the Cone-Rod Dystrophy category. Public access to de-identified clinical and genetic information is under development and will be available to researchers in the vision community upon NIH IRB approval. The eyeGENE™ Network will continue to serve the vision community by promoting accessibility of genetic testing to patients with ocular diseases and by promoting clinical and basic research for a better understanding and treatment of ocular diseases.

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## 1555/T/Poster Board #104

**Creating interest in autism genetics research among underrepresented populations through targeted outreach.** *J.L. Robinson, M.L. Cuccaro, R.J. Martinez, M.J. Gavier, M.A. Pericak-Vance.* Miami Institute for Human Geno, University of Miami, Miami, FL.

High throughput gene discovery efforts such as genome-wide association studies (GWAS) have substantially increased efforts to detect both common and rare genetic risk variants in complex diseases such as Alzheimers disease, multiple sclerosis, and autism. While critical for gene discovery, GWAS require large numbers of participants to ensure sufficient power. Increasing enrollment must also recruit from underrepresented populations. These populations have historically been challenging to recruit in large numbers for genetic studies of various complex diseases and autism in particular and are poorly represented in such studies. Given recent discoveries of common and rare risk variants in autism, it is crucial that we ascertain individuals and families of diverse ethnic and ancestral groups to identify population specific risks for autism. In this study we describe efforts to increase interest and participation of underrepresented populations in autism genetics research through a targeted outreach approach. This approach has two components: 1) increasing community education about autism and the importance of genetic and genomic research and 2) increasing community trust in our research group. Outreach methods include creating community partnerships with established service provider groups, offering targeted information sessions in communities where underrepresented populations are served, providing personalized and/or language matched follow-up, and focused advertising to reach underrepresented populations. We will present findings demonstrating the values of specific outreach activities and events on participation rates in autism genetic research in Hispanic Americans and Blacks/African Americans in the South Florida Area.

## 1556/T/Poster Board #105

**Consideration of Molecular Technology Issues Using an Ethics Values Game.** *R. Anderson, T. Schonfeld, A. Jameton, M. Godfrey.* Univ Nebraska Medical Center, Omaha, NE.

Is choosing traits for one's children a desired outcome of current progress in molecular genetics? In a recent national survey about 10% of people seeking genetic counseling said they would prefer a child with greater intelligence, height, or athletic ability. More than 50% would use genetic testing to avert blindness and more than 75% to avert mental retardation in a child. In contrast, when we asked high school students on six Indian Reservations whether people should be allowed to choose traits for their children or let nature take its course, more than 95% of the times the student response was to let nature take its course. But these students also believed that everyone should have equal access to health care reproductive technologies.

After our survey, these students played an "Ethics Values Game" we developed that allows players to choose traits for their "offspring." The game is played by dealing five "trait" cards to each player. The player can then keep all the cards or discard up to three cards and draw new ones. In addition, the roll of a die or the spin of a wheel gives players points to buy additional traits or modify their trait cards. The more points, the greater the choices. This allows students to experience the uneven distribution of opportunity as well as inborn characteristics. (Students were later asked to explain why they chose to retain, discard, or purchase traits.)

Even though students initially said they wanted to let nature take its course, most played the game with zeal. It was rare to find a student who simply kept the initial five cards, and declined to purchase additional traits if given the opportunity. These students were the ones who really let nature takes its course. On one occasion a fifteen year old student played the game while holding her infant daughter. By chance she received a trait card for low fertility for her "offspring." The student was elated and said, "that way I would not be a grandma too soon." This hands-on exercise invites participants to consider numerous questions about the Pandora's Box opened by advances in molecular and reproductive biology.

## 1557/T/Poster Board #106

**Assessing the Cost-Effectiveness of Genetic Testing.** *M. Bergman[1], H. Honoré[2], D. Regier[3].* 1) VA Puget Sound, Seattle, WA; 2) HCQCUS, MEDVAMC, Houston, TX; 3) National Perinatal Epidemiology Unit, University of Oxford, Oxford, England.

Background: Genetic testing has potential to advance quality and efficacy of care at many stages of disease. However, this promise does not come free; patients and doctors are presented with personal and economic choices concerning use of these tests. As these tests become more prevalent greater understanding of their cost-effectiveness is needed. Purpose: Here we systematically review studies analyzing cost-effectiveness of different human genetic tests. Data Source: PubMed search of English language papers published from 1975 to December 2008. Study Selection: Papers were included if they provided empirical results of an economic evaluation on the application of a specific genetic test, or set of genetic tests in the human population. Data Extraction: We categorized papers by type of test offered and divided tests into these categories: carrier screening, prenatal screening/diagnosis, predictive/diagnostic, prognosis, and reoccurrence. Data Synthesis: 42 of the 86 studies indicate that, for their particular test, genetic testing is cost-effective. 10 studies indicated that testing was not cost-effective, although 5 of these were analyzing tests that were found cost-effective in at least 1 other study. The remaining 5 encompassed 3 genetic tests and one method, with only 1 test having more than 1 study performed. 8 studies discussed willingness to pay rather than cost-effectiveness. 7 studies were strict cost studies, with no mention of effectiveness, or discussed technologies that are currently obsolete. 17 studies stated that cost-effectiveness depends on parameters that can change, and are changing, over time. Limitations: Each type of disease had only a limited amount of studies that included empirical economic evaluation. Conclusions: Genetic testing has been found to be cost-effective for a wide variety of test types and diseases. The majority of qualifying studies showed cost-effectiveness uniformly, or in certain circumstances. Those not found to be cost-effective represent a small portion of the populations tested, and typically have a non-genetic test to determine their presence. We can conclude then, that genetic testing can be a cost-effective method of determining health outcomes. However, each test should be analyzed on its own merits and within a specific population, because it is not possible to generalize the results of any study of an individual test.

## 1558/T/Poster Board #107

**Gaining Consent for Biobank Samples: the NW Biobank Experience.** *K. Goddard, S. Smith, C. Chen.* Ctr Hlth Res, Kaiser Permanente Northwest, Portland, OR.

Human tissues provide essential molecular data for basic and translational research. Large-scale population-based biobanks can provide large sample sizes for genomics research, and offer economies of scale, improved quality and reliability of samples, and widespread access through automated sample retrieval. However, it is challenging to adequately inform participants of the broad nature of the research and participation risks and benefits. In a pilot study to develop a biobank at Kaiser Permanente Northwest (KPNW), we wrote recruitment materials at an 8th grade reading level that were vetted by a communications team, focus groups of KPNW members, the IRB, and external advisory boards. We then surveyed 500 KPNW members to assess attitudes and response to the materials. We asked members in waiting rooms to review the recruitment materials then complete a brief survey on demographics, willingness to participate, reasons for non-participation, and knowledge of the written materials. The survey respondents were 39% male, 20% had a high school education or less, 16% minorities, and 50% over 49 years old. Overall, 69% agreed to contribute to the biobank, 20% were not sure, and 11% did not agree. Of those who agreed, 82% also agreed to have their information posted in a US government database, such as dbGaP. The primary reason for non-participation was concern about confidentiality of genetic and health-related information. Respondents who agreed to contribute were more likely to be male (75% males vs. 65% females, p=.01), older (75% highest age group vs. 62% lowest age group, p=.03), more affluent (77% highest income group vs. 58% lowest income group, p=.01), and white (70% vs. 56% of minorities, p=.005). Overall, 74% of respondents correctly answered ≥6 of 9 questions, indicating a high understanding of the information. Respondents who answered the questions correctly were white (77% vs. 58% of minorities, p=.0023), college graduates (83% vs. 52% of high school education or less, p=.0001), and more affluent (86% highest income group vs. 58% lowest income group, p=.0001). Respondents who agreed to contribute were more likely to correctly answer the knowledge questions (71% vs. 51% who did not agree or 55% who were not sure). Biobanks will be most beneficial for genomic research if they widely represent the underlying population. Our findings indicate that more communication methods may be needed to ensure the broadest participation possible.

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## 1559/T/Poster Board #108

**Some Remarkable Women in Medical Genetics.** *P.S Harper.* Institute of Medical Genetics, School of Medicine, Cardiff Univ, Cardiff, United Kingdom.

A series of highly talented women have played a major role in key discoveries in human and medical genetics, dating from the beginning of the 20th Century, a time when women rarely met encouragement and the possibility of making major contributions in science. Out of a large number of possibilities, three individuals are discussed here, two of whom have not received the credit due to them. The achievements of these three are described, Julia Bell (UK), Eva Melander (Sweden) and Marthe Gautier (France), the last two having made major, but largely unrecognised contributions to early human cytogenetics. It is instructive to compare their experiences with the situation in contemporary medical genetics, where women form a majority, in both clinical practice and laboratory research.

## 1560/T/Poster Board #109

**Increased participation of African Americans in genetic studies.** *S. Hartz[1], E. Johnson[2], N. Saccone[1], J. Rice[1], L. Bierut[1], COGEND Investigators.* 1) Dept Psychiatry, Washington Univ, St Louis, MO; 2) Division of Health, Social, and Economic Research, Research Triangle Institute International, Research Triangle Park, NC.

Although investigators select samples to preserve genetic homogeneity for association studies, differing genetic structures between ethnic groups may lead to improved identification of variants related to disease. In addition, the inclusion of minority subjects in genetic studies allows for a more accurate representation of the population. Unfortunately, there is a paucity of genetic data from minority subjects. In the attempt to preserve genetic homogeneity, community samples may not have adequate numbers of minority participants to analyze separately. Also, specifically sampling minority populations is considered to be particularly difficult because minorities are thought to be less likely to consent to genetic research. In fact, increasing numbers of Caucasian-only samples may perpetuate this belief. Therefore, we used previously collected population-based genetic data to test the hypothesis that minorities (specifically African Americans) are less willing to participate in genetic studies.

The Collaborative Genetic Study of Nicotine Dependence (COGEND) screened 42,289 community subjects. Based on preliminary data, 30,557 European Americans (EA) were screened, 3713 qualified for the study and 2081 (56%) participated in the study. Conversely, 11,732 African Americans (AA) were screened, 1115 qualified for the study and 757 (68%) participated. The difference in participation rates between EA and AA was statistically significant ($p<0.0001$).

These results strongly challenge the conception that minorities are less likely to consent to genetic studies. It suggests that standard protocols for population recruitment work at least as well on AA as EA.

## 1561/T/Poster Board #110

**Beyond Race: Utilizing genetic markers instead of racial categories to characterize diversity among children participating in clinical research.** *P. Payne[1,2], R. Freishtat[2,3], F. Suer[2,3], J. Devaney[2,3], K. Panchapakesan[2,3], S. Choudhry[4], S. Teach[2,3], E. Hoffman[2,3].* 1) The George Washington University School of Public Health and Health Services, Department of Health Policy, Washington, DC; 2) Children's National Medical Center, Center for Genetic Medicine Research, Washington, DC; 3) The George Washington University School of Medicine and Health Sciences; 4) University of California San Francisco School of Medicine, San Francisco, CA.

OBJECTIVE The use of racial/ethnic Census categories to characterize diverse populations in pediatric clinical research can create misleading findings and pose safety problems, such as inappropriate diagnosis or treatment, when this information is translated into clinical practice. Herein, we offer an alternative approach for characterizing diverse populations in clinical research by using ancestry informative markers(AIMs), single nucleotide polymorphisms(SNPs) associated with certain geographic regions. This approach may provide a better way to assess risk for disease or drug response in diverse populations of children. METHODS A panel of AIMs was developed from existing literature using a number of selection factors including the existence of an allele frequency difference of 60% between one geographically defined population and two other different populations. For this preliminary study, 11 of the selected SNPs were genotyped in the AsthMaP Project cohort which contained parent-identified African American children with asthma (n=48), and a related control group (n=92). Simpson's Biodiversity Index (SBI), a measure used to quantify habitat diversity, was used as a measure of diversity for the genotypes of each AIM. Chi square tests were used to assess differences between the observed and expected genotypes using three different models of expected genotype frequencies. RESULTS Based on the SBI, cases and controls were generally more diverse than their parent-identified racial group labels, with the SBI ranging from .39 (more diverse) to .92 in cases and .33 to .69 in controls. More diversity was seen in the cases and controls for AIMs linked to regions of Africa. Chi square tests revealed significant differences between expected and observed genotype frequencies for all models of expected genotype frequencies with p-values ranging from approximately 0 to 9.5 E -12. There were a few exceptions for the case group. These exceptions were probably due to a smaller population size in the case group which will change as the cohort grows. CONCLUSIONS This preliminary study demonstrated that a cohort of parent-identified African American children are more diverse with regard to ancestry than their parent identification assumes. This finding is particularly important, given the increasing genetic diversity of American children. The current approach of using race/ethnicity alone in clinical research appears outdated and deserving of policy change.

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## 1562/T/Poster Board #111

**Getting the Word Out: Genetic Medicine for the Health Care Provider through Continuing Medical Education.** *A. Gilbert[1], C. Wiener[2], D. Valle[1].* 1) Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 2) The Johns Hopkins Hospital Department of Medicine Baltimore, MD.

Since the completion of the Human Genome Project (HGP) the ongoing rapid progress in genetic medicine is producing new insights and vast amounts of information that will ultimately transform the practice of medicine. Increasingly, we are identifying genetic contributions to all human diseases, and the prospect of genetic testing for predisposition to disease, molecular diagnosis, prognosis and response to treatment is thus becoming a reality. Advances in our understanding of the unique interaction between individuals and their environment as the framework for the balance between health and disease has the potential to revolutionize prevention and treatment. These advances form the basis for personalized medicine/individualized care. Medical education regarding the genetic contribution to disease varies greatly in the content and extent provided. Though primary care physicians in academic centers may have access to genetic information through a variety of venues: genetic colleagues, lectures and seminars, there is, however, no single organization responsible for delivering and assessing this information effectively. Furthermore, clinicians in private practice do not have ready access to these resources and are increasingly faced with patient's who are better informed. Addressing this need, the Johns Hopkins University (JHU), School of Medicine has developed an entirely new medical school curriculum commencing in the fall of 2009: Genes to Society. The course content is embedded in the concepts of human individuality and variability, risk, and the ability to alter disease presentation and outcomes. The Institute of Genetic Medicine at JHU is also expanding the effort to bring genetic information to the practicing healthcare provider, as well as JHU house staff, by offering a one-and-a-half day continuing medical education (CME) course titled: Genes to Society: Genetic Medicine for the Healthcare Provider. This course, the first of its kind at JHU, will be given in the fall of 2009. Registration will be free for all JHU postdoctoral trainees. This 12 credit CME course contains 13 half-hour lectures that will offer up-to-date information applicable to today's clinical practice. The course will also provide information and instruction on the effective use of the WWW for obtaining vetted genetic information. The effectiveness of the course will be measured by the uptake and CME evaluation forms. If successful, the course will be offered every year.

## 1563/T/Poster Board #112

**Lysosomal Disease Network, and WORLD Symposium 2010.** *B. Kohler[1], J. Barranger[2], J. Muenzer[3], C. Eng[4], G. Grabowski[5], M. Patterson[6], S. Walkley[7], B. Davidson[8], R. Steiner[9], W. Wilcox[10], E. Shapiro[1], C. Whitley[1], the other LDN investigators.* 1) University of Minnesota, Minneapolis, MN; 2) Genzyme Corporation, Cambridge, MA; 3) University of North Carolina, Chapel Hill, NC; 4) Baylor College of Medicine, Houston, TX; 5) Children's Hospital Research Foundation, Cincinnati, OH; 6) Mayo Clinic, Rochester, MN; 7) Albert Einstein College of Medicine of Yeshiva University, New York, NY; 8) University of Iowa, Iowa City, IA; 9) Oregon Health & Science University, Portland, OR; 10) Cedars-Sinai Medical Center, Los Angeles, CA.

The Lysosomal Disease Network (LDN) is a consortium of basic researchers, clinicians, pharmaceutical industry professionals, and patient advocates devoted to promote and facilitate basic, translational and clinical research in lysosomal diseases. The network is a scalable multi-center consortium of geographically distributed expert medical centers, patient support and corporate partners. The infrastructure, mission and charter were developed through organizational committees; the collaborating medical centers, pharmaceutical and patient support organizations will direct the activities of the network through participation in the Steering Committee. Toward meeting its goals, the LDN has launched its web site www.LysosomalDiseaseNetwork.org to facilitate professional and public education, timely network communication, and develop a data entry mechanism for research projects. The web site has announced its 6th annual research meeting, We're Organizing Research for Lysosomal Diseases "WORLD Symposium 2010" (February 10-12, 2010) in Miami, FL, USA. Through the web site, participants are able to submit abstracts (October 1, 2009 deadine), register for the meeting, and join the growing membership list to receive regular communications. The Network is currently conducting longitudinal studies to understand the natural history of lysosomal diseases and treatment outcomes. Long term network goals include: 1) development of an information management infrastructure to encourage integration of shared clinical experience and relevant longitudinal studies, 2) investigator training, and 3) public education. Organizational and symposium support has been provided by NIH, NINDS, NIDDK, and ORD (U54-NS065768).

## 1564/T/Poster Board #113

**Newborn Screen and Beyond: An Outreach Education Initiative.** *A. Rajadhyaksha[1], E. Velis[2], S. Deshmukh[1], M. Perez[1], P. Jayakar[1].* 1) Dept of Genetics and Metabolism, Miami Children's Hospital, Miami Fl; 2) Master of Science Program in Health Services Administration Division of Health Management/College of Health Sciences, Barry University, Miami Fl.

**Introduction:** Delayed diagnosis of treatable metabolic disorders remains a healthcare problem due to lack of awareness. We initiated an outreach project to educate healthcare providers on early detection and intervention of treatable metabolic disorders. **Materials and Methods:** Seminars using algorithms based on "red flags" or clinical presentation for early detection and intervention of metabolic disorders were conducted. A general survey on demographics, attitudes and awareness of these disorders included questions as follows: Q1:Are you familiar and comfortable identifying suspected metabolic disorders Q2:Are you familiar with ordering metabolic tests when suspecting a disorder Q3:Are you familiar with medical intervention and treatment of metabolic conditions. Additionally, pre and post-seminar tests on specifics of various metabolic disorders was administered. Responses were analyzed based on the following groups: (a)Gender (b)Experience:<6 years, 6-14 years and ≥15 years (c)Position: attending, resident, medical student and allied health (d)Employment:teaching hospital, private office and non teaching hospital. Analyses of variance and independent and dependent t-tests were performed to identify mean differences between groups. Categorical variables were explored by Chi-Square test. **Results:** Of 65 surveyed participants: 32%, 57% and 31% stated familiarity to Q1, Q2 and Q3 respectively with no statistical difference between the groups. Pretest score analysis showed no significant difference except residents scored significantly higher(M=5.73,SE=3.29) than allied health and medical student(M=43.78,SE=3.39),(F2,80)=3.82,p<0.05). Significant increases in scores between pretest(M=54.14,SE=2.58) and post test(M=66.67,SE= 2.90) in all participants(t(48)5.11, p<0.01)) and groups was noted except for residents (t(14)=1.64,p>0.05) and those with 6-14 years experience (t(7)= 1.30,p>0.05). **Conclusion:** Our findings demonstrate a lack of awareness of metabolic conditions in over half of the care givers surveyed. Test scores significantly increased after seminars indicating the need for continued outreach education. The scores observed in Resident and 6-14 years Experience groups probably reflects the positive impact of current training and continued education for board recertification respectively. The study serves as a foundation for the development of public policy and long-term educational strategies.

## 1565/T/Poster Board #114

**What do the health professionals students know about cleft lip and palate?** *M.C. Silveira[1], R.J.N. Nogueira[2], A.E.S Lima[2], L.A. Magna[1], V.L. Gil-da-Silva-Lopes[1].* 1) Department of Medical Genetics, State University of Campinas, Campinas, São paulo, Brazil; 2) Equipe de Nutrição Enteral e Parenteral, Hospital de Clínicas, State University of Campinas.

Introduction: The cleft lip and/or palate (CLP) are the most common congenital craniofacial defects and may occur on their own or associated with other anomalies. Children born with this congenital defect are subject to diverse complications such as feeding disorders, oral communication disorders, otologic and/or hearing disorders as well as emotional and social problems that are mainly due to anatomical alterations of the face. Objective: The aim of this study, as part of the Brazilian Craniofacial Project, was to characterized the knowledgement of health professions students from the senior year about the management and primary care of cleft individuals. Methods: Students from senior year of medical (MD), nurse(N), speech therapist (ST) and dentistry(D)schools at State University of Campinas were interviewed using a structured questionnaire. After this, in a previous scheduled day for each course, the researcher offered a training to deal with the peculiar situations presented by children with CLP in primary care. The data was analyzed using the Epi Info program version 6.04d and the SPSS version 8.0. Results: From a total sample of 167 students, interviews (64 - Medicine; 38 - Nursery; 17 - Speech Therapist and 48 - Dentist). The data revealed that the future professionals were not preparing for care of CLP individuals. Main difficulties were related to 58,7% affirm to have reasonable slight knowledge on anatomical alterations and 50.3% declare the same reply for knowledge of functional alterations. 1.8% if they only consider completely apt for the accompaniment of these patients. Between that they had presented a reason for this ineptitude 65.7% had indicated unfamiliarity. Conclusion: This investigation should be extended for others universities. However, in view of the prevalence, morbidity of CLP, and others studies conducted by our team, it is possible to suggested that educational programs for non-specialized health professionals working at primary care should be part of health policy. Finnancial Support: CNPq, FAPESP.

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## 1566/T/Poster Board #115

**Results of a Needs Assessment to Inform a National Curriculum for Postgraduate Training in Clinical Genetics.** *G.E. Graham[1], S. Langlois[2], A.M. Innes[3], B. Chodirker[4], E. Lemyre[5], T. Costa[6], R. Mendoza-Londono[7].* 1) Children's Hospital of Eastern Ontario and University of Ottawa, Ottawa, ON; 2) University of British Columbia, Vancouver, British Columbia; 3) Alberta Children's Hospital and University of Calgary, Calgary, Alberta; 4) University of Manitoba, Winnipeg, Manitoba; 5) CHU Sainte-Justine, Université de Montréal, Montréal, Quebec; 6) McGill University, Montreal, Quebec; 7) Hospital for Sick Children and University of Toronto, Toronto, Ontario.

In Canada, Medical Genetics is a primary specialty with direct entry from medical school that requires 5-years of residency training under the auspices of the Royal College of Physicians and Surgeons of Canada (RCPSC). The first two postgraduate years are composed of rotations in Internal Medicine, Pediatrics, Maternal-Fetal Medicine and electives. The final three years provide core training in Genetics, including clinical and laboratory rotations, courses and up to 12 months of research to prepare the resident for consultancy practice in what is usually an academic setting. Individuals with a primary RCPSC specialty such as Pediatrics who wish to practice in Genetics instead complete a 3-year CCMG (Canadian College of Medical Geneticists) fellowship in Clinical or Biochemical Genetics. There are currently 39 residents and 10 fellows enrolled in seven MD training programs across the country. Historically the relatively small numbers of trainees in each center and their geographic separation fostered individual approaches, with each program "reinventing the wheel" to provide didactic teaching. This was problematic, particularly for centers with a small number of trainees and limited teaching staff already over-burdened by clinical demands. In 2008, recognizing the potential benefits of collaboration, the seven RCPSC Medical Genetics Program Directors embarked on a pilot project to deliver teaching sessions via TeleHealth video connection from rotating sites across the country. TeleGRaF (TeleHealth for Genetics Residents and Fellows) featured a monthly seminar highlighting genetic disorders in specific Canadian populations including the Anabaptist populations, Ashkenazi Jews, Native Canadians and French Canadians. The success of this project has encouraged us to further develop TeleGRaF as a cost effective strategy to achieve educational objectives and foster strong collaborations between sites. As the next step in the development of a national curriculum, we administered an online needs assessment to all physician trainees enrolled in RCPSC and CCMG programs in Canada, as well as their program directors, staff geneticists and recent graduates. Herein we present the results of our need assessment and their implications for the planning of TeleGRaF 2009-2010.

## 1567/T/Poster Board #116

**Getting Genetics Done: an educational productivity blog by graduate students for getting things done in human genetics research.** *E. Holzinger, W. Bush, S. Turner.* Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

A large portion of graduate students' and post-docs' time is spent trying to find solutions to procedural issues, e.g. finding the right software for a particular analysis, figuring out how to do a particular procedure in a given software, finding new relevant literature, etc. This is time that could be better spent on higher-level tasks, such as designing experiments and interpreting & publishing the ensuing results. There is a high likelihood, however, that someone else working in a related area of human genetics research has faced a similar problem, and has devoted a considerable amount of time to finding a satisfactory solution. Finding a way to obviate this duplication of effort is the motivation for Getting Genetics Done (URL: http://GettingGeneticsDone.blogspot.com/), an actively maintained blog created and written primarily by graduate students in a human genetics Ph.D. program. The target audience of Getting Genetics Done comprises graduate students, post-docs, and analysts working in human genetic epidemiology. Its purpose is to call attention to software, useful web tools, analytical tips, important recently published papers, and other subject matter that will enhance productivity by eliminating the unnecessary time spent finding workflow solutions that others have already discovered.

## 1568/T/Poster Board #117

**Teaching dysmorphology.** *S.M. Nikkel.* Dept of Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada.

The ability to recognize syndromic diagnoses is a major objective that must be mastered by residents and fellows in Genetic training programs. However there are no guidelines as to how this is to be accomplished. There is an assumption that the trainee will see a spectrum of diagnoses and learn by exposure. Case rounds are a method to provide further exposure, however "interesting" patients may not be available each week. For the extremely rare diagnoses, photographs of classic patients are available for review in texts and databases. Differential lists are learned for specific anomalies. However, it is uncertain as to how often the syndrome is thought of if this key feature is absent. "Theme of the week" was developed in order to expose trainees to a wide variety of syndromes and conditions. Each series is comprised of slides with pictures that have a unifying theme. However, the theme may not be demonstrated in the series. There are a variety of sources where appropriate images can be found. The trainees are asked what they think the theme may be prior to the discussion of the slides. This allows them to think about the conditions in an alternative manner, not just from the key feature. Further discussions of the slides include diagnosing the condition demonstrated, genes/pathways involved, describing the dysmorphology, and management issues. There is willing participation by staff members, as they too like to figure out the theme. Their participation broadens the experience for the trainees due to their input regarding the conditions and their personal clinical experiences. Examples of theme of the week will be demonstrated.

## 1569/T/Poster Board #118

**Alcohol abuse: an innovative multi-disciplinary scientific approach for an unsolved social problem.** *M. Borriello[1,2], E. Alloero[1], P. Schifano[1], M. Antola[1], R. Galuffo[1], A. Soricelli[1], B. Zanini[6], L. Viale[8], L. Canepa[3], M. Altamura[3], C. Saracco[3], M. Burlando[3], E. Andreozzi[4,5], R. Tafuri[7], O. Sansalone[7], G. Borrelli[7], M. Achilli[7], M. Giribaldi[7], M. Annaloro[7], A. Cancellieri[7].* 1) IPSSART "Marco Polo", Genova , Genova, Italy; 2) Faculty of Biotechnology - University Federico II of Napoli, Italy; 3) ITIS "E.Majorana", Genova, Italy; 4) ITCG "Archimede", Napoli, Italy; 5) CEINGE - Advanced Biotechnologies Naples Italy; 6) Faculty of Medicine, University of Genova, Italy; 7) Prefecture of Genova Italy; 8) Scientific High School "M. Champagnat", Genova, Italy.

"Although alcohol abuse is major health problems in many countries, the impacts of alcohol abuse have not always been appreciated. Biological and genetic studies clearly place alcoholism among diseases with both genetic and environmental influences, but persistent stigmas and attribution to moral failure have impeded recognition and treatment of alcohol problems" (Goodman&Gilman) An innovative approach to reduce unaware alcohol abuse is proposed, with a multidisciplinary scientific activity performed in high schools for deeper comprehension of facts, problems, needs: · FACT: increase of alcohol abuse of young Italian population · PROBLEM: increase of biological, psychological and behavioral damages linked to alcohol abuse · NEED: prevent alcohol abuse, building deeper knowledge in school population through a diversified, multi-actor program developed in: 1) Biological Sciences: a) Biochemistry: alcohol metabolism b) Genetics: functional variants in genes involved in alcohol metabolism and its variances attributed to genetic differences in alcohol - and aldehyde-metabolizing enzymes 2) Math: statistical analysis of population behavior to comprehend dimension of the problem and make, where possible, international comparative analysis 3) Law: laws to limit vehicles operation under alcohol influence - comparative analysis in European countries. A pilot study in several high schools of Liguria (north-west of Italy) has been activated in cooperation with University departments and has been supported by governmental entities like the Prefecture of Genova. In a two years period the activities have been: 1. a statistical analysis of data on alcohol consumption on more than 1000 students ranging between 14 and 18 2. a teaching activity in biochemistry of alcohol metabolism in the liver 3. an experimental genetic analysis with several selected classes of students, to study functional variants in ADH gene involved in alcohol metabolism, with lab activity in the field of: a. DNA extraction b. PCR of gene region of interest c. Statistical analysis on detected polymorphisms The pilot study is now developed in several other high schools in North and South of Italy, to enlarge the involved populations of students and teachers and make geographical studies in adolescent populations. The classes will be then followed along the years from the behavioral point of view, to assess the impact of teaching toward deeper knowledge of alcohol abuse at students and family level.

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## 1570/T/Poster Board #119

**DNA Day Experience in High Schools on Six Indian Reservations.** *M. Godfrey[1], K. Szarama[2], L. Bronner[1], R.A. Godfrey[1], R.L. Jokela[1].* 1) Univ Nebraska Medical Ctr, Omaha, NE; 2) Dev Neuroscience and Auditory Mechanics, NIDCD/NIH, Bethesda, MD.

One of the most effective ways to capture and retain students' interest in science is to have plenty of hands-on opportunities. Despite the best intentions of most teachers, interactive presentations are less common in places where students' needs are greatest. To help fulfill some of this need we developed a full day DNA Day program for high schools on six Indian Reservations. Our long-standing efforts in these communities have generated trust to enable us to provide educational outreach.

The program was structured to provide background information via visual material and discussion complemented with many hands on science activities. At the start of the program students received a DNA Day booklet to follow the topics and take notes. The program was planned to run an entire academic day with students taking part in all aspects. For the most part, our DNA Days did just that. In two of the schools, however, different students came for their regularly scheduled science class and were exposed to a single aspect of the program. While this was not ideal, we were able to provide some information about the significance of genetics and genomics. In one school the program was modified to fit a half-day schedule since the teacher was unsure of the attention span for most students.

The absence of American Indian role models is partially responsible for the lack of achievement in these disparate populations. To this end we produced a brief video presentation of two American Indian geneticists as role models for these students. More recently, as part of an ongoing program to highlight Native Americans in health and science professions, we have produced a poster and curriculum materials on a Navajo molecular biologist.

More than 400 students participated in DNA Day. An evaluation of their enjoyment, using a Likert scale, confirmed our impression that the hands-on activities were enjoyed more than slide shows or traditional lecture pedagogy. The video of the Native American geneticists was also well received. Evaluation from teachers who participated was also very positive.

These data and our experience shows that faculty of institutions of higher learning has an important role in promoting their profession and discipline to the public in general and to underserved populations in particular.

## 1571/T/Poster Board #120

**Model organisms in teaching Life Sciences.** *A. Pascucci, G. Forni.* ANISN National Association of Natural Science Teacher, Italy.

Basic life processes are conserved among species. As far back as Gregorio Mendel's use of the pea plant as a 'tool' conducive to the understanding of wider-reaching mechanisms, the strategy of employing 'model organisms' such as Drosophila melanogaster, Arabidopsis thaliana, Caenorhabditis elegans, Danio rerio, has been, and still is, extremely commonplace in scientific research. Each model organism has its own advantages and disadvantages. Choosing an appropriate model depends on the exploratory situation one has to face. Use of model organisms even in the didactic field has proved to be an interesting and versatile teaching tool in the classroom approach to both basic biological mechanisms and more recent complex bio-molecular studies. The didactic projects with model organisms such as bacteria, peas, fruit flies, worms and fishes, already been introduced in schools of different levels , are as follows: Inclusive of experimental protocols, student resource/observation files and teacher's guide. Organized in self-standing, graded experiments based on complexity of theme and laboratory techniques and the acquisition of cross-level skills. Inclusive of a number of 'classroom friendly' activities which require neither sophisticated instruments nor fully equipped laboratory facilities. The organisms presented, though greatly varied, have the advantage of being small, cheap and easy to cultivate and well suited to an experimental life indoors. The organisms employed are the very same as those used in research laboratories thus bringing the students 'closer' to science, especially if an attempt is made to create an opportunity for interaction with research centres. Experimental protocols have been developed with the advice of scientists from the Institute of Genetics and Biophysics of the CNR of Naples and University of Naples "Federico II".

## 1572/T/Poster Board #121

**Integrating an authentic research experience into an undergraduate recombinant DNA technology laboratory course.** *B. Quimby.* Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD.

National science education reform initiatives recommend that undergraduate biology programs integrate authentic research based laboratory activities into the curriculum. To answer this call, a project-based approach was employed for an undergraduate six week summer Recombinant DNA Technology laboratory course at the University of Maryland. The goals were 1) to apply recombinant DNA techniques to address a real-life problem, 2) to focus on student problem solving skills, and 3) to integrate team work throughout the process. Mutations in the human galactose-1-phophate uridylyl transferase (hGALT) gene result in the metabolic disorder galactosemia. For the majority of the more than 230 mutations in hGALT that have been identified in patients, it is unknown how they affect the protein function to result in disease. The yeast Saccharomyces cerivisiae has been used as a model system for analysis of hGALT. Yeast is an excellent model system for undergraduate work, because it is fast, easy and cheap to work with. To meet the goals set for the course, students were divided into 6 groups of three and each group performed PCR mutagenesis of the hGALT protein and analyzed the effects of the mutations isolated on the function of the hGALT protein using the yeast model system. As this is a multi-step process involving many recombinant techniques the groups had multiple opportunities to work on problem solving skills as the various steps failed for a variety of reasons. The project culminated with each working group presenting their results in a 20 minute presentation to the class that was assessed by their classmates and the instructor based on a defined rubric. Through these group presentations the students demonstrated their ability to assemble data into a coherent story and formulate hypotheses about the novel mutations they had isolated and analyzed. Student responses on surveys administered at the end of the course indicated that students valued ownership of their project and the unknown outcome of their work. Students also reported a better understanding of how the techniques learned in the course could be applied to address a specific scientific problem. Through the use of authentic research to teach recombinant DNA techniques students demonstrated mastery of basic skills and their ability to interpret experimental results. Most importantly, this approach gave students an experiential snapshot of the process of science.

## 1573/T/Poster Board #122

**The Impact of High School Genetics Education and Youth Risk Behaviors on Chronic Disease Prevention.** *J.L. Raiford.* Behavioral Sciences & Health Education, Emory University/Rollins School of Public Health, Atlanta, GA.

Given the effect genomics may have on the prevention of chronic diseases and the paucity of data on genetics education and disease prevention among youth, this study utilized the Utah Youth Risk Behavior Survey (YRBS) to assess (1) the impact of genetics testing awareness on youth risk perception and intention of behavioral change and (2) predictors of interest in family health history and intent to change health behaviors in instances of disease risk among youth. The 2005 Utah YRBS assesses risky health behaviors among youth (e.g., substance use, violence) and includes 4 questions developed by the Utah Tobacco Prevention and Control Program and the Utah Department of Health's Chronic Disease Genomics Program to assess genetics awareness and interest among Utah adolescents. 1,976 Utah high school students (51.3% male, 88.5% white) completed the 2005 YRBS. Study results show that, when controlling for age, those adolescents who were educated regarding genetic testing were 4 times more likely (AOR= 3.8, $p \leq .001$) than those without this education to perceive themselves as at risk for developing a particular disease (e.g., heart disease, diabetes, asthma or cancer) if one of their family members had the disease, and those reporting genetic testing education were more than twice as likely (AOR= 2.4, $p \leq .001$) to report that if a disease ran in their family they would change their lifestyle behaviors to decrease their chances of getting the disease. Moreover, tobacco and drug use and psychological distress reduced interest in learning about family health history and intention to change health behavior. Those adolescents reporting cigarette or marijuana use were less likely to be interested in learning more about their family's health history or report an intention to change health behaviors in the event a disease ran in their family than those adolescents not reporting tobacco or marijuana use. Also, adolescents reporting depressed affect, suicidal thoughts, plans, attempts, and/or injuries from a suicide attempt were less likely to report interest in learning about their family health history than those adolescents not reporting psychological distress. These results demonstrate the importance of educating youth about the nature of genetic testing and its benefits for prevention. This study also highlights individuals at-risk of not benefiting from genetic education, thereby strengthening the rationale for substance use and suicide prevention interventions.

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## 1574/T/Poster Board #123

**Culturally and linguistically appropriate genetics education materials for Hispanic/Latino students and families: testing and outcomes.** *L. Stark[1], R. Giles[2], J. Johnson[2], S. Eddings[3].* 1) Genetic Science Learning Center, Univ Utah, Salt Lake City, UT; 2) Utah Department of Health, Salt Lake City, UT; 3) Bach-Harrison LLC, Salt Lake City, UT.

We used a community-based participatory approach, working with teachers and community members, to develop two sets of culturally and linguistically appropriate genetics curriculum materials for Hispanic/Latino students. The bilingual fifth grade materials address inherited traits and the partially bilingual secondary materials address polygenic, multifactorial chronic diseases and family health history. Both curricula include bilingual take-home activities for students to share with their families. The materials were tested in Utah schools with Hispanic/Latino enrollments of 46-64%. The fifth grade materials were tested with 159 students and six teachers. Students showed a significant knowledge gain between the pre and post tests (p<.01) and a significant increase in answer confidence. Most students (77.5%) talked about genetic traits and inheritance with someone in their family. The largest number indicated that they talked to their parents (64.5%), followed by siblings (27.2%). Teachers reported that due to the Spanish-language family materials, students had good conversations with their parents about genetics. The secondary materials were tested with 499 middle and high school students and six Health Education teachers. Approximately half of the classes viewed an introductory movie in which Hispanic/Latino students learned and discussed how genetics, lifestyle and behavior influence one's risk of developing chronic diseases. All students showed a significant knowledge gain between the pre and post tests (p<.01) and a significant increase in answer confidence. Significantly more students completed a family health history if they were in classes that saw the movie (62.7%) than if they were in classes that did not see the movie (45.5%) (p<.001). Significantly more students indicated they talked to at least one family member about the family health history if they were in classes that saw the movie (86.4%) than if they were in classes that did not see the movie (75.6%) (p<.01). These data show that both sets of curriculum materials effectively impacted student learning, that bilingual take-home materials effectively engaged families in genetics learning, and that a culturally-appropriate movie can motivate students to complete a family health history. This project is supported by grant U33MC00157 from the Health Resources and Services Administration, Maternal and Child Health Bureau, Genetic Services Branch and the March of Dimes.

## 1575/T/Poster Board #124

**One example of Genetics Education in High School: obesity and the leptin gene.** *B. Zanini[1], V. Marini[1], M. Marini[1], E. Rovini[2], A. Corda[2], R. Ravazzolo[1].* 1) School of Medicine, University of Genoa, Istituto G. Gaslini, Genova, Italy; 2) National Institute for Cancer Research, Genoa, Italy.

Teaching genetics in high school undoubtedly represents one of the most challenging activity in the post genomic era. While the discoveries of molecular genetics, genomics and biotechnology have been transferred from research laboratories to the textbooks, the mendelian classical genetics remains at the core of the practical activity of genetic training of students. We realize the inadequacy and the insufficiency of the traditional didactics in teaching the new genetics and we describe here an activity which has been undertaken in the context of the official teaching programs in Biology in some Italian high schools, in the city of Genova. Since almost ten years, a working group of Biology teachers in different Italian cities has developed experience in extending teaching of Genetic issues with practical activities offered to high school students. A member of this working group, who is now attending a Research Doctorate course in genetics, has started a program in which a practical laboratory course in Molecular Genetics has been set up. The possibility to attend a doctorate course has been fundamental to allow a strict interaction between a person involved in high school teaching and a university research laboratory, by which an exchange of knowledge is then re-addressed to the school. The course has the objective to teach the students how a DNA variant in a gene of interest, in this case the Leptin encoding gene, can be identified and how genotypes can be tested. The students extract DNA from their own saliva, amplify a segment of the gene of interest by Polymerase Chain Reaction (PCR) and assess individual genotypes by digestion with a restriction enzyme. We emphasize that this type of analysis is simple enough to be followed step by step by each student, under the guide of their teachers who have had an appropriate training before in the same activity. This approach allows to introduce the students to the existence of common variants in human populations and to explain how these might be related to multifactorial conditions, such as obesity in this particular case. The statistical methods to verify whether a molecular variant can be considered as a susceptibility genetic factor are explained with the help of examples taken from known and published work both using a candidate gene approach and a genome wide approach. Moreover, variability in the human genome is also explained in more general terms related to population genetics.

## 1576/T/Poster Board #125

**Team Teaching Genetic Concepts to Ninth Graders.** *L. McCabe[1], E. Stanley[2], T. Huynh[2], J. Martinez[2], J. Thiel[2], J. Truong[2], E. Steja[2], E. McCabe[2].* 1) Dept Human Gen, David Geffen Sch Med UCLA, Los Angeles, CA; 2) University of California, Los Angeles, Los Angeles, CA.

Background: The UCLA Center for Society and genetics (CSG) attempts to anticipate the impact of developments in genetics on individuals and groups. CSG is intensely cross-diciplinary, and currently includes faculty members and students from seven professional schools, 29 departments, and three of the four instructional divisions of the College. CSG developed an undergraduate minor in Society and Genetics and one of the required courses in DNA: Promise and Peril (based on the McCabe's book by that title), which describes how genetics impacts your daily life. Objective: To determine if we could develop team teaching materials for ninth grade students. Methods: UCLA in LA provided grant funding and an introduction to Ms. Woods, Principal, King Drew Magnet High School of Medicine and Science. Ms. Woods welcomed us to her school and introduced us to three of her teachers: Ms. Kong (Life Skills); Ms. Reyes (Biology); and Mr. Zajc (Health). Ms. Kong and Ms. Reyes worked with us the first semester, and Ms. Reyes and Mr. Zajc worked with us the second semester. Results: The King/Drew teachers and UCLA team developed eleven, 90 minute discussions. After the UCLA team met to discuss materials for each discussion. Topics included: DNA forensics; epigenetics; pharmocogenomics; genetic discrimination; newborn screening; genetic bottlenecks; sample ownership; near relative DNA forensic testing; ancestry; gender testing; and designer babies. The students were actively and enthusiastically engaged by the debates, discussion questions, games, plays, powerpoints, videos, and writing exercises. Conclusions: Ninth graders are able to master genetic concepts and consider the impact of genetics in their lives. The stories we used provided a context for the science and encouraged the students to apply genetics to their own experience.

## 1577/T/Poster Board #126

**Are information websites of hereditary breast and ovarian cancer useful? : Evaluation based on web research in Japan.** *M. Izumi, K. Kaneko, S. Yokoyama, N. Gongo.* Genetic Testing Dept. Biomedical Business Div. FALCO biosystems Ltd.

FALCO biosystems (Kyoto, Japan) is a commercial laboratory providing wide range of clinical testing services, including genetic test of BRCA1/2 gene for diagnosing hereditary breast and ovarian cancer (HBOC). We have acquired an exclusive license in Japan for BRCA1/2 gene testing by Myriad Genetics, Inc. (Utah, U.S.). The genetic test of BRCA1/2 is available as a routine clinical test in the U.S. and other Western countries. However, the BRCA1/2 testing is still utilized only in 15 medical centers in Japan. The certified genetic counselors working in FALCO support clinicians and nurses to utilize the BRCA1/2 testing appropriately, for example with providing educational tools for genetic counseling of HBOC. The counselors also created the information website of HBOC for patients/the public to learn more about the hereditary cancer. All our activities follow some related guidelines for genetic testing in Japan. We performed the web research among 359 clinicians (gynecological oncologists or breast cancer specialists) and 1007 patients/families separately to evaluate the information website of HBOC and to survey their attitudes toward the BRCA1/2 testing. More than ninety percent of the clinicians evaluated the website as "good" or "rather good". 58%; of them answered "I am interested in utilizing the BRCA1/2 testing on their clinics/medical centers". On the other hand, about 90%; of patients/families did not browse the website. Once they accessed to the website, 95%; of them evaluated it as "very useful" or "useful". More than half of patients/families did not believe in hereditary breast cancer. However, 70%; of patients/families were "very concerned" or "have thought" about any risk of heritability of breast cancer. About 70% of them were interested in undergoing genetic counseling. Regarding the BRCA1/2 testing, 70%; of patients/families were interested in undergoing it if the conditions (costs etc) were met, 14%; of them answered "I won't undergo the testing". As the reasons, some answered "I feel scared to know the risk" and others answered "To know the risk is not useful". These results will serve as a basis for some improvement of the information website. We need to improve the website recognized by patients/families. They also help us to provide the BRCA1/2 testing more appropriately in Japan. It is necessary to take care of their attitude toward the BRCA1/2 testing to educate them.

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## 1578/T/Poster Board #127

**An Integrated Approach to Autosomal Recessive Genetic Diseases in High School Teaching.** *E. Andreozzi[1], M. Borriello[2], G. Esposito[3], A. Perreca[3], F. Salvatore[4].* 1) ITCG Archimede, Naples, Italy; 2) IPSSAR Marco Polo, Genoa, Italy; 3) University Federico II, Biochemistry and Medical Biotechnologies Dept., Naples, Italy; 4) CEINGE-Advanced Biotechnologies, Naples, Italy.

With a prevalence of 1/20,000 (Europe and North America) and an autosomal recessive mode of transmission, hereditary fructose intolerance (HFI) is an uncommon genetic disease. In affected individuals, ingestion of fructose produces such acute and chronic symptoms as hypoglycaemia and progressive liver damage. In recent years, attention has indirectly focused on HFI mainly because of the supposed correlation between fructose-rich diets and a series of metabolic diseases, and the obesity epidemic. Consequently, HFI is a hot topic that can arouse the interest of undergraduate students. Moreover, a discussion of HFI will allow us to apply the Punnett Square and to examine numerous different disease mutations that have been identified in the aldolase B gene in connection with HFI using the real-time polymerase chain reaction (PCR) technique. A series of lessons on HFI will thus lead to a discussion of both social aspects (the national programme of health education) and scientific aspects (genetic mechanisms). On a social level, the main aims are to spread knowledge/awareness about HFI and about the potential harmful effects of fructose-rich diets. On a scientific level, the aims are to learn to apply methods underlying the autosomal recessive mode of transmission of genetic diseases and to learn about the modern laboratory techniques used to identify modifications in the aldolase B gene. These aims can be pursued thanks to the knowledge acquired during the activities of a research doctorate during group work. The instruments used to pursue the aims on the social level of health education consist in a questionnaire designed to identify the students' eating with specific reference to the intake of fructose in the single diets and discussion of the questionnaire results. The discussion will show that exclusion of fructose from a diet can suggest fructose intolerance, and therefore the need for further tests to verify this condition. The instruments used at scientific level consist in the application of the Punnett Square to the transmission of HFI and in access to the PCR instrumentation thanks to the activities of the research doctorate during group work.

## 1579/T/Poster Board #128

**Who said it? When did they say it? How did they know?: Deciding when to trust or trash genetics health information.** *E. Reed[1], M. Weaver[2], A. Chappelle[3], P. Furlong[4], K. Clapp[5], R. Miller[6], M. Blitzer[2], C. Greene[2], S. Terry[3].* 1) NCHPEG, Lutherville, MD; 2) University of Maryland, Baltimore, MD; 3) Genetic Alliance, Washington, DC; 4) Parent Project Muscular Dystrophy, Middletown, OH; 5) FRAXA Research Foundation, Newburyport, MA; 6) National Fragile X Foundation, Walnut Creek, CA.

The Access to Credible Genetics Resources Network (ATCG) is a cooperative effort among researchers and advocacy professionals to develop approaches for addressing concerns about the quality of information available about genetic disorders. Existing evidence-based criteria in medicine provide standards of clinical care or treatment, but do not address standards for educational information about rare, single-gene disorders. To address this gap, the ATCG has developed the Quality Assessment Toolbox (Toolbox) to help developers of genetics educational information create quality materials. We are creating an additional online version of the Toolbox for the general public to serve as a guide in assessing the quality and completeness of educational materials. The development of these tools has raised issues about the definitions of quality, completeness, and accessibility of information, specifically as they pertain to conditions with a genetic basis. To advance these discussions, ATCG is holding a national meeting in September 2009 to bring together individuals who convey complex information to a variety of audiences, including those involved in education, evidence-based care, communication, and policy. Discussion will focus on issues relate to: defining information quality, choosing appropriate content, and utilizing new technologies effectively for education. The structure of the day will be organized around the three content areas of the Toolbox. The discussions of the meeting will be parlayed into tangible products, including publications, which will be distributed widely using the ATCG network. We will gather feedback and evaluations from conference participants. We will present both versions of the QAT and the outcomes of the national meeting.

## 1580/T/Poster Board #129

**The Coriell Personalized Medicine Collaborative: Examining the Utility of Genome-Informed Medicine.** *M. Christman.* President & CEO, Coriell Inst Med Res, Camden, NJ.

The Coriell Personalized Medicine Collaborative (CPMC) is a research study that employs an evidence-based approach to determine the utility of using personal genome information in health management and clinical decision-making1. The CPMC also aims to build a cohort with rich genotypic and phenotypic data with which to discover genetic variants that affect drug toxicity and efficacy, as well as to discover presently unknown gene variants that elevate a person's risk of cancer and other complex diseases. This forward-looking, collaborative effort involves physicians at several hospital partners, scientists, ethicists, genetic counselors, volunteer study participants, and information technology experts. Its goal is to better understand the impact of personalized, or genome-informed, medicine and guide its ethical, legal and responsible implementation2. All participants are genotyped on the Affymetrix 6.0 and D-MET genechips and potentially actionable items are returned to participants and physicians. The study will enroll 10,000 individuals by the end of 2009 with an ultimate goal of 100,000 participants. As of April 2009, there were ~4,000 participants enrolled in the study. There is no charge to study participants. 1. "Personalized Health Care: Pioneers, Partnerships, Progress" A report prepared by the Initiative on Personalized Health Care under Federal HHS Secretary Michael O. Leavitt. Found at: http://www.hhs.gov/myhealthcare/news/presonalized-healthcare-2008.html 2. Prainsack, P., Reardon, J., Hindmarsh, R., Gottweis, H., Naue, U., Lunshof, J.E. (2008) "Commentary on Personal Genome Tests", Nature 456, 34-35.

## 1581/T/Poster Board #130

**Group Genetic Education Sessions: An Alternative Approach to Traditional Genetic Counseling.** *V. Oliva[1], D. Durand[1], M. Johansen[1], M. Rabinowitz[2], J. Follmer[1].* 1) Genzyme Genetics, Miami, FL; 2) Miami Beach Community Health Center, Miami Beach, FL.

**Background:** Given the recommendation to offer prenatal genetic screening and diagnostic options to all patients, there is an increasing demand on obstetricians to provide their patients with appropriate education about their options. Many have decided to refer their patients to genetic counselors (GC) in order to provide this information. Consequently, GCs are inundated, providing education to patients who would not have previously been referred to them. **Objective:** To develop a group genetic education session as an alternative to traditional genetic counseling in order to meet the needs of obstetricians who routinely offer screening and diagnostic options as recommended by ACOG and ACMG. **Methods:** PowerPoint presentations were developed in English and Spanish for a genetic education session for obstetric patients of the Miami Beach Community Health Center. The education session included a discussion of age related aneuploidy risks, maternal serum screening, prenatal diagnosis, and ethnicity screening. The session did not include a family history evaluation and was not intended to replace traditional GC. Bi-monthly group sessions were performed by 2 GCs from Aug-Dec 2008. Following the sessions, patients were given satisfaction surveys which queried 6 areas: was the information easily understood, how well was the available testing explained, how appropriate was the amount of information, did the information help in decision-making, would the patient recommend such a session to others and what was the overall satisfaction. Each area was ranked on a 5-point scale (1-5 strongly disagree to strongly agree). **Results:** Based on survey responses, patients had an overall satisfaction rating of 4.7. Patients reported that the education sessions helped their decision making. When asked if the amount of information presented was acceptable, the overall response was 4.73 on the 1-5 scale. Furthermore, 83% of the patients strongly agreed that the information was easy to understand and 80% of the patients would recommend the education sessions to others. **Conclusion:** An alternative to traditional genetic counseling was developed to meet the demands of obstetricians and patients needing education regarding prenatal screening options. The group education sessions met this need and were seen as highly effective by patients. Although this was a small sample size, this model could be applied on a broader scale to meet the increasing demand for genetic education.

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## 1582/T/Poster Board #131

**The Genetic and Rare Diseases Information Center (GARD): Improving access to health information resources through collaboration.** *M. Snyder[1], S. Von Schuch[1], J. Lewis[1], M. Della Rocca[1], D. Lea[2], H. Hyatt-Knorr[3].* 1) Lockheed Martin, Rockville, MD; 2) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 3) Office of Rare Diseases Research, National Institutes of Health, Bethesda, MD.

In 2001, the Office of Rare Diseases Research (ORDR) and the National Human Genome Research Institute (NHGRI) at the National Institutes of Health (NIH) established the Genetic and Rare Diseases Information Center (GARD) to enhance the public's knowledge of genetic and rare disorders. Over the last 8 years, GARD has responded to nearly 24,000 requests for information on 6,313 specific conditions.

While the majority of GARD users are patients, friends, or family members, the Information Center has responded to more than 2,000 requests for information from researchers, physicians, genetic counselors, nurses, and social workers. Many of these individuals requested help finding clinical or research genetic testing, treatment information, or experts in a particular condition. In response, GARD provided more than 7,000 resources, including referrals to NIH resources and publications, high quality Web sites, advocacy organizations, experts, clinical trials, and genetic services.

In 2008, GARD introduced a new online portal (http://rarediseases.info.nih-.gov/GARD/) to its collection of information resources and questions answered by GARD Information Specialists. The GARD Web pages include an expanding variety of resources that can be used by genetic researchers and healthcare professionals. Information is now available on past and upcoming ORDR cosponsored scientific conferences, making it easy to locate conferences related to a particular disorder. GARD also continues to increase links to resources on clinical and research genetic testing laboratories in the United States and other countries.

Since the launch of the Web pages, GARD has partnered with government agencies to further expand Web content. Last year, GARD collaborated with the Centers for Disease Control and Prevention (CDC) to incorporate information about more than 90 newborn screening diseases from the CDC's GeT-EQuiP portal onto the GARD Web pages. GARD also worked with the Office of Orphan Products Development (OOPD) of the Food and Drug Administration (FDA) to include information about OOPD-approved orphan products for rare diseases. GARD's vast online collection of resources can be a helpful tool for genetics professionals.

## 1583/T/Poster Board #132

**Resource Repository: Revolutionizing access to information.** *A. Chappelle, J. Bialick, S.F. Terry.* Genetic Alliance, Washington, DC.

The genetics and health community needs an accessible and common forum in which to share the wealth of quality information the flows from parent and family groups, community organizations, disease-specific advocacy organizations, professional societies, educational institutions, corporations, and government agencies. Genetic Alliance launched a new and improved electronic Resource Repository in the summer of 2009 (www.resourcerepository.org) that provides the community with free access to a growing collection of documents, links, audio, and video files. Features include the ability to track new content tailored to a user's interests, a robust and intuitive indexing system, the capability to view the most recently uploaded and most often downloaded content, an auto-generated cover page with title, author, and date, and a user-friendly submission process for users to upload their own materials. Users can upload new resources at any time, so information is kept up-to-date. In addition, organizations will be able to host their own entries to the Resource Repository with the look and feel of the host organization's website (through Application Programming Interfaces). These diverse access points will lead to the same vast knowledge network and result in shared information across genres and industries. The ease of use and accessibility of the Resource Repository makes it an invaluable tool for the entire genetics and health community.

## 1584/T/Poster Board #133

***Celestial3D*: A tool for 3D visualization of familial data for genetics education.** *E. Lam[1], R.J. Webster[1], J. Emery[2], L.J. Palmer[1].* 1) Centre for Genetic Epidemiology and Biostatistics, The University of Western Australia, Crawley, Western Australia, Australia; 2) School of Primary, Aboriginal and Rural Health Care, The University of Western Australia, Crawley, Western Australia, Australia.

Traditionally, paper-drawn pedigrees have been represented in two-dimensions (2D) and many current software packages remain true to this standard. However, the representation of large and/or complicated pedigrees in only 2D is cumbersome and reduces the ease of comprehension. The Celestial3D project started with the concept of arranging pedigrees in three-dimensional (3D) space, with the primary purpose of improving the visualization of large/complex pedigrees. The original pilot software for Celestial3D stemmed from applications in epidemiological and pedigree research and offered visualization capabilities only. We have taken Celestial3D to the next stage, using a 3D games engine (Unity) for rendering the pedigrees, which opens new opportunities for added interactivity and enhances functionality for serving its original purpose. These include features such as: on-demand searching/filtering capabilities, dynamic environmental changes and freedom to incorporate building of new pedigrees within 3D space. A primary application focus for this tool has been established in consultation with secondary-school teaching representatives and collaborators in the field of science education, where we have specifically identified the potential for Celestial3D to be used in introducing the concepts of genetics at secondary-school and undergraduate biology classes. Our goal is to develop a complete educational package to instruct students, via a student-friendly 3D environment offered by Celestial3D, to conduct exercises in reading pedigrees, explore and investigate Mendelian genetics that may parallel in-class experimental data and look at complex genetic matters. This renewed approach is hoped to assist educational facilitators in increasing the appeal for students studying subjects involving genetics and encourage future interest at higher education levels. We are also exploring potential clinical applications of Celestial3D.

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## 1585/T/Poster Board #134

**Megalourethra: Prenatal Diagnosis, postnatal outcome and autopsy findings. Report of 7 cases.** *H. Amsalem[1], G. Ryan[1], B. Fitzgerald[2], S. Keating[2], P. Salle[3], A. Toi[4], H. Berger[5], D. Chitayat[6].* 1) Department of Ob & Gyn, The Fetal Medicine Unit Mt Sinai Hospital, University of Toronto, Toronto, Ontario, Canada; 2) Department of Pathology and Laboratory Medicine Mt Sinai Hospital, University of Toronto, Toronto, Ontario, Canada; 3) Department of Pediatrics, Division of Urology, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; 4) Department of Diagnostic Imaging, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada; 5) Department of Ob & Gyn, St. Michael Hospital, University of Toronto, Toronto, Ontario, Canada; 6) The Prenatal Diagnosis and Medical Genetics Program Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada.

Megalourethra is a rare form of functional obstructive uropathy, caused by maldevelopment of the penile mesodermal tissue resulting in functional lower urinary tract obstruction. It is subdivided into two types, fusiform with deficiency of both corpus spongiosum and cavernosum and scaphoid with absence of corpus spongiosum. Both types result in severe dilatation of the penile urethra. Postnatally, urination and sexual function are significantly impaired. We report the prenatal diagnosis, postnatal outcome and autopsy findings in 7 cases diagnosed prenatally at 18-24 wk gestation. The main sonographic features were those of partial lower urinary tract obstruction including distended bladder accompanied by cystic dilatation and elongation of the penile urethra. In three of the fetuses, talipes was also diagnosed. Only one case was complicated by mild early oligohydramnios at diagnosis. Three of the pregnancies were terminated and the autopsy confirmed megalourethra and distended bladder. All 4 live born babies needed several urologic procedures which will be described in detailed. Two of them had normal renal function, one had impaired renal function and one developed end stage renal disease at the age of 2 currently awaits renal transplantation. None of the live borne babies was able to urinate normally. CONCLUSIONS: In cases of megacystis, diagnosed by antenatal ultrasound, particular attention should be paid to the appearance of the penis, especially if the amniotic fluid volume is not severely decreased. Parents should be counseled regarding the long term urology outcome which is different than that of most other forms of lower urinary tract obstruction.

## 1586/T/Poster Board #135

**Association of early fetal megacystis with trisomy 18.** *M. Kambich[1], S. Weiss[2], L. Geibel[1], S. MacGregor[1], I. Salafsky[3].* 1) Department of Obstetrics & Gynecology, Center for Maternal and Fetal Health, NorthShore University HealthSystem Evanston, IL; 2) Department of Medicine, Center for Medical Genetics, NorthShore University HealthSystem Evanston, IL; 3) Department of Pediatrics, NorthShore University HealthSystem Evanston, IL.

Fetal megacystis is defined as an abnormal enlargement of the fetal bladder. First trimester diagnosis requires a longitudinal bladder diameter of 7mm or more. The incidence of fetal megacystis is approximately 1 in 1500 pregnancies. Commonly recognized associations include posterior urethral valves, prune belly syndrome, and urethral atresia. We report several cases of early fetal megacystis noted on ultrasound in fetuses who had a subsequent diagnosis of trisomy 18. A retrospective chart review was conducted of all cases of trisomy 18 at our institution in which a ultrasound had been performed on fetuses between 11-21 weeks of gestation for a five year period from January 2003 to December 2008. Six fetuses out of forty-eight affected with trisomy 18 (12.50%) were initially identified with megacystis occurring in the first and second trimester of pregnancy. Three of the five fetuses were identified with megacystis at the time of the first trimster Down syndrome screening examination. The remaining three fetuses (identified in the second trimester) had not undergone first trimester Down syndrome screening, but the extent of the megacystis suggests that the process may have been present in the first trimester. Subsequent to the abnormal ultrasound findings, prenatal diagnosis of trisomy 18 in all fetuses was achieved through cytogenetic analysis utilizing either chorionic villus sampling or amniocentesis, depending on the appropriate gestational age. This review suggests that trisomy 18 should be included in the differential diagnosis of early fetal megacystis. Cytogenetic analysis should be initiated at the time of diagnosis of fetal megacystis. This will allow for accurate genetic counseling and pregnancy management.

## 1587/T/Poster Board #136

**Functional analysis of ATG9B in the human placenta.** *R. Shono[1], T. Yamada[1], I. Furuta[1], M. Morikawa[1], T. Yamada[1], H. Yamada[2], H. Minakami[1].* 1) Department of Obstetrics; Hokkaido University Graduate School of Medicine; Sapporo, Japan; 2) Department of Obstetrics and Gynecology, Kobe Univ., Kobe, Japan.

We previously reported that ATG9B (NOS3AS), the overlapping antisense transcript with endothelial nitric oxide synthase (NOS3) is involved in its post-transcriptional regulation, and encodes an autophagy protein highly expressed in placenta. ATG9A and ATG9B are identified as human homologue of yeast Atg9p. ATG9A is ubiquitously expressed and evolutionarily conserved from yeast to mammalian, whereas ATG9B is tissue-specifically expressed in adult tissues (pituitary gland and placenta) and exists only in vertebrate species but not in lower eukaryotic species. ATG9B has a function to complement ATG9A. The NOS3 locus at chromosome 7q36 has been suggested to be associated with a number of diseases including preeclampsia (PE). In this report, we hypothesized that ATG9B may play important roles in the development of placenta and the pathophysiology and etiology of perinatal disease including PE and placentas after delivery were analyzed to investigate the hypothesis of the function of ATG9B with related genes. A total of 114 placentas (80 from normal pregnancies (NP) and 34 from pregnancy-induced hypertension: PIH) were examined under approval of ethical committee of the institute. After the extraction of RNA, quantitative RT-PCR was performed to measure the expression levels of ATG9A, ATG9B, NOS3, and sONE (short variant of ATG9B). Autophagy activity was determined by the detection of LC3-II shifted from LC3-I through immunoblotting using anti-LC3 antibody. Among samples of NP, the expression of ATG9B increased with advancing GA and those of ATG9B and sONE were higher in the samples with labor onset (LO) than those without LO. ATG9A and NOS3 were not influenced by GA or LO. ATG9A showed positive correlations with NOS3 and inverse correlations with ATG9B. ATG9B showed inverse correlations with NOS3. The autophagy activity was not enhanced along with GA nor LO, suggesting that the increased ATG9B expression with advancing GA and LO in NP does not play a role in the activation of autophagy but may have other function. Next, PIH (PE and gestational hypertension) samples were analyzed and compared with those of NP. The gene expressions were not influenced by GA in PIH. The expression of ATG9B was higher in preterm PIH samples than those in preterm NP without LO. The autophagy activity was more enhanced in PIH than in NP, suggesting that the increased ATG9B expression might have played a role in the activation of autophagy in preterm PIH.

## 1588/T/Poster Board #137

**Abnormal prenatal ultrasound findings and one Ashkenazi Jew parent that carries the R2478_D2512del in *NEB*, as an early warning sign for Nemaline Myopathy.** *H. Yonath[1, 2], M. Berkenstadt[1], H. Reznik-Wolf[1], S. Eisenberg-Barzilai[1], L. Peleg[1,2], V.L. Lehtokari[3], M. Frydman[1,2], E. Pras[1].* 1) Inst Human Gen, Sheba Med Ctr, Ramat Gan, Israel; 2) Sackler Faculty of Medicine, Tel Aviv University, Israel; 3) The Folkhälsan Institute of Genetics, University of Helsinki Finland.

Nemaline Myopathy (NM) is a devastating neuromuscular disease, which is caused by mutations in several genes. One of the genes is Nebulin (NEB), the most common cause for autosomal recessive NM. A common founder mutation, R2478_D2512del was found in Ashkenazi Jews (AJ) with a carrier rate of 1:108. This mutation is included in the prenatal screening in AJ in Israel. We have recently encountered 3 pregnancies with abnormal findings on ultrasound (US) all of which resulted in babies suffering from NM. Only one of the parents in these families, however, carried the deletion mutation in NEB. Case #1: Prenatal US revealed club feet and polyhydramnios. On carrier screening the mother was found to be a carrier of the deletion, and the father was not, both are AJ. Soon after birth the girl was noted to be severely hypotonic and a diagnosis of NM was made on muscle biopsy. DNA testing revealed the second mutation in intron 108, a splice site mutation 17118+1G>A. On RT-PCR the mutation was found to cause skipping of exon 108. Case #2: A newborn girl was diagnosed with severe hypotonia. During the pregnancy there were decreased fetal movements and polyhydramnios. Muscle biopsy was compatible with NM. Molecular testing revealed heterozygosity for the R2478_D2512del in NEB, inherited from her AJ father. The deletion was not detected in the mother who is of Greek and Yemenite origins. Prenatal testing in their next pregnancy with polymorphic markers close to NEB, revealed the same haplotype in the fetus and in the affected child. The pregnancy was terminated. Case #3: Polyhydramnios, decreased fetal movements and arthrogryposis were noted during pregnancy. A previous child had similar findings and died soon after birth due to respiratory failure. The father and the fetus were found to be carriers of the deletion, the mother not, both parents are of AJ. A common haplotype was not identified in the parents that did not carry the R2478_D2512del mutation. A second mutation was found in one of the families, we are currently trying to identify the other two mutations. Ultrasonographic features such as polyhydramnios, decreased fetal movements, club feet and arthrogryposis should promote genetic NM screening. Hence, in Ashkenazi couples with such a presentation both parents should be screened for the deletion mutation and even if only one of them is found to be a carrier, NM should be highly suspected.

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## 1589/T/Poster Board #138

**Attitudes of recent and prospective mothers about newborn genetic screening: a survey of 2,266 U.S. women.** *D. Kaufman[1], N. Bonhomme[2], K. Hudson[1], S.F. Terry[2], J. Scott[1].* 1) Genetics & Public Policy Ctr, Johns Hopkins University, Washington, DC; 2) Genetic Alliance, Washington, DC.

INTRODUCTION: Studies of public attitudes about genetic newborn screening (NBS) have focused on high-risk parents and parents of affected children. However, understanding the lay public's opinions on NBS may be useful in the design of expanded NBS programs. To this end, recent and prospective mothers were surveyed about their experiences and opinions on genetic newborn screening. METHODS: An online survey was conducted in two random samples of U.S. women aged 18-45. One sample included women who had given birth in the past three years (recent mothers). The other included women planning to have a biological child in the next three years (prospective mothers). We measured the influence of a disease's severity, age of onset, and a test's positive predictive value (PPV) on support for NBS for a condition. Recent mothers also were asked about experiences with their last child. RESULTS: The response rate was 60%: 1,258 recent mothers and 1,008 prospective mothers participated. A total of 67% of recent mothers and 38% of prospective mothers had heard of NBS before. Among recent mothers, 10% received no information about NBS during their last pregnancy and another 30% felt they did not receive enough information. Although large majorities would prefer to learn about NBS before going to the hospital to deliver, 55% of recent mothers said they received information during or after delivery. After reading a definition of NBS, nearly all women said the goals of NBS were important and supported NBS to improve a baby's health, while 5% of both groups felt NBS should never be done. In both groups, 52% said parental consent should not be required but parents should be able to decline NBS, and 47% said parents should be able to choose which diseases to screen for. The largest concern in both groups was that NBS might not provide accurate results. Majorities expressed interest in NBS for adult onset diseases like colon cancer, and there was low but measurable interest in testing for traits like adult height or artistic ability. Disease severity and age of onset had little influence on support for screening, but there was 10% higher support for use of tests with a higher PPV. CONCLUSIONS: The public both needs and wants information on genetic NBS programs earlier than it is generally being delivered. There is support for NBS for a range of conditions. However, accuracy of tests results is important, as evidenced by the influence of PPV on support for use of a given test.

## 1590/T/Poster Board #139

**Newborn Screening of Congenital Hypothyroid in Shanghai Area.** *G. Tian, H. Xu, Y. Wang, W. Zhu.* Dept. Newborn Screen, Shanghai Children's Hospital, Shanghai, China.

Newborn screening for congenital hypothyroidism has been carried out for 24 years in Shanghai, China. and the prevalence of CH was investigated . A 1:1 matching case control study was performed on two groups of women that delivered normal babies and CH babies, respectively for probing the nosogenesis. Family conditions, conditions in pregnancy and other indicators of two groups of pregnant women were investigated to probe the influence factors on the delivery of CH babies. The incidence of CH tended to increase in the past 24 years, the average is 32.11/100,000 and the average annual increase rate of the incidence was 12.50 %; After iodine complement for the whole population, the incidence of CH tends to increase; Six factors are the main impacting factors for CH, in which the family history of the thyroid disease and the extent of seafood intake during pregnancy are the influence factors are statistically significant. Genetic factor plays important role in the development of CH. It should be set safe upper limit for the amount of iodine taking-in in pregnant women.

## 1591/T/Poster Board #140

**Study on interaction of eNOS and LCHAD in apoE-/- mice onset preeclampsia-like at multiple gestational stages.** *R. Ma, M. Sun, Z. Yang, Corresponding author: Zi Yang zi_yang@email.com.* Peking University Third Hospital, Beijing, China.

Objective: This study aimed to investigate interaction of fatty acid oxidation with nitric oxide in apoE-/- mice onset preeclampsia-like at multiple gestational stages, and to explore the correlation in pathogenesis in early onset preeclampsia. Methods: preeclampsia-like model were established in apoE-/- and wild-type pregnant mice at early, mid and late gestational stages by injecting nitric oxide synthase (NOS) inhibitor L-arginine methyl ester (L-NAME) subcutaneously. Control groups were received normal saline (NS) simultaneously. Groups were subdivided into L-NAME and NS in early, mid and late gestational stages in apoE-/- and WT mice respectively. Plasma lipid levels and production of NO were measured, and histological examinations of placenta were performed. The expression of endothelial nitric oxide synthase (eNOS) and long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) expression were analyzed by RT-PCR and western blot respectively. Data were treated statistically. Results: Compared with NS control groups, total cholesterol (TC), triglyceride (TG) and free fatty acid (FFA) concentration were significantly increased in early and mid subgroups in L-NAME of apoE-/- and WT groups($p<0.05$). But there was no significant difference among the late L-NAME of apoE-/- and WT subgroups and the NS control subgroups ($p>0.05$). A lower NO production were found in early, mid L-NAME subgroups in apoE-/- and WT groups than those of in the NS controls($p<0.05$), whereas no significant difference among the late L-NAME subgroups in apoE-/- and WT and the NS controls ($p>0.05$). The LCHAD expression levels of mRNA and protein showed significantly down-regulated in early and mid subgroups in L-NAME of apoE-/- and WT groups compared with NS controls ($p<0.05$). The eNOS expression levels of mRNA and protein were significantly down-regulated in early and mid subgroups in L-NAME of apoE-/- and WT groups compared with NS controls ($p<0.05$). A positive correlation was found between eNOS and LCHAD at mRNA expression in early and mid L-NAME subgroups in apoE-/- and WT groups. Conclusions: These results suggested that in preeclampsia-like mouse model by L-NAME in early and mid gestational stages, the inhibition of NO may cause hyperlipidemia through the reduction of fatty acid oxidation, which may lead to endothelial dysfunction. Whereas the changes of preeclampsia-like onset at late gestational stage may be maternal vascular response to inhibition of NO.

## 1592/T/Poster Board #141

**Chromosomal aneuplodies other than Down syndrome and trisomy 18 detected through integrated prenatal screening.** *W.S. Meschino[1, 2], T. Huang[1].* 1) Genetics Program, North York General Hospital, Toronto, ON, Canada; 2) Dept. of Paediatrics, University of Toronto, Toronto, ON, Canada.

Objective: To review Integrated Prenatal Screening (IPS) results for trisomy 13, Turner syndrome, triploidy, and other rare chromosomal anomalies. Methods: IPS for Down syndrome was introduced at North York General Hospital (NYGH) in 1999. IPS is carried out in two steps. The first step involves ultrasound nuchal translucency (NT) and maternal serum pregnancy associated plasma protein A (PAPP-A) at 11-14 weeks gestation. The second blood sample is taken at 15-20 weeks for the measurements of maternal serum alpha fetoprotein (AFP), unconjugated estriol (uE3), and total human chorionic gonadotropin (hCG). The final result is based on the integration of the values from both stages. This study reviews IPS results of trisomy 13, Turner syndrome, triploidy and other rare chromosomal anomalies identified in our screening program and assesses whether these anomalies can also be detected by IPS. Results: Between December 1999 and November 2007, 71,477 women were screened using IPS at NYGH. Ten cases of trisomy 13, 11 cases of Turner syndrome (including variants and mosaicism), 8 cases of triploidy, 3 cases of autosomal mosaicism and one case of ring chromosome 7 and monosomy 7 mosaicism were identified among women who completed IPS. IPS detected 7 cases of trisomy 13, 7 cases of Turner syndrome and 7 cases of triploidy. All three cases of autosomal mosaicism and the case of ring chromosome 7 and monosomy 7 mosaicism were also screen positive using IPS. Two cases of trisomy 13, one case of Turner syndrome and the case of ring chromosome 7 and monosomy 7 mosaicism would be screen negative using conventional second trimester triple marker screening. Conclusion: IPS can detect most cases of trisomy 13, Turner syndrome, triploidy, and rare chromosomal aneuploidies that are viable in the second trimester. Some of these anomalies would have been missed by conventional triple marker screening. This study provides useful information for genetic counselling.

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### 1593/T/Poster Board #142

**Prediction of preeclampsia by analysis of cell-free messenger RNA in maternal plasma.** *A. Sekizawa, Y. Purwosunu, A. Farina, H. Saito, T. Okai.* Showa University, Tokyo, Japan.

Objective: The purpose of this study was to predict the occurrence of preeclampsia in a series of patients at gestational week 15-20 weeks, with the use of a panel of messenger RNA markers. Materials and Methods: Data from 62 patients with preeclampsia who were asymptomatic at the time of blood testing and 310 control subjects were analyzed. Multivariable analysis was performed with discriminant analysis. Results: Univariable analysis identified vascular endothelial growth factor receptor 1 as the marker with the highest detection rate; placenta-specific 1 with the lowest. Mean estimated score for preeclampsia was 9.4 for control subjects and 72.5 for subjects who experienced preeclampsia. A receiver operating characteristic curve that was obtained with the estimated score for preeclampsia as a test variable yielded a detection rate of 84 percent (95 percent CI, 71.8-91.5) at a 5 percent false-positive rate with an area under the curve of 0.927 ($P < 0.001$). Again, detection rate and score for each patient for classification as preeclamptic correlated with severity. Conclusion: A panel of messenger RNA is able to detect subjects who will experience preeclampsia.

### 1594/T/Poster Board #143

**ADAM12: its utility in first and second trimester maternal serum aneuploidy screening.** *M. Macek[1], H. Kluckova[1], A. Lashkevich[1], R. Vlk[2], I. Spalova[2], D. Chudoba[1], D. Novotna[1], S. Vilimova[1], M. Simandlova[1], M. Turnovec[1], M. Macek Jr[1].* 1) Dept of Biology & Medical Genetics; UH Motol and Charles Univ Prague, Czech Republic; 2) Dept of Obstetrics and Gynecology, UH Motol and Charles Univ Prague, Czech Republic.

The aim of the study was to evaluate the utility of ADAM12 as aneuploidy marker in the first and second trimester maternal serum screening. ADAM12 was measured by DELFIA ADAM12 Research Kit (Perkin Elmer; USA). The control 11-17th week levels were ascertained in frozen (-20ºC) maternal sera from our screening programme from years 2001-2008. For each day at least 30 samples were used for control percentile (P) calculations. Altogether, 59 and 26 sera from first and second trimester, respectively, with different types of chromosomal aberrations were tested. The levels lower than P25 and higher than P75 were stratified in subgroups in order to express the degree of deviation from P50. The P50 levels within 11-17th week were 456-1133 μg/l. Trisomy 21 had highest prevalence in the first trimester within P25-50 range, while in the second trimester it was within P50-75. The 2/5 cases with trisomy 18 were under P5-10 and 3/3 cases with trisomy 13 were under P25. All triploidy cases were under P5. The 5/6 cases with 47,XXX were under P5, while 1/6 was under P25. All 47,XXY and 47,XYY were within the P50-95 range. The 5/11 cases with Turner syndrome (including mosaicism) were within P25-50 and 3/11 cases were within the P75-95 range. In 4 cases with structural chromosomal aberrations and in one mosaic trisomy 10, levels of ADAM12 were under P25. Further prospective studies will determine additional value of ADAM12 for the first and second trimester maternal serum screening. Supported by VZFNM00064203 and NR9448-3/2007.

### 1595/T/Poster Board #144

**Prenatal Screening for Aneuploidies and Neural Tube Defects in a newly set up Genetic Centre at GMCH, Chandigarh, India, 160031 - Results & Experiences.** *G. Kaur[1], B.S. Chavan[1], J. Srivastav[1], R. Atwal[1], G. Randhawa[1], A. Sehgal[2], R. Kaur[3], A. Huria[2], P. Goel[2].* 1) Genetic Centre, Government Medical College and Hospitall, Chandigarh, India 160031; 2) Department of Gynecology & Obstetrics, Government Medical College and Hospitall, Chandigarh, India 160031; 3) Department of Radiodiagnosis, Government Medical College and Hospitall, Chandigarh, India 160031.

The burden of genetic disorders and physical and mental handicap is excessively high in India due to preferential consanguineous marriages in some communities, absence of screening facilities, and lack of awareness among the masses. In a developing country like India, the cost of management of genetic disorders also gets substantial, not only in terms of monetary expenditure but also associated emotional and social distress. However, focus has only recently shifted towards prevention and eradication of genetic abnormal disorders through initiation of early intervention in the form of screening. Introduction of screening facilities on a large scale in any developing country is not easy unless authorities appreciate the necessity of screening for all and integrate it with sustained financial support. This becomes imperative as majority of the population cannot afford these facilities in spite of their availability.

This study was carried out to provide early interventional measures in the form of prenatal maternal serum screening of biomarkers (AFP, hCGβ, uE3) to all pregnant women and establish prevalence of aneuploidies and neural tube defects along with providing pre-test and post-test genetic counseling to all enrolled women.

Prenatal screening was introduced in December'2006. Triple test was offered at nominal charges between 15-19 weeks of gestation. All pregnant women visiting GMCH were enrolled after an informed and written consent; 5ml of blood was taken and tested for a high risk for Trisomy 21 &18 and Neural Tube Defects (NTDs). Around 3500 pregnant women were screened in the period extending December'06 to March'09 and 195(5.57%) high risk cases were segregated; who were further referred for level II fetal ultrasound for all soft markers and amniocentesis.

A single case of Trisomy 21 (DR-0.61) was diagnosed apart from one of gastroschisis and three of anencephaly. The data obtained has also been used to obtain local medians. Prenatal screening proved to be effective because of its early intervention and prevention based approach. Apart from picking up aneuploidies, high risk triple test report also helped in picking up many other prenatal conditions like oligohydamnios, intrauterine fetal demise and other congenital malformations.

### 1596/T/Poster Board #145

**Ancillary benefits of prenatal maternal serum screening achieved in the California program.** *F. Lorey, N. Kazerouni, B. Currier, C. Hodgkinson, S. Goldman, M. Roberson.* Genetic Disease Screening Program, California Department of Public Health, Richmond, CA.

Aim: To evaluate the extent of fetal structural abnormalities, other than neural tube and abdominal wall defects, identified by California's prenatal screening program. Methods: The quad marker prenatal screening records of 539,782 women were examined for both the screening interpretation and the diagnosis of structural abnormalities detected via programmatic follow-up. The time period was from July 16, 2007, to January 16, 2009. Women that were screen-positive for trisomy 21, trisomy 18, neural tube defects (NTDs) or Smith-Lemli-Opitz syndrome (SLOS) received follow-up services at State-authorized prenatal diagnosis centers. Detailed reports of services and diagnostic information were submitted on each patient in order to obtain reimbursement. These reports were linked in a database to the original screening results. Results: A total of 26,324 women received follow-up ultrasound services at State-approved prenatal diagnosis centers in the study time period. Of these women, 1082 (4.1%) were identified as having fetuses with significant structural abnormalities, other than NTDs or abdominal wall defects. In addition to the structural abnormalities, 225 cases of fetal demise, 65 cases of severe oligohydramnios, 1 case of severe polyhydramnios, 1 case of conjoined twins, 20 cases of twin-twin transfusion, 4 molar pregnancies, 4 acardiac twins and 92 cases with placental abnormalities were identified. The total number of women identified with fetuses affected by these structural defects and pregnancy complications far exceeds the number of women identified with fetuses affected by NTDs (n=202) and abdominal wall defects (n=254). The primary focus of maternal serum screening programs (other than aneuploidies) has always been considered to be the detection of NTDs and abdominal wall defects. Conclusion: While prenatal screening programs do not explicitly screen for structural abnormalities other than NTDs and abdominal wall defects, clearly many other structural abnormalities may be associated with a screen-positive status. Thus, the detection of these additional structural defects can be considered an ancillary program benefit. The California Program's follow-up ultrasounds must meet very stringent standards with respect to the practitioner's qualifications and the exam details. These high standards may also contribute to the detection of these abnormalities.

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## 1597/T/Poster Board #146

**Preimplantation genetic diagnosis for chromosome reciprocal translocation carriers by triple-color or repeat fluorescence in situ hybridization.** *Y. Tan, D. Cheng, C. Lu, F. Gong, H. Liao, L. Li, G. Lu.* Institute of Reproduction and Stem Cell engineering, Central South University, Changsha, China.

Objective To analyze the outcome of preimplantation genetic diagnosis(PGD) conducted for carriers with chromosome reciprocal translocation by multi-colour or repeat fluorescence in situ hybridization(FISH). Methods A total of 22 couples were performed for PGD after accepting genetic counseling and signing informed consent. Ovarian hyperstimulation, oocyte retrieval and ICSI were carried out as usual. Blastomere biopsy was performed at the 6-8cell stage. Triple color or repeat FISH were performed to ensure to exclue the all unbalanced embryos. Normal or balanced embryos were transferred into uterus on d5. Results A total of 24 PGD cycles were carried out. 282 oocytes were retrieved. The success rate of biopsy, fixation and hybridization were 97.7%, 96.06% and 84.85%, respectively. A total of 22 embryos were transferred and 7 clinical pregnancies were achieved. All clinical pregnancies resulted in healthy babies. No spontaneous abortion occurred. Conclusion Triple-color or repeat FISH was an effective PGD strategy to enhance normal pregnancy for reciprocal translocation carriers.

## 1598/T/Poster Board #147

**Chromosome X-inactivation and FISH studies in a rare case of familial analphoid X-centromere variant.** *PN. Rao[1], F. Nooraie[2], F. Quintero-Rivera[1].* 1) Pathology & Lab Medicine, David Geffen UCLA Sch Med, Los Angeles, CA; 2) Genoptix Medical Laboratory, Carlsbad, CA.

We report a rare case of an analphoid centromere variant of the X chromosome, in a three generation family. A 36-year-old Persian woman was referred for amniocentesis, at 17 wks gestation (G1P0). Prenatal ultrasound was unremarkable, with normal fetal movement and cardiac activity. Interphase FISH on uncultured cells with the Aneuvysion probe-set for chromosomes X,Y,13,21, and 18, exhibited only one X-chromosome signal in all 50 nuclei examined; the results were reported as abnormal consistent with Turner syndrome. A repeat ultrasound was normal, and the couple waited for karyotype studies for pregnancy management. G-banding analysis of 15 in situ colonies from 4 cultures showed a 46,XX karyotype, and no evidence of 45,X cells. In the light of the discrepancy between the interphase FISH and G-banding, FISH investigation using the X/Y-centromere probes was performed on unbanded in situ slides. All metaphase and interphase cells revealed only one X centromere signal. There was no evidence of any signals with variations in intensity or size of the X centromere. Parental chromosomes and X/Y FISH revealed the mother to carry the X-centromere variant. A healthy baby girl was delivered at 40 weeks (apgar 8,9). X/Y FISH on the maternal grandparents' metaphase cells identified the grandfather to carry the variant X-centromere. Replication banding studies were done on the mother and the infant to determine any possible relationship between X inactivation status and the variant X-centromere. This was followed by sequential FISH with the X/Y probes on the same metaphases. These studies showed a skewed X-inactivation, in both the mother and daughter. The X chromosome with the variant centromere (DXZ1 FISH negative) was always active, while the inactive/late-replicating X carried the normal centromere signal. This observation may be purely coincidental and unrelated, since the grandfather with one active X exhibited the centromere variant. It is known that most centromeres are largely made up of alpha satellite sequences, but not required for centromere function. Neocentromeres, which are functional centromeres, located in non-centromeric regions, lack alpha satellite DNA, and form to confer mitotic stability to chromosome fragments. However, an X-centromere variant as observed in this family has never been reported before and the possibility of false positive results due to variations in centromere sequences should be discussed with patients in genetic counseling.

## 1599/T/Poster Board #148

**Altered expression of mRNA and proteins for trisomy 21 affected amniocytes.** *C.-K. Cho[1,2], J. Bayani[1,2], S. Dason[1,2], E.P. Diamandis[1,2,3].* 1) University of Toronto, Toronto, Ontario, Canada; 2) Mount Sinai Hospital, Toronto, Ontario, Canada; 3) University Health Network, Canada.

Down syndome (DS), caused by an extra chromosome 21, affects 1 in 750 live births, and is characterized by cognitive impairment as well as congenital defects and increased risk for several diseases such as leukemia. Three major objectives of DS research include: 1) discovery of more sensitive and specific markers for prenatal screening, 2) understanding molecular mechanisms of DS phenotypes, and 3) finding therapeutic targets. Unfortunately, little is known about the molecular pathogenesis of DS, and no direct genotype-phenotype relationship has yet been confirmed. Proteomic analysis based on mass spectrometry (MS) allows discovery of a large number of proteins in complex biological samples. We have recently generated the most comprehensive list of proteins present in human amniotic fluid (AF) by using high resolution MS, since AF contains the most information of the developing fetus. Based on this result, we hypothesize that quantitative analyses of proteins from amniocytes will reveal novel biomarkers and/or clues to altered molecular mechanisms of a developing fetus affected by DS. We compared the proteome of amniocytes from chromosomally normal and trisomy 21 affected pregnancies (n=3 ea.), including quantitative analysis of secreted, intracellular, and membrane proteins from amniocytes by Stable Isotope Labeling of Amino acids in Cell culture (SILAC). Also, we examined microRNA expressions from these amniocytes by TaqMan Arrays, and performed qRT-PCR for proteins of interest. Over 1200 proteins from normal and DS amniocyte conditioned media were identified and quantified by LTQ-Orbitrap mass spectrometer based on MS/MS spectra ratios of peptides containing isotope-labeled amino acids. At least 5 of these quantified proteins consistently show differential expression between euploid and trisomy 21 affected amniocytes, indicating that they may play a role in DS phenotypes. Candidate proteins were selected based on the quantification between the two conditions and consistency between the triplicates of each condition, and a few have been confirmed by immunoassay and/or multiple reaction monitoring. .

## 1600/T/Poster Board #149

**Array CGH has a 13% yield for clinically significant chromosome imbalances in prenatal samples of pregnancies that eventually resulted in loss or therapeutic termination.** *S. Alliman, B.A. Bejjani, B.A. Torchia, R. Schultz, L.G. Shaffer, A.N. Lamb, J. Coppinger.* Signature Genomic Laboratories, Spokane, WA.

The use of array-based CGH (aCGH) for diagnosis of chromosome imbalances in pediatric patients is increasing and has led to exploration of aCGH applications in the prenatal setting. We evaluated 23 prenatal specimens from ongoing pregnancies that eventually resulted in stillbirth, termination, or miscarriage to address the diagnostic utility of aCGH. Of these, 17/23 (73.9%) were cultured amniocytes, 2/23 (8.7%) were DNA specimens extracted from cultured amniocytes, and 4/23 (17.4%) were cultured CVS specimens. High-resolution oligonucleotide-based aCGH was performed on 22/23 specimens and whole genome BAC-based aCGH was performed on one specimen. Indications for study included multiple congenital anomalies (MCA) in 11/23 (47.8%), cystic hygroma with or without hydrops in 5/23 (21.7%), arthrogryposis or clubbed feet with family history of previous affected pregnancy in 2/23 (8.7%), fetal loss (one with two-vessel cord) in 2/23 (8.7%), and microcephaly, skeletal anomalies, and sex discrepancy in one case each. Karyotypes were reportedly normal for 20/23 cases, with no karyotypic information provided in 3/23 cases. aCGH identified 3/23 (13.0%) clinically significant abnormalities including a de novo 4.8 Mb deletion of 7q35q36.1 in a prenatal specimen submitted for fetal loss and two-vessel cord, a de novo 3 Mb deletion of 17q24.3q25.1 encompassing the SOX9 gene in a pregnancy with MCA suspected to have campomelic dysplasia, and a de novo 6.6 Mb deletion of 9q21.33q22.32 in a pregnancy with MCA. Results of unclear significance were identified in 1/23 (4.3%) cases. These preliminary results demonstrate that high-resolution aCGH is a useful diagnostic tool for detection of submicroscopic chromosome imbalances in these pregnancies, with a 13.0% yield for results of clinical significance. Care should be taken to ensure that a sample type that allows for DNA extraction and FISH analysis is preserved in karyotypically normal pregnancies undergoing loss or termination so that aCGH can be performed.

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## 1601/T/Poster Board #150

**Detection rates for fetal anomalies in first versus second trimester ultrasound.** *A. Kang[1], P. Miny[2], H. Struben[1], I. Filges[2], S. Tercanli[1].* 1) Ultrasound Unit, University Women`s Hospital Basel, University Basel, Switzerland; 2) Division of Medical Genetics, University Children's Hospital and Department of Biomedicine, Basel Switzerland.

Objective: The aim of this study was to evaluate the performance of ultrasound screening for fetal anomalies indicating chromosomal anomalies in a two-stage screening concept comparing the detection rates of the 11 to 14 weeks scan and the routine anomaly scan in the second trimester. Methods: In this prospective study a total of 8074 fetuses were examined between 11 and 14 weeks and received a detailed scan of the fetal anatomy in addition to nuchal translucency (NT) measurement for risk assessment of aneuploidy by well trained sonographers. The first ultrasound evaluation had been carried out early between 11+0 13+6 and the second scan late between18 to 24 weeks of gestation performed in the same center. Results: In the study group a total of 128 (1.6%) chromosomal defects were diagnosed. Using NT and maternal age for risk assessment the detection rate for chromosomal anomalies at 11-14 weeks was 83% (106/128) including detection of fetuses with trisomy 21 in 93% (54/58), trisomy 18 in 96% (25/26), Turner syndrome in 80% (8/10), trisomy 13 (6/6) in 100% and other rarer chromosomal defects in 46%(13/28). The overall incidence of fetal malformations (other than increased NT-findings) detected by ultrasound was 3,6% (n=288), 40% of which were diagnosed between 11 and 14 weeks (116/288). The following 2nd trimester scan revealed 102 (35%) supplementary structural anomalies including 5% with additional chromosomal defects. Conclusion: The 11-14 weeks scan detects the vast majority of chromosomal anomalies and about 40% of all structural defects, primarily the severe and lethal forms. These results demonstrate the changing role and growing impact of the 11-14 weeks scan.

## 1602/T/Poster Board #151

**Prenatal molecular diagnostics of trisomy 21 using quantitative APEX-2 assay.** *E. Oitmaa[1], M. Peters[1,3], K. Vaidla[2], R. Andreson[2], R. Mägi[2], N. Tõnisson[6], M. Remm[2], M. Schneider[4], A. Salumets[2,3,5], K. Õunap[6], A. Metspalu[1,2,7].* 1) Biotechnology, University of Tartu, EBC, Tartu, Estonia; 2) Institute of Molecular and Cell Biology, University of Tartu, Riia 23b, Tartu 51010, Estonia; 3) Department of Obstetrics and Gynecology, University of Tartu, L. Puusepa 8, Tartu 51014, Estonia; 4) University of Ulm, Steinhövelstr. 9, ULM 89075, Germany; 5) Nova Vita Clinic, Centre for Infertility Treatment and Medical Genetics, Kaluri tee 5A, Haabneeme, 74001 Viimsi, Harjumaa, Estonia; 6) Department of Genetics, United Laboratories, Tartu University Hospital, L. Puusepa 2, Tartu 51014, Estonia; 7) The Estonian Genome Project of University of Tartu, Tiigi 61b, Tartu 50410, Estonia.

**Objective:** Trisomy 21 is the most frequent chromosomal aneuploidy affecting approximately 1 in 700 live births. Here, we demonstrate a new, fast and reliable method based on one tube multiplex PCR followed by modified Arrayed Primer Extension-2 (APEX-2) for the prenatal diagnosis of trisomy 21 (T21).

**Design & Method:** The T21 APEX-2 assay is based on the comparison of signal intensities of allelic fraction of heterozygous SNPs to discriminate between trisomy and euploid DNA samples after APEX reaction. We used 143 SNPs with high heterozygote frequency (according to HapMap genotype frequency data) from Down Syndrome Critical Region (21q21-21q22) for the analysis. For validation of the T21 assay we used blood samples obtained from T21 patients and euploid individuals and for evaluation of the T21 assay we used DNA samples derived from cultured and uncultured amniocytes (AC). APEX-2 method enables the simultaneous amplification of multiple loci of the template in two phases: the one tube multiplex PCR with SNP- specific primers in the first phase and PCR with the universal primer in the second phase. The multiplex PCR was followed by ExoI/sAP purification step and APEX reaction. APEX microarrays were imaged with the Genorama QuattroImager and genotyping of SNPs was performed by the Genorama Genotyping Software (Asper Biotech Ltd). Differences between allelic ratios of heterozygous SNPs of normal individuals and T21 patients were verified by t-test statistics.

**Results:** We developed APEX-2 microarray assay for the detection of trisomy 21. Analysis of the APEX results revealed that 90 SNPs out from initial 143 SNPs were sufficient for the reliable discrimination between T21 and euploid DNA samples ($p$-value ≤ 0.05 from one or both strand). Fifty three SNPs were excluded from the final set of APEX-2 primers during the assay development due to low call-rate and reproducibility or false signals. In the blind study using 109 clinical samples, the sensitivity and specificity of the T21 assay were both 100%. All T21 assay results were confirmed by conventional cytogenetic study.

**Conclusion:** Our experiments demonstrated that this new approach is suitable for the rapid detection of trisomy 21 in diagnostic laboratories.

## 1603/T/Poster Board #152

**Application of a validated BAC-based array CGH platform for prenatal diagnosis in South Korea.** *J. Park[1], J. Woo[2], S. Shim[3], S. Yang[2], Y. Choi[4], K. Yang[2], D. Cha[3].* 1) Obstetrics and Gynecology, Bundang CHA General Hospital, CHA University, Seongnam-si, Kyunggi-do, Korea; 2) Bio Chip Service, Macrogen Inc., Seoul, Korea; 3) Obstetrics and Gynecology, Kangnam CHA General Hospital, CHA University, Seoul, Korea; 4) Obstetrics and Gynecology, Seoul National University, Seoul, Korea.

Introduction: While conventional G-banded karyotyping still remains a gold standard in prenatal genetic diagnoses, the widespread adoption of array CGH technology for postnatal genetic diagnoses has led to increasing interest in the use of this same technology for prenatal diagnosis. Objective: Here, we describe the development and application of a BAC-based DNA chip that is being used for prenatal diagnosis in South Korea. Method and Result: We have designed a target BAC-based array CGH platform (MacArrayTM M-chip). This array CGH platform specifically targets submicroscopic deletions and duplications for 26 known genetic syndromes of medical significance as well as frequently-observed common chromosomal aneuploidies that are observed prenatally. To validate the array, we obtained genomic DNA from 132 cell lines containing relevant genomic imbalances and specific chromosomal aneuploidies. Experiments were performed in a blinded manner and all known genomic alterations were successfully identified. We then applied this array CGH platform to 98 amniotic fluid specimens that were also subjected to conventional karyotyping. Due to the targeted nature of this array CGH platform, certain chromosomal aberrations could not be detected by array CGH, but were identified via conventional karyotyping including balanced chromosomal rearrangements: 2 cases of inv(9) and 1 case of t(8;11) and two cases of trisomy (trisomy 10 and trisomy 16). However, certain chromosomal aberrations were detected with this array platform that were not oberserved by G-banded chromosomal analysis. For example, we identified eight cases of microdeletions in the Yq11.23 chromosomal region, which harbors the DAZ gene, and may lead to non-obstructive spermatogenesis. FISH analysis confirmed the deletion in all eight cases. Conclusions: We have successfully designed and applied a BAC-based array CGH platform for prenatal diagnosis. This array CGH platform can be used in conjunction with conventional karyotyping and will provide rapid and accurate diagnosis for the targeted genomic regions while eliminating the need to interpret clinically-uncertain genomic regions - certain copy number variants (CNVs).

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## 1604/T/Poster Board #153

**Large scale application of QF-PCR for rapid prenatal diagnosis of common chromosome aneuploidies, results of ten years clinical experience.** *V. Cirigliano[1,2], G. Voglino[3], E. Ordoñez[1,2], A. Marongiu[3], P. Cañadas[1], L. Rueda[1,2], E. Lloveras[4], C. Fuster[2], M. Adinolfi[5].* 1) Departament de Genètica Molecular. General Lab. 08029 Barcelona, Spain; 2) Unitat de Biologia. Departament de Biologia Cellular, Fisiologia Immunologia. Universitat Autònoma de Barcelona. E-08193 Barcelona, Spain; 3) Molecular Genetics and Cytogenetics Lab. Promea-Day Surgery, 1026 Turin. Italy; 4) Departament de Citogenètica. General Lab. 08029 Barcelona, Spain; 5) The Galton Laboratory. University College London. NW1 2HE London. U.K.

The QF-PCR test has been developed to allow prenatal diagnoses of chromosome abnormalities in a few hours after sampling. The main advantages of the assay are low cost, speed and automation allowing its application to all pregnancies undergoing prenatal diagnostic procedures. We developed a QF-PCR assay that was employed to test 50.000 clinical samples with results issued in 24 hours. Up to 10 markers were included on the sex chromosomes, 8 on chromosomes 21 and 18 and 6 markers on chromosome 13. The most common referral indications were raised biochemical risk (32%), advanced maternal age (30%), parental anxiety (22%) abnormal ultrasound (7%) and increased nuchal translucency (6%). All samples were also tested by conventional cytogenetic analysis and the results compared. All non mosaic aneuploidies involving chromosomes 21, 18, 13 X and Y were detected with 100% specificity. Several cases of partial trisomies and mosaicism were also identified. Overall 95% of clinically relevant abnormalities were readily detected and termination of the affected pregnancies could be performed without waiting for results of the cytogenetic analyses. When applied in CVS samples the molecular assay allowed discriminating true fetal abnormalities from CPM in several cases thus avoiding further investigations by amniotic fluid sampling. QF-PCR showed 100% sensitivity in detecting clinically relevant chromosome abnormalities in samples referred for advanced maternal age and increased biochemical risk; in fetuses with abnormal ultrasound sensitivity was 95%. Despite being deliberately targeted to chromosomes 13, 18, 21, X and Y the rapid QF-PCR test detected the great majority of chromosome abnormalities in a few hours from sampling. Our results support the possibility of reducing the load of prenatal cytogenetic analysis if pregnancies are carefully monitored by first or second trimester non invasive screening. As a result, invasive procedures should only be performed in high risk pregnancies. In case of abnormal QF-PCR results, medical action can be made available in a few hours after sampling. In cases of negative QF-PCR results cytogenetic analyses might only be performed for fetuses with clear evidence of abnormalities based on ultrasound results. In Countries, where large scale conventional cytogenetic tests are hampered by high cost and lack of technical expertise, QF-PCR may be used as the only prenatal diagnostic test.

## 1605/T/Poster Board #154

**Genetic diversity of three STR Loci for rapid prenatal diagnosis of Down syndrome in Korean Population.** *MH. Lee[1], SY. Park[1], DJ. Kim[1], MJ. Kim[1], HM. Ryu[1,2], YH. Cho[3].* 1) Laboratory of Medical Genetics, Cheil General Hospital & Women's Healthcare Center, Kwandong University College of Medicine, Seoul, Korea; 2) Department of Obstetrics and Gynecology, Cheil General Hospital & Women's Healthcare Center, Kwandong University College of Medicine, Seoul, Korea; 3) Department of Medical Genetics, College of Medicine, Hanyang University, Seoul, Korea.

In order to increase the number of STR loci for rapid prenatal diagnosis of Down syndrome as well as for forensic DNA analysis in Korean population, we have studied three tetranucleotide STR loci on chromosome 21 (D21S1435, D21S1411, and D21S1412). Two hundred genetically unrelated individuals were tested in this study. The loci showed no significant deviations from Hardy-Weinberg equilibrium. The nomenclature is based on the sequence data of the polymorphic region of the microsatellite markers according to the International Society for Forensic Haemogenetics (ISFH) recommendations. The alleles consisted of simple tetra-nucleotide repeat patterns and highly complex repeat patterns. Several alleles revealing the same fragment size but different repeat structures were found. The combined polymorphism information content for three STR loci was 0.991 and combined heterozygosity was 1.0, which is highly informative. The obtained data may be useful for rapid prenatal diagnosis of Down syndrome and also for population genetics research and individual identification in forensic science.

## 1606/T/Poster Board #155

**Prenatal Diagnosis of Tanatophoric Dysplasia Type 1 with Array CGC.** *P. Tavares[1], S.L. Pereira[3], J. Anderson[3], R. Nogueira[1], P. Rendeiro[1], H. Santos[2], A. Palmeiro[1].* 1) CGC Genetics, USA and Portugal; 2) Servico Genetica Medica, Hospital S. Maria, Lisboa, Portugal; 3) Foetal Medicine Unit, Hospital S. Teotonio, Viseu, Portugal.

Skeletal Dysplasias account for over 350 different genetic diseases with bone involvement but variable clinical characteristics (Superti-Furga and Unger, 2007; Krakow,2008), that can be identified in the foetus during the 2nd trimester, usually around 20 weeks. In most cases there is no previous history of skeletal dysplasia, and the foetal diagnosis, based on ultrasound, is difficult and, in approximately 60%, non conclusive (Krakow, 2008). Only the accurate diagnosis of the type of skeletal dysplasia allows a correct approach for physicians and parents about the evolution and natural history of the genetic disorder identified in the foetus, and also the evaluation of the recurrence risk. The early molecular characterization of genes responsible for skeletal disorders, is of main interest for establishing a more precise diagnostic evaluation, namely during the prenatal period. The Molecular Diagnosis of Skeletal Dysplasia by Array CGC test was specially designed for the diagnosis of the most common pathologies referred above, and contains a panel of 26 point mutations, identified in the 6 main genes involved on these skeletal dysplasias: FGFR3 (Achondroplasia, Tanatophoric Dysplasia), COL2A1 (Achondrogenesis type II), SLC26A2 (Achondrogenesis type IB), CRTAP (Osteogenesis Imperfecta recessive type), LEPRE1 (Osteogenesis Imperfecta recessive type) and SOX9 (Campomelic Dysplasia). We present a case of a Prenatal Diagnosis of Tanatophoric Dysplasia through the Molecular Diagnosis of Skeletal Dysplasia by Array CGC test. Second trimester anomaly scan performed at 20 weeks showed all long bones well below the 3rd centile, with telephone receiver shaped femurs; very small chest with a narrow thorax and a normal connected heart. The analysis of the foetal DNA with Molecular Diagnosis of Skeletal Dysplasia by Array CGC detected a c.742C>T mutation (p.Arg248Cys) on FGFR3 gene. This mutation is associated with Tanatophoric Dysplasia Type 1, thus confirming the type of skeletal dysplasia observed in the ultrasound examination. Turnaround time for detection of the mutation and confirmation by sequencing was completed in 1 week. The parents opted for a termination of the pregnancy, which took place uneventfully. This case confirms the importance of this new molecular diagnostic tool in rapid molecular characterization of skeletal dysplasia detected in prenatal diagnosis, allowing genetic counseling based on molecular diagnosis.(www.cgcgenetics.com).

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## 1607/T/Poster Board #156

**Hypospadias in males with intrauterine growth restriction due to placental insufficiency: The placental role in the embryogenesis of male external genitalia.** *T. Uster[1], Y. Yinon[2], J. Kingdom[2], L. Proctor[2], E. Kelly[3], J. Salle[4], D. Wherrett[5], S. Keating[6], O. Nevo[7], D. Chitayat[1].* 1) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital University of Toronto, Toronto, Ontario, Canada; 2) Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, The Placenta Clinic, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada; 3) Department of Pediatrics, Neonatal Intensive Care Unit, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada; 4) Department of Pediatric Urology, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; 5) Department of Pediatrics, Division of Endocrinology, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; 6) Department of Pathology, Mount Sinai Hospital and The University of Toronto, Toronto, Ontario, Canada; 7) Department of Obstetrics and Gynecology, Sunnybrook Health Centre, University of Toronto, Toronto, Ontario, Canada.

Objective: To define the association between early onset intrauterine growth restriction (IUGR) due to placental insufficiency and hypospadias in male offspring. Methods: Prospective cohort study of small-for-gestational age (SGA) male infants with hypospadias managed by a multidisciplinary team over a 5-year period. Antenatal charts and delivery information were reviewed for evidence of IUGR due to placental insufficiency. Results: Thirty SGA male infants were diagnosed with hypospadias/abnormal genitalia after birth, and 4 of them were diagnosed antenatally. Five cases occurred in the smaller pair of discordant IUGR twins, where the larger co-twin appropriate for gestational age in each case, had normal male genitalia. Serial ultrasounds demonstrated features of early-onset IUGR in all cases at a median gestational age of 21 weeks (range 14-31weeks). Twenty-three (79%) pregnancies were subsequently complicated by absent/reversed end-diastolic flow in the umbilical arteries indicating severe IUGR, and 15 (50%) women developed severe pre-eclampsia. Twenty-seven (90%) live births occurred at a median gestational age of 31 weeks (range 27-37); 23 (77%) of the neonates had birthweights <3rd centile. All newborns had normal male karyotypes. In 62% (18/29) the hypospadias was severe. A correlation was found between the severity of the IUGR and the severity of hypospadias as significantly more infants with severe hypospadias were less than the 3rd centile compared to the mild-moderate hypospadias group: 94%(17/18) vs 55%(6/11) respectively, (p=0.02). Other external genital abnormalities apart from hypospadias included micropenis (8), bifid scrotum (9), penoscrotal transposition (6), severe chordee (11) and cryptorchidism (14). Conclusions: This study demonstartes an association between early placental dysfunction and abnormal development of male external genitalia. Careful sonographic evaluation of the genitalia is advised when early-onset placentally-mediated IUGR is found.

## 1608/T/Poster Board #157

**Low Estriol Levels in the Maternal Quadruple-Marker Screen in a Fetus with Cornelia de Lange Syndrome and a Partial Deletion of the *NIPBL* Gene.** *J.R. Goodman[1], P.L. Wilson[1], E.D. Cole[2], J.J. Mulvihill[2], J. Lee[2], S. Li[2], A.F. Wagner[1].* 1) OB/GYN, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2) Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

We present a 26yo Caucasian female $G_3P_{1102}$ who was referred due to an increased risk for Trisomy 18(1:11) on a maternal quadruple marker screen at 18-6/7wks. Unconjugated estriol(uE3) levels were decreased at 0.30 ng/mL and 0.30 MoM. Her initial U/S did not reveal any abnormalities but was limited by maternal body habitus and fetal positioning. She returned 4wks later. Micrognathia, overlapping fingers on the right hand, bilateral cleft feet with hyperextended 1st toes, and growth restriction were seen. She underwent amniocentesis which revealed a 46,XY karyotype. Smith-Lemli-Opitz (*DHCR7*) sequencing due to the low uE3 level was normal. She was hospitalized due to IUGR and had a repeat C/S due to a non-reassuring BPP at 31wks.

Postnatal genetic evaluation of the fetus revealed a generous posterior fontanelle, synophrys, long eyelashes, micrognathia, cleft palate, hypospadias, unilateral cryptorchidism, left single palmar crease, absent right 2nd finger, bilateral cleft feet with incomplete development of toes on the right. DDx included ectrodactyly ectodermal dysplasia and Cornelia de Lange syndrome (CdLs). Postnatal karyotype confirmed the prenatal findings. Postnatal diagnosis of CdLs was determined by whole genome oligonucleotide microarray analysis that revealed an approximately 312kb loss on the short arm of chromosome 5p13.2 involving the *NIPBL* gene. Annular pancreas was found causing duodenal stenosis. He underwent ex-lap and gastroduodenostomy due to a partial rotational anomaly with emergent tracheostomy at 33d and expired at 37d.

In CdLs, prenatal evaluation is difficult due to non-specific prenatal findings, normal karyotype, and sporadic de novo mutations. Studies have suggested that low 1st and 2nd trimester levels of pregnancy associated plasma protein-A (PAPP-A) are an indicator for this heterogeneous condition that is characterized by pre/postnatal growth deficiency, limb abnormalities, hirsutism, characteristic facial features, developmental delay, and mental retardation. However, PAPP-A can be non-specific. Here, we suggest the addition of estriol to the prenatal evaluation of CdLs. This steroid hormone is produced by the placenta from fetal 16OH-DHEA. It can serve as an indicator for intrauterine growth retardation, fetal distress, and placental function. Low uE3 levels have been associated with trisomy 18, Smith-Lemli-Opitz syndrome, and placental sulfatase deficiency.

## 1609/T/Poster Board #158

**Blinded Analysis of Cultured Amniotic Fluid Samples: Conventional Cytogenetics vs Affymetrix 6.0 Microarray vs Array Comparative Genomic Hybridization (aCGH).** *T.A. Maher[1], J.M. Milunsky[1,2], A. Anguiano[3], R. Owen[3], C.M. Strom[3], A. Milunsky[1,2].* 1) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 2) Department of Pediatrics, Boston University School of Medicine, Boston, MA; 3) Quest Diagnostics, Nichols Institute, San Capistrano, CA.

Single Nucleotide Polymorphism (SNP)/Copy Number Variation (CNV) analysis using oligo probe microarrays, and BAC array-based comparative genomic hybridization (aCGH), are now routinely used in the detection of deletions/duplications to elucidate genomic imbalances of patients with mental retardation, dysmorphic features and/or multiple congenital anormalies. These platforms are capable of finely defining chromosomal regions, amenable to high throughput and able to detect CNVs below the resolution of conventional cytogenetics. At Boston Universtiy, we had previously validated the Affymetrix 500K, 5.0 and 6.0 SNP/CNV microarrays on more than 1000 clinical samples. We now sought to validate the Affymetrix 6.0 SNP/CNV microarray on 34 prenatal samples previously tested by a national reference cytogenetics laboratory to determine the feasibility of SNP/CNV microarray analysis on these samples. The reference cytogenetics laboratory was also testing the samples by aCGH to provide a comparison of the different platforms available. Samples selected by the reference cytogenetics laboratory were received blinded to our laboratory and the 6.0 microarray assay was performed. All the samples were analyzed at a 50 probes/200kb resolution. By SNP/CNV microarray, we determined 15 of the samples to be normal. The quality of DNA in 4 of the samples was insufficient to run the assay. 14 of the samples were determined to contain pathogenic copy number alterations. One sample passed all the QC checkpoints but produced an uninterpretable result. The aCGH analysis produced 18 normal, 15 abnormal and 1 failed results. 14/14 normal and 14/14 abnormal samples showed concordance on both platforms. A normal finding on both platforms was determined for 1 sample with a balanced translocation. Two mosaic samples were discordant on both platforms when compared to the final karyotype. Mosaicism and balanced rearrangements represent important limitations on both platforms. Both the SNP/CNV and aCGH platforms were able to determine the size and physical location of the alterations with slight differences. We report our experiences in the quality of samples, type of results generated and limitations of the different platforms.

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## 1610/T/Poster Board #159

**WHOLE GENOME MICROARRAY CGH VS. TARGETED MLPA ANALYSIS IN PRENATAL DIAGNOSIS: A BALANCE BETWEEN SENSITIVITY AND INFORMATIVITY.** *C. Pangalos.* InterGenetics-Diagnostic Genetic Center, Athens, Greece.

Introduction: Our experience, using three different high resolution array CGH platforms in high risk prenatal cases, revealed the benefits but mostly the problems associated with the application of the technique during prenatal diagnosis. For 3 years, we have also applied a targeted approach for the detection of a number of known, recurrent deletion/duplication syndromes. We present data and discuss benefits and potential shortcomings of both approaches. Material & Methods: Fetal DNA was extracted, labeled and hybridized to either an Agilent 4X44K microarray slide (4 samples), Agilent 244K microarray slide (1 sample) or 19K Human BAC Empire Genomics AccuArray (6 samples). Results were evaluated using the Agilent CGH Analytics 3.4.40 software. Also, 799 prenatal samples were analyzed by MLPA for the detection of microdeletion syndromes and subtelomeric rearrangements. Results: Multiple CNV's were identified in 4 prenatal samples/cases, referred due to ultrasound findings and/or karyotypic abnormalities. In one case and after analysis of the parents, the variants were classified as de novo and pathogenic, involving both a subtelomeric deletion and subtelomeric duplication. Common copy number polymorphisms (CNP's) were observed and reported as such. Following MLPA analysis, we detected 2 samples with abnormal findings, both involving well characterized microdeletion syndromes. Conclusions: Although it is obvious that high-resolution array CGH will uncover more abnormalities, there are severe limitations prohibiting their routine use in a clinical prenatal setting: (a) the burden on clinicians and parents, who have to deal with ambiguous information, due to copy number variants of unclear clinical significance, (b) serious time constraints, due to the fact that prenatal testing is increasingly performed at a later gestational age and (c) the high cost associated with the test. In contrast, our experience leads us to favor a targeted approach, by interrogating multiple, recurrent, clinically defined (and constantly updated) pathogenic regions, via standard and custom MLPA as a routine procedure.

## 1611/T/Poster Board #160

**Diagnostic Utility of Array-Based Comparative Genomic Hybridization in a Prenatal Setting.** *M. Shohat[1,2], I. Maya[1], Y. Zalzstein[1], E. Taub[1], B. Davidov[1].* 1) Raphael Recanati Genetic Institute, Rabin Medical Center, Beilinson Hospital, Petah Tikva, Israel; 2) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Array-based comparative genomic hybridization (aCGH) is a new technique for detecting submicroscopic deletions and duplications across the entire genome. There is limited information regarding aCGH in prenatal settings. Here we present our experience of 239 prenatal aCGH analyses in the Recanati Genetic Institute between 2006 and 2009. During this period there was a 6-fold increase in the number of tests performed per month (from 5 to 30). The indications for testing were: 38% major anomalies on ultrasound (U/S), 24% advanced maternal age (AMA), 17.5% family history of unknown etiology, 16% parental concern, 4.1% abnormal karyotype and 0.4% abnormal serum biochemical screening. Ten cases of abnormal karyotype with unknown significance were sent for aCGH: 4 with de novo translocations, 2 with a marker chromosome, 2 with additional chromosomal material and 2 with partial trisomy. A normal aCGH in 8 of these cases enabled us to reassure the families and the pregnancies were continued, and in 2 cases an abnormal aCGH enabled us to confirm the presence of an unbalanced karyotype and the pregnancies were terminated. Of the remaining 229 cases with a normal karyotype, 86.5% had normal aCGH results and 13.5% had abnormal aCGH results. 12.2% were familial copy number variants (CNV) and three cases (1.3%) had a de novo microduplication by aCGH and were terminated (1 had post axial polydactyly on U/S and AMA, 1 had a heart defect, ventriculomegaly and AMA, and 1 had no abnormality). Our results suggest that prenatal aCGH helps to clarify abnormal karyotype findings and non-specific U/S malformations and therefore should be offered particularly in these cases. In low risk pregnancies (when no indication exists) the rate of detecting an abnormality by aCGH was 1:76, but more cases are needed in order to expand our knowledge and validate our conclusions.

## 1612/T/Poster Board #161

**Preimplantation genetic diagnosis (PGD) in 35 couples with familial chromosome rearrangement: FISH results and outcome analysis.** *S. Weremowicz[1,3], A. Nureddin[2], D.J. Sandstrom[1], K.V. Jackson[2], E. Ginsburg[2,3], C. Racowsky[2,3].* 1) Dept Path-CAMD, Brigham & Women's Hosp, Boston, MA; 2) Dept Obstetrics & Gynecology, Brigham & Women's Hosp, Boston, MA; 3) Harvard Medical School, Boston, MA.

Preimplantation genetic diagnosis (PGD) can allow avoidance of transfer of embryos affected with specific mutations or chromosome abnormalities, so couples at risk avoid the trauma of recurrent pregnancy loss or affected offspring. Since February 2002 - January 2009, PGD was performed at Brigham and Women's Hospital for 35 couples in which one partner had a balanced chromosome rearrangement. Our patients included 28 balanced reciprocal translocation carriers, five Robertsonian translocation carriers, and two inversion carriers. Embryo biopsy was performed in 74 cycles in these 35 couples. FISH analysis, using commercially available probes, was performed on 767 blastomeres from 728 embryos. A single blastomere was assessed for 689 embryos (94.6%). FISH RESULTS: Informative FISH results were obtained for 653 embryos (90%) including 166 embryos (23%) assessed as chromosomally normal or balanced and 487 (67%) assessed with an abnormal/unbalanced FISH pattern. Inconclusive FISH results were reported in 14 embryos (1.9%), and in 61 (8.4%), no FISH result was obtained (32: no nucleus found; 28: fragmented nucleus; 1: failed hybridization). PREGNANCY OUTCOMES: Of the 74 cycles, embryo transfer was canceled in 11 (15%) due to absence of any embryo with a normal/balanced hybridization pattern. In the remaining 63 cycles, 113 embryos were transferred. A positive pregnancy test was obtained in 34 cycles (46% per cycle; 54% per embryo transfer) including six chemical pregnancies, two ectopic pregnancies, 17 term pregnancies with 20 babies delivered and three ongoing viable pregnancies (i.e., beyond 12 weeks) for an overall ongoing/delivered pregnancy rate 27% per cycle and 31.7% per embryo transfer. Spontaneous abortion occurred in five pregnancies with fetal karyotypes: 46,XY and 47,XY,+21, and unknown in 3 cases). One pregnancy was electively terminated (trisomy 21). The overall ongoing/delivered pregnancy rate per couple treated was 54% (20/35). CONCLUSION: Our data compares favorably with published literature and further documents the great value of PGD as an important laboratory diagnostic option for at-risk couples.

## 1613/T/Poster Board #162

**Disocordant increased nuchal translucency thickness in monochorionic twin is a possible predictor of severe TTTS.** *S. Abe, K. Miura, K. Yamasaki, Y. Ikeda, H. Masuzaki.* OB/GYN, Nagasaki Univ. School of medicine, Nagasaki, Japan.

Objectivs ; Twin to twin transfusion syndrome (TTTS) is seen in 15-20% of monochorionic diamniotic twins. However, we are not able to predict which cases have TTTS later, because the pathophysiology of TTTS remains unknown. In clinical, the conditions in cases of TTTS were assessed by the Quintero classification, and the fetoscopic laser photocoagulation (FLP), which is performed in cases of 16-26 weeks of gestation, obtains satisfactory results in Japan. Meanwhile, in utero surgeries such as FLP are invasive approach and always have serious risk of both mother and baby. Therefore, the prediction and the prevention of TTTS later have a great significance in cases of monochorionic twins.Increased nuchal translucency thickness (NT) is used as one of the factors for prenatal screening of chromosomal abnormalities. A few were reported association between increased NT and twin-to-twin transfusion syndrome (TTTS). We studied whether discordant NT at early second trimester predicted affecting severe TTTS. Patients and methods ; We defined discordant NT as different NT thickness which is over 30% discordance between inter-twins and increased NT observed in at least one of the twins. We recruited 13 pairs monochorionic-diamniotic twins (MD twins), 6 cases of discordant NT and 7 cases of concordant NT that we measured NT thickness at 10 to 14 gestational weeks in 2002 to 2008. Other MD twins we have not measured NT thickness at those gestational weeks were excluded. We also evaluated each CRLs at the NT measurement, amnion pocket measurement (AFV) and doppler wave forms of fetus retrospectively. Results ; All 6 cases of discordant NT except 1 case would result in TTTS. One discordant NT case which would not develop TTTS showed that increased NT observed in one of the twins disappear at the 4th day of admission and bed rest. Other discordant NT cases were followed up as outpatients. None of the cases represented chromosomal abnormalities. Conclusions ; Although discordant NT may be an early form of TTTS, at that stage TTTS is still reversible state. Increased NT can be utilized only as a prenatal screening tool for chromosomal abnormalities but also as a useful predictor for TTTS. Discordant NT cases of MD twins should follow up strictly and there is possibilitys that stop developing TTTS.

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## 1614/T/Poster Board #163

**First trimester prenatal diagnosis of β-thalassemia through chorionic villus sampling in Pakistan.** *S.M. Bakhtiar[1], D. Sabih[2], K. Ibrahim[2], A. Azhar[1], S.A. Baig[3], M. Tariq[1], M.S. Hussain[1], S.M. Baig[1].* 1) Human Molecular Genetics Lab, National Inst for Biotech and Genet Eng (NIBGE), Faisalabad, Punjab, Pakistan; 2) Multan Institute of Nuclear Medicine and Radiotherapy (MINAR), Multan, Pakistan; 3) Department of Pathology, Children Hospital, Pakistan Institute of Medical Sciences (PIMS), Islamaabad, Pakistan.

β-Thalassemias are a group of heterogeneous recessive disorders common in many parts of the world. Despite the great advances in the treatment of thalassemia, there is so far no cure, but perhaps bone marrow transplantation (BMT) is a possibility. Prevention, using prenatal diagnosis and selective abortion in the cases where the fetus is found to be affected, may be considered as a sensible alternative. β-Thalassemia is the most common monogenic disease in the Pakistani population with an overall carrier frequency of 6%. The objective of our study at National Institute for Biotechnology and Genetic Engineering (NIBGE) was to evaluate the beta-globin gene mutations in cell-free fetal DNA in the first trimester (8-12th weeks' gestation) for the prediction of thalassemia risk at chorionic villous sampling (CVS). Monoplex and multiplex Amplification Refractory Mutation System (ARMS) PCR was used for detection of known mutations and sequencing of the β-globin gene was carried out in case of rare or unknown mutations. In the last one year, CVS from 48 pregnant women were collected for retrospective PND of β-thalassemia in the first trimester of pregnancy. The mutation detection through Monoplex and/or Multiplex ARMS-PCR and sequencing revealed 9 (19%) fetuses affected i.e homozygous and all the 9 couples opted for termination of the pregnancies. The mutations detected in 48 families include IVS-I-5 (G-C), FSC-8/9 (+G), FSC-41/42 (-TTCT), IVS-I-1 (G-T), 619 bp deletion, FSC-5(-CT) and CD-15 (G-A) with frequencies of 39.4% 39.4%, 9%, 7.5%, 1.3%, 1.3% and 0.66% respectively. Only four fetuses were homozygous normal and the remaining 35 were carrier/heterozygote for one parental mutation. There were consanguineous marriages in 39 couples (81%). True homozygosity was detected in 39 couples and nine couples with two different mutations (compound heterozygosity). Compound heterozygosity was detected in three couples who were first degree cousin. Four unrelated couples were also having the same mutation which reflects high frequency and prevalence of β-thalassemia alleles in the local population. Establishment of mutation detection, genetic counseling and prenatal diagnostic facility may facilitate similar programs for other monogenic diseases to control affected births in the Pakistani Population.

## 1615/T/Poster Board #164

**A Case Report of SC Phocomelia and Illicit Drug Use.** *J. Davalos.* Gynecology, Area of health N° 1, Quito, Pichincha, Ecuador.

INTRODUCTION SC phocomelia syndrome (SC) is a rare autosomal recessive disease, its relationship with illicit drug use is not well established.This report is an approach to this problem. First we mention some reports related with SC, birth defects associated with illicit drug use and then we report the case itself. Roberts syndrome (RBS) is characterized by tetraphocomelia, growth deficiency of prenatal onset, craniofacial anomalies, microcephaly and mental deficiency1,2,3.In contrast SC has a milder phenotype with survival to adulthood4. Heterochromatic repulsion (HR) is characteristic for RBS and SC5,6,7.Vega et al. reported a study of RBS confirming linkage to 8p21.2-p12.They also reported eight different mutations in a gene called 'ESCO2' ( establishment of cohesion 1 homolog 2) that causes RBS8. Francke et al. established that RBS and SC are caused by mutation in the same gene (ESCO2)9. The use of methamphetamine, marijuana and cocaine has been associated with cardiovascular, oral clefts and limbs malformations10. Cocaine exposure in utero caused neurobehavioral abnormalities11, placental abruption and premature rupture of membranes12, lower weight (536 g), and smaller head circumference (1.5 cm)13. Prenatal use of methadone and cocaine produced congenital malformations in 6% of offsprings14. METHODS We are reporting a pregnancy in which the fetus showed phocomelia in the 21.6 weeks by ultrasonograph examination. The father uses marijuana, cocaine and alcohol since 10 months ; the mother has a partialy septated vagina. They do not have consanguinity ; they have another normal two years old child. The family history is negative for RBS,SC, other congenital malformations, and illicit drug use . The SC child was born at 32 weeks of pregnancy, with premature rupture of membranes two weeks before the delivery, with respiratory distress, and died on the fourth day of live by cardiorespiratory arrest. We have performed a clinical history, laboratory examinations,pelvic ultrasound and a caryotype. RESULTS The mother's blood and urine examination were normal, her cariotype in lynfocytes was 46.XX. CONCLUSIONS The father's drug addiction could be related with a mutation in his genes and probably the mother's genes could be altered too, because she has a partial septated vagina ; as a result a probable mutation in the gene ESCO2 affected the child with SC.

## 1616/T/Poster Board #165

**Prenatal diagnosis of scalp congenital hemangiopericytoma.** *C. Forrest[3], H.Y.B. Chung[1,2], R. Silver[2], A. Toi[4], S. Blaser[5], G. Taylor[6], S. Viero[6], D. Chitayat[1,2].* 1) Department of Pediatrics, Division of Clinical and Metabolic Genetics, Hospital for Sick Children; University of Toronto, Toronto, Ontario, Canada; 2) Department of Obstetrics and Gynecology, The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital; University of Toronto, Toronto, Ontario, Canada; 3) The Craniofacial Program, Division of Plastic Surgery,Department of Pediatrics, Hospital for Sick Children; University of Toronto, Toronto, Ontario, Canada; 4) Department of Medical Imaging, Mount Sinai Hospital and University Health Network, University of Toronto, Toronto, Ontario, Canada; 5) Division of Neuroradiology, Department of Pediatrics, Hospital for Sick Children; University of Toronto, Toronto, Ontario, Canada; 6) Division of Pathology and laboratory Medicine, Department of Pediatrics, Hospital for Sick Children; University of Toronto, Toronto, Ontario, Canada.

Hemangiopericytoma (HPC) is a rare form of vascular tumour. It consists of extensive proliferation of pericapillary cells (pericytes). The most common locations are the head and neck, lower extremities and retroperitoneum. HPC can be benign or malignant. Malignant HPC can metastasize to other areas of the body, usually to the lungs. Up to 5-10% HPCs present in childhood and 5-40% occur in the 1st year of life. We report a fetal case of HPC presenting as an ultrasound finding of a forehead mass. Case:The proband was a 23y P1 Caucasian woman and her husband was 22y and of same descent. The couple was healthy and non-consanguineous. Early fetal ultrasounds were normal. IPS was negative. Ultrasound at 19w showed bilateral pelviectasis. A follow-up ultrasound at 32w confirmed the pelviectasis, and also showed a midline mass over the forehead just above the nasal bridge, with slightly amorphous content measuring 4.1 x 4.0 x 3.1 cm. The mass did not appear to penetrate the scalp or skull and no gross abnormalities in the brain were seen. The nose and eyes appeared normal. The findings were confirmed by fetal MRI. The couple decided to continue the pregnancy. Delivery was at 38.8w via CS and was uncomplicated. The birth growth parameters were all normal. The APGAR scores were 9(1) and 10(5). A pedunculated tumor with a large base which measured 4 cm, originating in the right side of the forehead was noted. There were areas of necrosis and excoriation over the skin and no thrill over the tumor. The baby was otherwise not dysmorphic. Complete surgical resection was performed and histopathology was consistent with HPC. Discussion:Congenital HPC behaves in a distinct manner and has excellent prognosis. Etiologically, congenital HPC may arise from a pluripotent cell capable of differentiating into smooth muscle cells, pericytes and retains its capabilities to differentiate into more mature cells, resembling infantile myofibromatosis. A single entity called composite myofibromatosis has been proposed. From our knowledge, this case is the 7th case of congenital/ infantile HPC reported in the literature. Previous cases affect the lip, nose, temporal bone, meninges, the cerebrum and the neck region. Complete surgical resection is the ideal treatment of choice. Since excellent response to chemotherapy has been seen, extensive surgical excision and radiotherapy are to be avoided. Long-term follow-up is recommended as relapse may occur late.

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## 1617/T/Poster Board #166

**Attitudes of genetic counselors toward array comparative genomic hybridization (aCGH) testing in a prenatal setting.** *S. Jeddi[1], J. Schmidt[2], V. Vandergon[1], C.G.S. Palmer[3].* 1) California State University-Northridge, Northridge, CA; 2) Cedars-Sinai Medical Center, Los Angeles, CA; 3) University of California-Los Angeles, Los Angeles, CA.

Array Comparative Genomic Hybridization (aCGH) detects chromosomal imbalances at higher resolutions than traditional cytogenetic methods. As a result, aCGH has been recently applied to the prenatal diagnostic field despite the lack of official guidelines governing its use. The purpose of this study is to identify factors explaining genetic counselors' perceptions of the appropriateness of prenatal aCGH testing for 10 scenarios reflecting common prenatal clients' referral indications and situations cited by laboratories offering prenatal aCGH testing. Genetic counselors were eligible to complete an anonymous survey posted on the NSGC ListServ; 238 completed surveys were analyzed. An aCGH appropriateness score [0 (never appropriate) - 20 (always very appropriate)] was computed for each respondent and examined for relationships with genetic counselor and patient characteristics. Five factors were significantly associated with perceptions of aCGH appropriateness: practice setting ($p<0.01$), use of aCGH in own pregnancy ($p<0.01$), perceived knowledge of aCGH ($p=0.01$), patient anxiety ($p=0.03$), and provision of comprehensive care ($p<0.01$). Specifically, working in a prenatal practice setting and having no interest in personally using aCGH were associated with lower aCGH appropriateness scores, whereas higher perceived knowledge of aCGH testing, decreasing patient anxiety, and providing more comprehensive care were associated with higher appropriateness scores. Of these factors, area of practice had the largest effect on genetic counselors' appropriateness scores. Additionally, 91% of genetic counselors indicated that it is very important to consider possibly finding a variant of uncertain significance before discussing this testing with patients. We conclude that variations in genetic counselors' perceptions of appropriateness of prenatal aCGH testing, and, hence, practice patterns, are determined by genetic counselor and patient characteristics. As a promising diagnostic tool, prenatal aCGH testing will continue to be incorporated in patient care, and the development of future guidelines and recommendations regarding its use will be imperative.

## 1618/T/Poster Board #167

**Prenatal diagnosis of a de novo partial trisomy 13q (q14→qter) presented as a translocation at chromosome 4q identified by SKY and aCGH.** *I.N. Machado[1], J.K. Heinrich[1], C. Campanhol[1], R.M. Rodrigues-Peres[1], F.M. Oliveira[2], R. Barini[1].* 1) Institution: Cell Culture and Cytogenetics Laboratory, Fetal Medicine Service, CAISM. Department of Obstetrics and Gynecology. Faculty of Medical Sciences State University of Campinas - UNICAMP 13083-970, Campinas, SP, Brazil; 2) Medical Clinics Department, National Institute of Science and Technology in Stem Cell and Cell Therapy. Faculty of Medical Sciences, Ribeirão Preto, SP, Brazil.

Partial trisomy 13q (q14→qter) is an uncommon chromosomopathy. It has been described with a variable phenotypic expression. We report a 19-year-old pimigravida with a fetus presenting partial absence of the cerebellar vermis, partial agenesis of callosum corpus, hydrops and polyhydramnios at ultrasound evaluation at 28 week's gestation. The cytogenetic result on fetal blood obtained by cordocentesis was 46,XX,add(4) (q28). The parents' karyotypes were normal. A girl was delivery at 34 week's gestation and died within 2 hours, showing several dysmorphic features. An autopsy confirmed the prenatal findings and completed it with the finding of agenesis of diaphragm. Spectral Karyotype (SKY) identified the additional material's origin as to be from chromosome 13. Array-CGH was carried out using a 1Mb resolution BAC array and showed amplification of distal regions of the long arm of chromosome 13 from region 13q14 to qter. This is the first report of a fetus with molecular characterization of a partial trisomy 13q (q14→qter), present as de novo unbalanced translocation at chromosome 4q. Our results confirm the usefulness of molecular characterization of malformed fetuses for prenatal diagnosis and counseling. Also, emphasize the importance of parental karyotyping.

## 1619/T/Poster Board #168

**Microarray-based analysis using cell-free mRNA in pregnant women has a potential to estimate a placental status.** *K. Miura[1], S. Miura[1], K. Yamasaki[1], T. Shimada[1], A. Higashijima[1], S. Abe[1], K. Yoshiura[2], N. Niikawa[3], H. Masuzaki[1].* 1) Dept OB/GYN, Nagasaki University School of Medicine, Nagasaki, Japan; 2) Dept Human Genetics, Nagasaki University School of Medicine, Nagasaki, Japan; 3) Research Institute of Personalized Health Sciences, Health Sciences University of Hokkaido, Hokkaido, Japan.

Objectives; Although the discovery of cell-free placental mRNA in maternal plasma provides new possibilities in prenatal monitoring a heterogeneous condition of placental status, a high-throughput approach is necessary for the clinical application of this novel particle. To resolve this technical issue, we investigated a clinical possibility of microarray-based analysis using cell-free mRNAs in maternal plasma. Materials and Methods; The study protocol was approved by IRB in Nagasaki University. Three each of first trimester, second trimester and third trimester placental tissue samples were obtained from normal pregnant women immediately after termination of pregnancy, respectively. Same amounts of RNA from three placenta or blood samples at each trimester were mixed and applied to GeneChip® analysis (Affymetrix, Santa Clara, CA). Top of the fifty highest placenta specific transcripts, whose signal intensities were more than 2,500 times higher in the placenta tissues as compared to the corresponding whole blood samples, were selected as the placenta specific cDNA in maternal circulation. Comparative cDNA hybridization analysis of cell-free placental mRNA in maternal plasma using the developed cDNA microarray panel, which included placenta specific cDNAs, was performed between 12 pregnant women with preeclampsia and 12 normal control pregnant women who were gestational age matched. Results; First, 50 placenta specific transcripts were identified by comparison between the expression levels in placenta tissues and the corresponding maternal bloods, and the custom cDNA microarray panel was developed. Subsequently, the custom microarray-based comparative cDNA hybridization analysis of cell-free mRNAs in 1.2 mL of maternal plasma was performed in 12 preeclamptic women and 12 normal control pregnant women. The scatter plots of signal intensities showed no changed pattern (pattern A) in 7 cases of mild hypertension and the aberration pattern (pattern B) in 5 cases of severe hypertension. Conclusions; The changes of cell-free mRNA levels could be detected by microarray-based analysis, and the pattern B included a total 17 placenta specific genes, which might be associated with the pathophysiology of hypertension in preeclampsia. Non-invasive overall assessments of cell-free mRNA in maternal plasma became a feasible scheme and have a potential to monitor the placental status during pregnancy.

## 1620/T/Poster Board #169

**Prenatal diagnosis of a fetus with ring chromosome 21 characterized by molecular cytogenetic methods.** *I.D. Papoulidis[1], E. Siomou[1], E. Manolakos[2], T. Liehr[3], A. Vetro[4], A.P. Athanasiadis[5], C. Malamaki[1], O. Zuffardi[4], M.B. Petersen[1].* 1) Eurogenetica SA, Thessaloniki, Greece; 2) Bioiatriki SA, Athens, Greece; 3) Friedrich-Schiller-University, Jena, Germany; 4) Universita di Pavia, Pavia, Italy; 5) Aristotle University, Thessaloniki, Greece.

Ring chromosome 21 is a rare structural chromosomal abnormality resulting, most of the times, from breakage in both arms of the chromosome and subsequent fusion of the two ends. There is a wide spectrum of phenotypes in ring 21 carriers that seems to be associated with different breakpoints of 21p and q arms, but also with somatic loss of the ring. Here we report a case of a fetus that was diagnosed with mos46,XY,r(21)(p11.2q22)[34]/45,XY,-21[4]/46,XY[14] karyotype. Ultrasound examination at 21 weeks' gestation showed no abnormalities and amniocentesis opted due to advanced maternal age. Cytogenetic analysis was performed in the parents and showed the presence of the ring chromosome in 1 out of 100 metaphases in the father, indicating a possible familial transmission. Analysis by Fluorescent In Situ Hybridization (FISH) and comparative genomic hybridization (aCGH) with resolution of 144 kb showed no deletion or duplication. After genetic counseling, the parents decided to continue the pregnancy and postnatal examination just after birth found no congenital abnormalities.

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## 1621/T/Poster Board #170

**Prenatal diagnosis and therapy development in harlequin ichthyosis.**
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Mutations in the ABCA12 gene encoding the ABC transporter underlie the most severe and often lethal form of recessive congenital ichthyosis, harlequin ichthyosis (HI). Affected foetuses display abnormal sonographic features late in the second trimester but identification of these is technically challenging. To improve the clinical care of HI families we have established a major referral centre for HI genetic screening and devised a programme of research to develop new therapeutics for this condition. We have identified mutations in ABCA12 in over 70 families and used this information for prenatal diagnosis both in the UK and in 2 HI families living in Turkey and Pakistan. Chorionic villus DNA was sequenced and compared to the known founder mutation. If termination of pregnancy is being considered, this test is desirable as it offers an earlier and more accurate result than ultrasound.

The barrier function of the skin is severely compromised in HI and approximately 55% of affected babies die in the neonatal period from respiratory distress and sepsis. To assess signalling pathways influenced or regulated by ABCA12, the Illumina array platform was used to compare global gene expression between keratinocytes with/without ABCA12 shRNA. Following Ingenuity Pathway analyses, it was found that components of the nuclear hormone receptor pathway such as RXR-alpha were amongst those dysregulated when ABCA12 expression was ablated. Many of these nuclear receptors are already used as therapeutic targets in hyperproliferative skin disorders. By manipulating components of the nuclear hormone receptor pathway, we aim to target abnormalities in the HI keratinocyte differentiation programme to reduce epidermal thickness, enhance desquamation and improve the lipid balance in HI skin. Towards therapy development we have generated an in vitro model of HI skin with a combination of shRNA targeting ABCA12 and a three-dimensional organotypic co-culture (OTCC) system. This replicates many features of HI skin including epidermal thickness, premature terminal differentiation and aberrant protease expression. Endogenous and synthetic ligands of the nuclear receptor signalling pathway are being tested in the OTCC system and characterised for improved terminal differentiation and lipid content.

## 1622/T/Poster Board #171

**Prenatal diagnosis of monozygotic twins with karyotypes discordant for a dicentric chromosome 13 resulting in functional trisomy 13.**
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A number of monozygotic twins with discordant karyotypes for sex chromosomes or other autosomal aneuploidies have been reported: to the best of our knowledge, this is the first report of monozygotic twins with discordant karyotypes resulting in trisomy 13. Our case involves an isodicentric chromosome 13, as opposed to the more common free trisomy 13. This was a spontaneously conceived first pregnancy in a non-consanguinous Caucasian 35 year old mother and 46 year old father. An ultrasound at 22 weeks gestation showed a monochorionic-diamniotic twin pregnancy. No structural anomalies were reported in Twin A. Twin B had a small omphalocele, suspicion of two vessel cord and two echogenic intracardiac foci. Amniocentesis with sampling of both gestational sacs was performed. Twin A had a normal female karyotype (46,XX) from 24 colonies, thus excluding mosaicism of 12% or greater, at a 95% confidence level. Twin B's karyotype was 46,XX,idic(13)(p1?3), in metaphase from 5 colonies examined. Interphase FISH studies using a probe specific for a locus on 13q14 showed 3 signals in 55 nuclei examined, further supporting the presence of trisomy 13. The twins were identical at all 15 STR loci studied (zygosity studies). The couple was counseled about the possibility of mosaicism in either twin and offered a termination of both fetuses vs a selective termination of Twin B using cord occlusion; they elected not to intervene and continue with the pregnancy. Twin A was phenotypically normal at birth and is now doing well at 1 month of age. Twin B passed away at 4 days with typical features of trisomy 13 such as omphalocele, clefting of the palate and polydactyly. Parental karyotypes were declined for the moment. Different mechanisms have been described to explain discordant karyoytpes in monozygotic twins and a number of reports have described multiple events leading to the observed karyotypes. Some have postulated that genetic heterogeneity likely promotes cleavage of embryos after the two cell masses become discordant, but this does not offer insight into whether the unbalanced karyotype was post-zygotic or not, which would have important implications for counseling this couple regarding the risk of mosaicism. This report will review the clinical presentation, possible cytogenetic etiologies and their implications regarding counseling for this couple.

## 1623/T/Poster Board #172

**Cornelia de Lange Syndrome: germline mosaicism, prenatal, and pregnancy findings.**
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Cornelia de Lange Syndrome (CdLS) is a genetic disorder associated with delayed growth, mental retardation, and characteristic facial features. Most cases are sporadic and are due to mutations in the gene NIPBL. We present a case of a 23 year-old G10P2264 woman who has had four affected children with CdLS with identical splice site mutations in exon 2 of the NIPBL gene, 64+1 G>A. Of the living children, two children occurred with one partner, and two children occurred with a separate partner. Maternal blood testing for NIPBL gene mutations was negative, gonadal and/or other tissue testing was not obtained. Although, previously reported as a potential form of inheritance, limited molecular data exist on germline mosaicism in CdLS. This presentation adds to the literature on germline mosaisicm in CdLS and reviews previously reported cases. Additionally, we also report the abnormal prenatal findings such as low pregnancy associated plasma protein A (PAPP-A), increased nuchal translucency (NT), severe intrauterine growth restriction (IUGR), abnormal fetal Doppler studies, as well as pregnancy complications that are not commonly known to be associated with CdLS, such as maternal preeclampsia. In conclusion, germline mosaicism should be part of pregnancy recurrence risk counseling for patients with a previous child affected with CdLS. Presently, the risk is estimated to be 1.5%; however, this figure may need to be increased if similar cases are reported in the future. In addition, specific prenatal parameters when combined with pregnancy course features may aid in the prenatal diagnosis of CdLS.

## 1624/T/Poster Board #173

**A First Report of Paternal UPD 4 Associated with Prenatal Trisomy 4 Mosaicism.**
*T.L. Prosen[1,2,3], S.A. Berry[1,5,6], C. Whitley[1,5,6], M. Mahoney[1,2,4], K. Baker-Lange[1,6], M. Dolan[1,7].* 1) University of Minnesota, Minneapolis, MN; 2) Department of Obstetrics, Gynecology and Womens Health; 3) Division of Maternal-Fetal Medicine; 4) Division of General Obstetrics and Gynecology; 5) Department of Pediatrics; 6) Division of Genetics; 7) Cytogenetics.

A 36-year-old G2P1 female was referred for genetic counseling at 13 wks gestation. The family history was positive for a paternal balanced t(4;12)(q25;q24). G-band analysis performed after CVS revealed trisomy 4 due to 3:1 segregation of the t(4;12). ISCN: 47,XX,+4,t(4;12)(q25;q24). FISH with a centromere 4 probe revealed 81.5% of interphase cells with trisomy 4. A follow-up amniocentesis at 18 wks gestation revealed low level mosaicism. By G-banding, 47,XX,+4,t(4;12)(q25;q24.1) in 3/168 metaphases; 46,XX,t(4;12)(q25;q24.1) in 165/168 metaphases. Interphase FISH similary showed low level mosaicism for trisomy 4 in 13.5% of cells. Uniparental disomy testing further demonstrated paternal UPD. The couple opted to continue the pregnancy. Serial ultrasounds revealed a unilateral choroid plexus cyst at 18 wks gestation that resolved by 21 wks gestation; additionally, although the cerebellum initially appeared somewhat small, measurements improved later in gestation. A fetal echocardiogram was normal. The pregnancy progressed uneventfully until preterm premature rupture of membranes at 35 4/7 wks gestation. After induction of labor, the patient delivered a 1880g (3%ile) female infant with Apgars of 81 and 85. The infant was admitted to the NICU for observation due to prematurity. Transient tachypnea resolved within 24 hrs. Five days of phototherapy were required for hyperbilirubinemia. She was noted to have micrognathia, low, posteriorly rotated ears and small nails. The placenta was remarkable for its small size (204g, <3%ile) and large, somewhat fibrotic stem villi with irregular outlines that lacked a correspondingly increased number of intravillous capillary vessels. Cytogenetic studies of 9 separate placental biopsies showed the predominant cell line to be the unbalanced 3:1 segregation product throughout the placenta (83.5-90.5% trisomic cells by both G-banding and FISH), whereas G-banded analysis of the umbilical cord and G-band and FISH analysis of peripheral blood from the newborn revealed only the balanced t(4;12). A few cases of maternal UPD have been published in the literature and have suggested the absence of maternally imprinted loci on chromosome 4. Continued clinical evaluation of this infant will be helpful for extrapolating to paternal UPD, and to delineate further phenotype-genotype correlations.

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## 1625/T/Poster Board #174

**Independent effect of Brain-Derived Neurotrophic Factor gene polymorphism (Val66Met) on attention problems and growth in early life.** *D.O. Mook-Kanamori[1 2 3], M. Zeegers[4], F.C. Verhulst[4], A. Hofman[1], E.A.P. Steegers[5], H. Tiemeier[1 4], V.W.V. Jaddoe[1 2 3].* 1) Epidemiology, Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands; 2) The Generation R Study Group, Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands; 3) Pediatrics, Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands; 4) Child and Adolescent Psychiatry, Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands; 5) Obstetrics and Gynaecology, Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands.

Background: The Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) is associated with both attention-deficit hyperactivity disorder and body mass index (BMI) in adulthood. We hypothesized that this gene is also associated with attention problems and growth in childhood. Design and methods: This study was embedded in the Generation R Study, a population-based prospective cohort study. Problem behavior was assessed by Child Behavior Checklist (CBCL) questionnaires at the age of 18 and 36 months. Fetal growth was assessed by estimated fetal weight (ultrasound in second and third trimester) and birth weight. Anthropometrics (weight, height, head circumference) were assessed at 9 time-points between birth and 2 years. BDNF Val66Met genotyping was performed in 3071 children of Caucasian ethnicity. Results: Genotype distribution was 65.8% (ValVal), 30.5% (ValMet), and 3.6% (MetMet). The Val-allele was associated with increased reporting of attention problems at the ages of 18 and 36 months. No associations were observed with fetal growth. Postnatally, the Val-allele was associated with increased weight gain (44.4 grams/year (95% CI: 21.2, 67.5) and height gain (0.062 cm/year (95% CI: -0.003, 0.128) until 24 months of age. Head circumference also tended to be increased in Val-allele carriers until the age of 11 months. Furthermore, weight-to-height ratio (SDS) was increased in Val-allele carriers, leading to an effect of 0.07 SDS (95% CI: 0.01, 0.15) per Val-allele at 24 months. There was no apparent association between attention problems and anthropometric measurements. Stratified analyses showed an independent effect of BDNF genotype on both attention problems and early growth characteristics. Conclusions: This study indicates that the previously found associations of the BDNF polymorphism Val66Met with behavior problems and BMI may start as early as in the first years of life. Furthermore, BDNF Val66Met was independently associated with both attention problems and body composition. Further longer follow-up studies are necessary to examine whether these effects persist throughout childhood.

## 1626/T/Poster Board #175

**Candidate Gene Analysis of Cholesterol Metabolism Genes and Intra-Uterine Growth Restriction.** *K. Borowski[1], J. Murray[2].* 1) Dept OB & GYN, Univ Iowa, Iowa City, IA; 2) Dept Pediatrics, Univ Iowa, Iowa City, IA.

Intra-uterine growth restriction (IUGR) is a complex trait mediated by multiple environmental and genetic contributors. Significant morbidity and mortality are associated with IUGR particularly in the preterm infant. Few genetic association studies have been performed evaluating genetic causes of IUGR although a significant role for genetic factors has been demonstrated in IUGR of unknown etiology. A previous study found a maternal association with *LRP8* and fetal growth. A family based candidate gene study of cholesterol metabolism genes was performed utilizing 129 pedigrees. Twenty-six SNPs in 15 genes were evaluated utilizing tagged SNPs from HapMap#24. Subjects were included in the study if they had a birthweight for gestational age at less than the 10th centile using the Lubchenco growth curve, had no major anomalies and were enrolled at the University of Iowa from 2/2001 until 3/2009 with available DNA. Allelic discrimination was performed using TaqMan genotyping assays from Applied Biosystems. The results were analyzed with a transmission disequilibrium test (FBAT) to determine association with the affected child. In perinatal disorders it is unknown if the risk is in the mother or the child. To determine maternal association a case control analysis was performed with Fisher's exact test, utilizing the father's genotypes as controls. FBAT was performed for all SNPs with none reaching significance. *ABCA1* rs2066716 showed overtransmission of the rare minor allele, with p=0.102. Further analysis of *ABCA1* will be performed in the future. In addition, the prior association described with maternal *LRP8* and fetal growth was not seen in our study. The previous study involved 204 black women of which only 34 had fetal growth restriction, compared to our predominantly Caucasian population of 129 pedigrees all with IUGR. Planned future work includes increasing the number of IUGR subjects, further cholesterol gene analysis as well as analysis of copy number variants in cases of IUGR.

## 1627/T/Poster Board #176

**The heritability of neonatal death in a Utah population.** *K. Ward[1,2], H. Albertsen[2], G. Frech[2], P. Farrington[2], T. Maness[2], S. Dintelman[2].* 1) Lucina Foundation, Salt Lake City, UT; 2) Juneau Biosciences, LLC, Salt Lake City, UT.

Purpose: Neonatal death (death within the first 28 days of an infant's life) is most commonly caused by preterm birth, birth defects, complications arising during pregnancy, infection and asphyxia. The collective literature provides compelling evidence that there may be a genetic component underlying neonatal death, regardless of the specific cause or complication. The present study utilizes an extensive population-based genealogical database to estimate the heritability of neonatal death. Methods: A genealogy database (GenDB) was constructed using more than 50,000 genealogy sources in the public domain. GenDB documents the relationships between more than 30 million ancestors and 3.5 million descendants of approximately 10,000 individuals who moved to Utah in the mid 1800s. Because of privacy issues, most individuals in the genealogy have birth dates prior to 1930. Within this database, we identified two groups of neonatal deaths to compare. We identified a set of about 8,000 infants who died as neonates. We also identified a subset of about 1,000 neonatal deaths who had one or more siblings also die as neonates. For each set of cases we computed the average pairwise kinship coefficient. Controls for each set of cases were matched for gender, birth year, birth place, and ancestry. Summary of Results: We demonstrate that the ancestors of both sets of neonate deaths were more closely related than matched controls, with the set of multiple deaths being more closely related than the full set. The average pairwise kinship coefficient for overall neonate deaths was significantly higher than the matched controls. For the set of neonates with sibs who also died as neonates, the average kinship coefficient was also significantly higher than matched controls. For both sets, the minimum number of founders required to account for the cases was significantly smaller than for the controls. Conclusion: A genetic component contributes to neonatal death and is even more significant in families with more than one neonatal death.

## 1628/T/Poster Board #177

**The importance of accurate gestational age in detecting the association between gene:environment interactions and birth weight.** *J.A. Marsh[1], C.E. Pennell[2], N.M. Warrington[1], S.E. Bolt[1], J.P. Newnham[2], L.J. Palmer[1].* 1) Centre for Genetic Epidemiology & Biostatistics, The University of Western Australia, Perth, Australia; 2) School of Women's and Infants' Health, The University of Western Australia, Perth, Australia.

Background Genome-wide association studies (GWAS) are revolutionising the search for the genes underlying gene:environment (G:E) interactions in early life. Recent data have demonstrated G:E interactions between smoking, polymorphisms in CYP1A1, GSTT1, GSTM1 and FTO and intrauterine growth restriction. Interaction analyses between common environmental exposures and common genetic variants thus have a growing role in early life investigations of chronic disease etiology. It is critical to account for gestational age (GA) in analyses of birth weight (BW). This research assessed the impact of the accuracy of GA estimation on the ability to detect associations of complex gene:environment interactions with BW using an exceptional birth cohort resource with extensive antenatal data. Methods The Raine Pregnancy Study recruited unselected women from the general population at 16-18 weeks gestation. Analyses focused on 2,065 full-term, singleton-births. GA at birth was recorded (1) based on the date of the last menstrual period (LMP) alone and (2) based on extensive ultrasound biometry throughout pregnancy. Multivariate linear regression models of BW were constructed, including parity, sex, maternal smoking and GA as covariates. G:E power calculations were based on 80% power, a MAF≥5%, a continuous environmental exposure, a dominant genetic model, and an $\alpha$ of 10-4. Results GA based on the date of LMP alone and based on ultrasound biometry accounted for 7% and 16% of the total phenotypic variance in BW, respectively. The correlation between the two methods of GA measurement was 0.69. Using LMP-estimated GA alone, studies ~10% larger would be required to detect a true association between BW and a gene:environment interaction compared to ultrasound-estimated GA. In terms of precision of a continuous outcome measure, decreases in BW or GA precision across larger studies would have an even greater impact on power. Conclusion Our results suggest that smaller birth cohort studies with more precise measures of gestational age will be as powerful as larger studies in detecting gene:environment interactions for continuous traits. These findings should be helpful when designing or interpreting analyses of pregnancy cohorts which have GA based only upon LMP.

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## 1629/T/Poster Board #178

**Gene-environment interactions define the relationship between the FTO gene, fetal growth and childhood obesity.** *N.M. Warrington[1], J.A. Marsh[1], C.E. Pennell[2], D. Mook-Kanamori[3], L. Briollais[4], S.J. Lye[4], L.J. Beilin[5], J.P. Newnham[2], E. Steegers[6], A. Hofman[3], V.WV. Jaddoe[3], L.J. Palmer[1].* 1) Centre for Genetic Epidemiology and Biostatistics, The University of Western Australia, Perth, Australia; 2) School of Women's and Infants' Health, The University of Western Australia, Perth, Australia; 3) Department of Epidemiology, Erasmus Medical Centre, Rotterdam, the Netherlands; 4) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Canada; 5) School of Medicine and Pharmacology, The University of Western Australia, Perth, Australia; 6) Department of Obstetrics and Gynaecology, Erasmus Medical Centre, Rotterdam, the Netherlands.

Background: FTO gene variants have been consistently associated with childhood and adult obesity across multiple populations and racial groups. This study examines the influence of the rs9939609 variant in the FTO gene on fetal and early life growth, which has not yet been explored, and investigates the gene environment and gender interactions. Methods: Participants in the Raine Birth Cohort study were recruited at 16-18 weeks gestation. Analyses focused on 1,079 singleton-birth Caucasians with complete data in antenatal life and childhood. Associations between the rs9939609 variant, fetal growth trajectories and anthropometric measures at birth and throughout childhood were assessed. Analyses were repeated in 3,512 singleton-birth Dutch Caucasians (Generation R Birth Cohort). Results: The allele frequency for obesity risk allele (A) of the rs9939609 variant was 0.39 in both the Raine and Generation R cohorts. In non-smoking mothers the rs9939609 AA genotype was associated with intrauterine growth restriction; however, this effect was reversed in mothers who smoked during pregnancy. This effect, although small, accumulates over time and is consistently modified by maternal smoking for head circumference (HC) (Interaction P=0.007), abdominal circumference (AC) (Interaction P=0.007), femur length (FL) (Interaction P=0.02), and estimated fetal weight (EFW) (Interaction P=0.001). No association was detected between HC/AC ratio and the rs9939609 AA genotype indicating symmetrical growth restriction. The pattern of modification by maternal smoking of the association between the rs9939609 AA genotype and birth anthropometrics was consistent across birth-weight (BW) (P=0.01) and birth length (P=0.04); a similar trend was observed in all neonatal day 2 anthropometry including skinfold thicknesses. Postnatally, the AA genotype was associated with increased BMI by age 14 years, although the effect size was greater in males (Interaction P= 0.0001). Consistent effect sizes and directions from birth to the 1st year of life were replicated in the Generation R Cohort. Conclusion: FTO influences fetal growth trajectories in the 3rd trimester and early life growth. Maternal smoking during pregnancy consistently modified (reversed) the effect of FTO on fetal growth. This study provides evidence of gene-environment interactions influencing the relationship between fetal growth and adult obesity and raises the prospect of early life interventions.

## 1630/T/Poster Board #179

**Microarray Analysis of altered gene expressions in peripheral blood of severe preeclampsia.** *D. Cha[1], J.Y. Kim[1], J.H. Kang[1], K.J. Lee[1], W.B. Han[1], S.H. Kim[2], D.Y. Park[2].* 1) Dept OB/GYN, Kangnam CHA Hosp, Seoul, Korea; 2) Digital Genomics, Seoul, Korea.

Objects : To compare the gene expression profiles between the peripheral blood with preeclampsia and normal pregnancy Material and methods : To investigate how the expression of peripheral blood genes contributes to the mechanisms of preeclampsia, we have analyzed differentially expressed genes using peripheral blood from 10 pregnant women who have preeclampsia and from 10 normal pregnancies. We performed genome-wide expression profiling using high-density oligonucleotide microarrays. The data obtained from 55,000 human genes were normalized and analyzed to identify genes with statistically significant changes in expression. All the analysis was done in FDR (false discovery rate) < 0.01. RT-PCR was randomly done for validation with 12 genes. Results: Among the 55,000 genes that were screened in the microarray, 676 genes were up-regulated and 153 genes were down-regulated. The up-regulated genes included CRIP1, LGTN, OXGR1, NEU3, GALK2, HBG2, and the down-regulated genes included GPI , PTGS2, TMED7, LTA4H. Validation was confirmed by RT-PCR. Conclusions : Differentially expressed genes in the peripheral blood may be associated with the pathophysiology of preeclmpsia in additional to the placenta.

## 1631/T/Poster Board #180

**Maternal and fetal haplotypes in immunity and stress-related genes are associated with preterm birth.** *M.J. White[1], K.K. Ryckman[1,2], N. Morken[3], S. Myking[3], S.M. Williams[1], B. Jacobson[3].* 1) Human Genetics, Vanderbilt University, Nashville, TN; 2) Dept Pediatrics, University of Iowa, Iowa City, IA; 3) Perinatal Centre, Department of Obstetrics and Gynecology, Institute for the Health of Women and Children, Sahlgrenska Academy, Götenburg, Sweden.

Preterm birth (PTB) is a common condition that accounts for 50% of perinatal morbidity and 75% of perinatal mortality. The main aim of this study was to investigate the associations of maternal and fetal haplotypes in candidate genes within the four major proposed PTB pathways in order to identify effects that may not have been detected in single locus association analysis. Methods: After data quality control and pre-processing, 424 maternal DNA samples (217 controls, 207 cases) and 419 fetal DNA samples (216 controls and 203 cases) were analyzed from the Norwegian Mother Child Cohort for haplotype association using Unphased. Haplotype analysis (1,339 SNPs in maternal samples and 1,3333 SNPS in fetal samples) spanning 126 genes was performed on fetal and maternal samples separately. Results: Overall, there were 266 significant haplotype associations discovered in 33 genes in fetal samples and 207 associations in 38 genes were detected in maternal samples. The most significant haplotype in maternal samples was in thyroid stimulating hormone receptor (TSHR) gene ( p= $1.7x10^{-3}$ ) and in fetal samples the interleukin 10 receptor beta (IL10RB) gene had the most significant haplotype association (p=$1.2x10^{-4}$). Pathway analysis of select haplotype associations revealed a significant overrepresentation of haplotype associations driven by multiple genes in the complement/coagulation pathway for maternal samples. In fetal samples there were more haplotype associations than expected by chance in the inflammation and infection pathways. Conclusion: This study identified maternal and fetal haplotype effects not detected by single locus association analysis. This study also validates the assertion that associations in the complement coagulation pathway of PTB are driven by the maternal genome, whereas associations found in the inflammation and infection pathways of PTB are influenced primarily by the fetal genome.

## 1632/T/Poster Board #181

**Maternal and fetal genetic associations in PTGER3 and PON1 with preterm birth.** *K.K. Ryckman[1,2], N. Morken[3,4], M. White[2], D.R. Velez[5], R. Menon[6,7], S.J. Fortunato[6,7], P. Magnus[4], S.M. Williams[2], B. Jacobsson[8,9,10].* 1) Dept Pediatrics, University of Iowa, Iowa City, IA; 2) Center for Human Genetics Research and Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 3) Department of Obstetrics and Gynecology, Haukeland University Hospital, Bergen, Norway; 4) Norwegian Institute of Public Health, Oslo, Norway; 5) University of Miami, Dr. John T. Macdonald Foundation Department of Human Genetics and Miami Institute of Human Genomics, Miller School of Medicine, Miami, FL; 6) The Perinatal Research Center, Nashville, TN; 7) Emory University, Department of Epidemiology, GA; 8) North Atlantic Neuro-Epidemiology Alliances, Department of Epidemiology and Social Medicine, University of Aarhus, Aarhus, Denmark; 9) Perinatal Center, Department of Obstetrics and Gynecology, Institute of Clinical Sciences, Sahlgrenska Academy, Gothenburg, Sweden; 10) Department of Obstetrics and Gynecology, Rikshospitalet, Oslo, Norway.

Objective: The purpose of this study was to identify associations between maternal and fetal genetic variants in candidate genes and spontaneous preterm birth (PTB) in a Norwegian population and to determine the effect size of those associations that corroborate a previous study of PTB. Methods: DNA from 434 mother-baby dyads (214 cases and 220 controls) collected from the Norwegian Mother and Child Cohort (MoBa) were examined for association between 1,430 single nucleotide polymorphisms (SNPs) in 143 genes and PTB. These results were compared to a previous study on European Americans (EA) from Centennial (Cenn) Women's Hospital in Nashville, TN. Odds ratios for SNPs that were corroborated in the Cenn study were determined on the combined MoBa and Cenn studies. Results: In maternal samples the strongest results that corroborated with the Cenn study were in the prostaglandin E receptor 3 gene (PTGER3; rs977214) (combined genotypic p = $3x10^{-4}$). The best model for rs977214 was the AG/GG genotypes relative to the AA genotype and resulted in an OR of 0.56 (95% CI = 0.38-0.82, p = 0.003), indicating a protective effect. In fetal samples the single strongest association in the combined data was rs854552 in the paraoxonase 1 gene (PON1) (allelic association p = 0.001, genotypic p = $7.6x10^{-4}$). The best model was the TT genotype relative to the CC/CT genotypes, and resulted in an OR of 1.69 (95% CI = 1.26-2.27, p = $5.1x10^{-4}$). Conclusions: These studies identify single locus associations with PTB in two populations of European ancestry.

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## 1633/T/Poster Board #182

**Cell-free fetal DNA trafficking increases in pregnant women who conceive by *in vitro* fertilization using egg donors.** *K.L. Johnson[1], K. Koide[1], Z. Jarrah[1], I. Peter[2], G. Lambert-Messerlian[3], D.W. Bianchi[1].* 1) Pediatrics, Floating Hospital for Children at Tufts Medical Center, Boston, MA; 2) Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 3) Pathology, Women and Infants' Hospital, Providence, RI.

**Objective.** To determine if pregnancies conceived by *in vitro* fertilization using egg donors (ED-IVF) result in increased levels of circulating cell-free DNA compared to spontaneously conceived pregnancies.

**Methods.** We obtained archived maternal serum samples from routine second trimester screening. Sixteen case-control sets were created, matched for gestational age (within 1 week) and time in storage prior to DNA extraction. Each set consisted of a sample from a woman who had a singleton ED-IVF pregnancy with a male fetus, 5 women with spontaneously conceived male fetuses, and 1 woman with a spontaneously conceived female fetus (total=112). Cell-free DNA was isolated and measured by real-time PCR amplification using *GAPDH* and *DYS1* as markers of total and fetal DNA, respectively. Samples were coded and analyzed blindly. Fetal gender was verified at delivery.

**Results.** The sensitivity of *DYS1* detection in women carrying male fetuses was 99.0% (95 of 96), with a specificity of 100% (no *DYS1* detection in 16 women carrying female fetuses). Due to the single false negative result in an ED-IVF case, this set was excluded from subsequent analyses (n=105). The average amount of fetal DNA in maternal plasma from ED pregnancies was 48 genome equivalents (GE)/mL, compared to 30 GE/mL in controls. This was statistically significantly increased (p=0.010), as determined by linear mixed model. *GAPDH* DNA levels were not significantly different between ED-IVF pregnancies and controls (p=0.45).

**Conclusions.** Elevated levels of fetal DNA in serum from women who conceive by ED-IVF may be the result of characteristic placental abnormalities observed in ED-IVF, such as dense fibrinoid deposition in the basal plate and severe chronic deciduitis, which have been previously reported by our group (Gundogan et al. Fertil Steril 2009; Feb 25). Further research is needed to understand the relationship between placental pathology, circulating cell-free DNA levels, and immunogenetic differences between the egg donor and recipient.

## 1634/T/Poster Board #183

**TURNER SYNDROME MOSAICS, CARDIAC RISK AND DONOR OOCYTE IN VITRO FERTILIZATION (IVF).** *A. Kittai, R. Pen, A. Benner, W.G. Kearns.* Shady Grove Center for Preimplantation Genetics, Rockville, MD.

Turner's Syndrome is characterized by short stature and ovarian failure. Cardiac and/or aortic abnormalities are present in a significant percentage of Turner syndrome patients and if spontaneously pregnant or by IVF, these patients are at significant risk of sudden death due to an aortic rupture or a cardiac event. The aortic / cardiac risks and outcome following a donor oocyte IVF cycle of Turner mosaics is unknown. Nineteen infertile female patients with a questionable mosaic Turner syndrome karyotype were enrolled in this study. The standard cytogenetic karyotypes ranged from 46,XX[19]/45,X[1] to 46,XX[44]/45,X[6]. We isolated white blood cells (wbcs) from peripheral blood using a modified microspin/phosphate buffered saline (PBS) wash. Wbc's were fixed using a modified Carnoy's method and fluorescence in situ hybridization (FISH) was performed for chromosomes X and Y. Approximately 500 cells were scored for the sex chromosomes to determine the actual percentage of sex chromosome mosaicism of each woman. All couples received counseling regarding the medical risks of beginning a donor oocyte IVF cycle. All women required a cardiology release for potential cardiac and aortic risks. Seven of the 19 couples enrolled in the study, underwent 13 IVF cycles. Clinical outcomes (embryo transfer, clinical pregnancies and delivery rates) of these cycles were determined. Eighteen of 19 women with questionable Turner syndrome mosaicism by traditional cytogenetics were shown to be true Turner mosaics by somatic cell wbc analysis. The average wbc sex chromosome mosaicism was 5.8% (1-18%). Of the 13 donor egg IVF cycles completed by the 7 couples, one couple had no transfer due to poor embryo quality, there were 2 healthy deliveries and 4 miscarriages (one couple had 2 miscarriages). The women with the healthy deliveries had 3 and 11% wbc mosaicism. In contrast, the women with miscarriages had 3, 4.4 and 6% wbc sex chromosome mosaicism. This preliminary study demonstrates that Turner syndrome mosaics can successfully undergo a donor oocyte IVF cycle following a cardiology release. However, the observed 67% miscarriages (four miscarriages out of six pregnancies from six couples) is 6 times higher than the expected donor oocyte IVF cycle miscarriage rate of 10% from this clinic. This preliminary data suggests that Turner syndrome mosaics, regardless of their percent XO cells, might consider a gestational carrier.

## 1635/T/Poster Board #184

**New insights into the biology of acid ceramidase and Farber disease.** *E. Schuchman, N. Shtraizent, X. He, E. Eliyahu.* Dept Genetics and Genomic Sciences, Mount Sinai Sch Medicine, New York, NY.

Acid ceramidase (AC), the lysosomal hydrolase deficient in Farber Lipogranulomatosis (Farber disease; FD), plays a central role in the metabolism of ceramide, a pro-apoptotic signaling lipid. We have recently shown that the complete knockout of AC expression in mice leads to apoptotic embryo death by the 4-cell stage (Eliyahu et al., FASEB J., 2007). Normal oocytes also expressed high levels of AC, likely providing the enzyme to the newly formed embryos and thereby sustaining survival until expression of the embryonic genome (4-cell stage in mice). AC levels in unfertilized oocytes gradually declined, leading to apoptosis. Based on these findings we hypothesized that AC is an important factor required for oocyte and early embryo survival, and that addition of recombinant AC (rAC) to culture media could sustain their survival in vitro and improve the outcome of in vitro fertilization (IVF). We tested this hypothesis using murine, bovine and human oocytes and/or embryos. In mice and cows, inclusion of rAC in IVF culture media markedly improved the quantity and quality of embryos formed. rAC also slowed apoptosis of unfertilized mouse and human oocytes. Notably, the levels of AC in preimplantation embryos were further correlated with their quality (i.e., high AC in high quality embryos). To extend these findings, we also investigated the use of rAC in other primary cell cultures, and found that as with oocytes and embryos, the enzyme could sustain survival by altering sphingolipid metabolism. Finally, to investigate the role of AC in mammalian development, we have recently constructed conditional AC knockout mice that can be used to inactivate the enzyme at various developmental stages and/or in a cell-specific manner. These mice, together with "hypomorph" knock-in mice we are developing, should provide the first viable models of FD. Funding by NIH grant R01 DK54830.

## 1636/T/Poster Board #185

**Genome-wide methylation analysis in women with trisomic pregnancies.** *CW. Hanna[1], MS. Peñaherrera[1], MD. Stephenson[2], WP. Robinson[1].* 1) Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) University of Chicago Recurrent Pregnancy Loss Program, University of Chicago, Chicago, IL, USA.

Trisomy occurs in 6% of clinically recognized pregnancies and accounts for >40% of miscarriages. Aging is associated with a decline in ovarian function and responsiveness to hormonal signals and is the strongest independent risk factor for trisomy. Hormonal balance has been shown to be essential for oocyte development, chromosome segregation, and follicular growth, and thus disruptions in this balance, due to endocrine disorders or exposure to endocrine disrupting chemicals (EDCs), may be associated with meiotic nondisjunction. Endogenous and exogenous changes in maternal hormone environment may result in altered gene expression as a consequence of abnormal DNA methylation. We hypothesize that maternal meiotic nondisjunction will be associated with altered DNA methylation, detectable in blood cells, due to the systemic effects of hormones and EDCs. DNA was extracted from whole peripheral blood from 21 fertile control women (>37 years of age with successful delivery and no history of miscarriage) and 20 women with a history of a trisomic pregnancy at an age of <35 years and/or ≥2 heterotrisomic miscarriages. To identify candidate methylation changes, samples were analyzed using the Illumina GoldenGate Methylation Cancer Panel I, which interrogates 1,505 CpG sites in 807 genes. Candidate methylation changes were identified using Significance Analysis of Microarrays with a false discovery rate of <20% and an absolute difference of 5% methylation. Five CpGs were found to be differentially methylated between cases and controls and are being validated with pyrosequencing in a larger set of 163 samples (63 controls and 100 women with ≥1 trisomic miscarriages). A candidate CpG of interest was located within the promoter of the Myeloid Leukemia Factor 1 (*MLF1*) gene, which was found to be significantly hypomethylated in women with ≥1 trisomic miscarriage (t-test, $p$=0.007), independent of age and blood cell composition. *MLF1* could play a role in risk for nondisjunction through its interaction with MLF1-interacting protein, which is highly expressed in the oocyte and is involved in centromere assembly and chromosome segregation. This study may improve our understanding of maternal meiotic nondisjunction and identify potential epigenetic markers which may be associated with meiotic nondisjunction in humans.

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## 1637/T/Poster Board #186

**A diverse phenotypic spectrum in a Chinese family with a novel micro-duplication of 15bp in human steroidogenic factor 1 (SF-1).** *H. Li[1], Y. Chen[1], Y.P. Lei[2], W. Wang[1], J. Ou[1], K.W. Choy[3].* 1) Center for Reproduction and Ge, Nanjing Medical University Affiliated Suzhou Hospi, Suzhou, China; 2) State Key Laboratory of Genetic Engineering and MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai, 200433, P.R. China; 3) Department of Obstetrics and Gynaecology, and Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, P.R. China.

Objective: Chromosomal imbalance or sub-microscopic copy number variation and some sex-related genes including sex determining region Y (SRY) and steroidogenic factor 1 (SF-1) play important role in the development of both the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes. The phenotypic spectrum associated with heterozygous loss of function mutations in SF-1 can range from complete testicular dysgenesis to mild genital ambiguity. This study is to designed to disclose the etiology of a Chinese family with two members affected by 46,XY sex-reversal phenotype. Methods: G-banding karyotyping, whole chromosome painting and microarray-based comparative genomic hybridization (array-CGH) were performed. Sequencing of SRY and SF-1 genes was performed to clarify the etiology. Results: G-banding karyotyping, whole chromosome painting were all confirmed that the two sisters are 46,XY. To exclude the presence of any sub-microscopic chromosomal imbalance, we performed high-resolution array CGH analysis on the patients' blood samples. Using the 244K array platform from Agilent Technologies, we detected 3 single copy number loss and 2 sing copy number gain of chromosomal materials ranging from 24Kb to 428Kb, including a chromosome region X:134605794-134737947 (within Xq26.3) was detected in the blood sample. Besides a novel 15 bp micro-duplication at c.1075-1089 in the SF-1 gene was identified in the two 46,XY sex-reversal sisters which is inherited from their fertile mother.. Conclusion: This observation comprises the evidence showing that micro-duplication may be the etiology of sex-reversal of 46,XY females.

## 1638/T/Poster Board #187

**CORRELATION PHENOTYPE-KARYOTYPE AND DETERMINATION OF REPRODUCTIVE RISK IN THE WOMEN WITH X CHROMOSOME MOSAICISM.** *R. Baez-Reyes[1,3], JC. Baez -Reyes[2], G. Razo-Aguilera[1,3].* 1) Dept Genetics, Instituto Nacional de Perinatología; 2) Dept Histophatology, Universidad Nacional Autonoma de Mexico; 3) Dept Morphology, Escuela de Ciencias Biologicas, IPN. MEXICO.

INTRODUCTION. Frequency of 11% of cytogenetic abnormalities is calculated in the patients with disturbance of the reproduction, among those the mosaics of sexual chromosomes are included. Mainly of the "X" that have not been studied appropriately until the moment. Reports exist for these patients with abnormal sexual development, gestational loss or newly born with chromosomophaty or children with congenital defects. OBJECTIVE. To know in a sample of mexican women the frequency of the "X" chromosome mosaicism and to correlate it with alterations of the reproduction. MATERIAL AND METHODS. We assisted the couples that went with reproduction alterations, the genetic clinical history, the pedigree and physical exploration having as selection approaches: 1)amenorrhoea primary or secondary 2)sterility primary or secondary 3) recurrent gestacional loss 4)previous children with birth defects 5)previous children with cromosomopathy 6) premature ovarian failure. To the couples that gathered the requirements they were carried out the karyotype in blood GTG Bands with the Moorhead technique, for analysis so much numeric as structural with a resolution of 450 bands. When she was a cell aneuploide in 25 metafases the study it was completed with the technique of FISH in 100 cells in metafase analyzed by case. RESULTS. We study 87 women that were detected with the X mosaicism, with a 19 year-old minimum age and 44 year-old maxim age, more frequent cause to go to the consultation are: 1) recurrent abortion(56), 2) amenorrhoea(12), 3)sterility(7), 4)children with birth defects (7) and 5)abortion and children with birth defects (5), we finding that the "X" chromosome mosaic more frequent it was: 45,X/46,XX/47,XXX(37); 45,X/46,XX(29) and 46,XX/47,XXX(19); 45,X/46,XX/47,XXX/48;XXXX(1) and 46,XX/47,XXX/48,XXXX(1). We gave the resulst and throught their consent they began into the protocol of integral study. CONCLUSIONS. 1)This alteration had not been studied in adequated form., 2)The more frequent cause of consultation is recurrent abortion, 3)The mosaicism that more is present is 45,X/46,XX/47,XXX, 4)The women can to present associate alterations, 5)The risk for the descendant is variable, 6) It's very important the study of the patients into of the protocol, 8)It's very important too to realize methods invasive for the prenatal diagnosis in the event of pregnancy.

## 1639/T/Poster Board #188

**Marked deviations from Hardy-Weinberg Equilibrium suggest the presence of a common variant with recessive lethal effect at 17q21.** *A. Vine, D. Curtis.* Centre for Psychiatry, Queen Mary's School of Medicine and Dentistry, London E1 1BB, UK.

We have recently reported regions in which groups of neighbouring markers exhibit marked deviation from HWE using genotypes in control subjects typed with the Affymetrix 500K marker set made available by the Wellcome Trust Case Control Consortium (WTCCC) (Vine and Curtis, 2009). The most striking of these results was a group of 6 markers on chromosome 17, each of which showed evidence for decreased homozygosity significant at $p<10^{-190}$. Because these deviations were observed in groups of neighbouring markers it does not seem plausible that these results could be explained as genotyping errors of individual markers. The markers were located within 4kb around position 41009000 on chromosome 17.

We have further investigated this phenomenon by studying the same markers typed in 7 cohorts of WTCCC cases, comprising a total of approximately 14,000 subjects, and markers in the same region typed in the HapMap subjects using the Perlegen platform.

The results we obtained consistently show a marked reduction in homozygous genotypes compared to what would be expected under HWE. This is manifest across all sets of samples and across different genotyping platforms.

We conclude that there is a real and marked deviation from HWE at this position on the human genome. The results are consistent with the presence of a common variant having a recessive lethal effect, meaning that subjects carrying two copies of this variant are unlikely to survive to adulthood. Given that this variant seems to be very common, we speculate that its effect is manifest very early in embryogenesis, resulting in early loss, so that overall it has only a modest impact on the reproductive fitness of parents carrying it. Thus, two parents carrying the variant might lose one embryo in every four reproductive cycles, leading to only moderately impaired fertility. Identifying this variant and others with similar effects might prove helpful in understanding genetic facts impacting on human fertility.

## 1640/T/Poster Board #189

**Long Polyglycine Tracts (GGN Repeats) of Androgen Receptor Gene in Cryptorchidism and Hypospadias.** *R. Asadollahi, R. Radpour, Z. Barekati, C. Kohler, XY. Zhong.* Department of Biomedicine, University of Basel, Basel, Basel, Switzerland.

Cryptorchidism and hypospadias are among the most frequent congenital abnormalities in male births affecting 0.3%-0.7% and 2%-4% of births, respectively. In this study, the association between CAG/GGN trinucleotide repeats of androgen receptor gene and the two mentioned congenital malformations was evaluated. We compared 76 cryptorchid and 92 hypospadiac patients (divided into subgroups of glanular, penile, and penoscrotal hypospadias) to 190 normal controls. Length of the CAG/GGN repeat segment was evaluated in exon 1 by using polymerase chain reaction (PCR) sequencing and in exons 2-8 by PCR-single-strand conformation polymorphism (PCR-SSCP). No significant difference was seen in the CAG length of patients versus normal controls. However, GGN numbers were found to be significantly higher (median, 24 vs. 22) in both patients with penile hypospadias (P=.018) and those with a history of cryptorchidism (P=.001) compared to the controls. In addition, GGN number of patients with penile hypospadias was significantly different compared to the other subgroups of hypospadias (P=.001). We were able to identify 12 different CAG alleles and 8 different GGN alleles in the cryptorchid group. Our data suggest the possible association between polyglycin tract polymorphism of androgen receptor gene and cryptorchidism. Further studies are needed to elucidate the possible role of specific CAG/GGC combinations as a susceptible factor.

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## 1641/T/Poster Board #190

**CYTOGENETIC ASPECT IN REPRODUCTIVE FAILURE.** *M. GULTOMRUK, E. AKCAY, H. UCAR, R. ULUOCAK, D. DOGAN, H. UNAL, L. ERKAN, E. TUTAR, G. KILIC, N. ERCELEN.* Genetics & Genomic Sciences Center, American Hospital, Istanbul, TURKEY.

To evaluate the cytogenetic study results of 1586 patients with reproductive failure. 1586 patients were analyzed cytogenetically. Peripheral blood cultures were carried out for chromosome preparations and 20 metaphases were analyzed for each patient by at least a biologist and two specialists. 50-100 metaphases were counted in case of mosaicism. 450-550 band level GTG banding technique was used, additionally, CBG-banding, NOR-banding or FISH techniques also used if necessary. We categorized reproductive failures patients into two groups according to their reproductive history: repeated pregnancy loss&poor obstetric history, infertility&repeated implantation failure. In this study, 1586 patients [937 men (59.1%), 649 women (40.9%)] with reproductive failure were analyzed cytogenetically. A total of 138 aberrant karyotypes (8.7%) were diagnosed, corresponding to an abnormality frequency of 9.7% (91/937) for men and 7.2% (47/649) for women. The observed chromosomal abnormalities were structural (40.6%,56/138), numerical (0.7%,1/138) and sex chromosome abnormalities (58.7%,81/138). The following frequencies of abnormalities were found 34.8% (48/138) for translocations, 4.3% (6/138) for inversions and 1.4% (2/138) for other chromosomal abnormalities; 57.2% (79/138) for numerical sex chromosome abnormalities; 1.4% (2/138) for other sex chromosome abnormalities. Abnormalities seen in both groups were translocations, inversions, sex chromosome abnormalities and marker chromosomes. In the first group, karyotyping were performed for 752 patients (382 women and 370 men) and 698 (92.8%) of them were analyzed as normal, 54 (7.2%) of them were analyzed as abnormal. In the second group, 750 (89.9%) patients were found as normal, 84 (10.1%) patients were found as abnormal in 834 patients (267 women and 567 men). 70 of 370 men evaluated for infertility had cytogenetic abnormalities of whom 32 had azoospermia, 3 had oligooospermia. Infertility - the inability to achieve conception or sustain a pregnancy through to live birth - is very common and affects about 15% of couples. The male and female with infertility accurately have higher risk to carry a chromosomal anomaly. The karyotype knowledge enables us to detect the individual affected couple with an increased risk for transmitting an unbalanced chromosomal complement to the embryo which leads comprehensive genetic counselling.

## 1642/T/Poster Board #191

**Sperm mtDNA mutations and oxidative stress- an important pathology in male fertility.** *K. Ihtisham[1], S. Venkatesh[1], R. Kumar[1], MB. Shamsi[1], R. Kumar[2], NP. Gupta[2], RK. Sharma[3], P. Talwar[3], R. Dada[1].* 1) Anatomy, All India Institue of Medical Sciences, New Delhi, Delhi, India; 2) Department of Urology, AIIMS, New Delhi-29; 3) ART center , Army Research and Referral Hospital, N Delhi.

Aim: To screen mtDNA mutations in semen of infertile men with elevated ROS levels. Methods: The study included 50 idiopathic infertile men and 50 fertile controls. Semen analysis was performed according to WHO criteria (1999). ROS in the semen was measured by chemiluminescence assay. Whole sperm mtDNA was sequenced by standard PCR-DNA sequencing method. Results: Infertile group showed significant difference in the sperm count (74.10±15.5 Vs 11.9±5.2), percent sperm motility (68.5±14.6 Vs 14±5.2) and percent normal morphology (62.9±13.3 VS 11±2.2) compared to control men. Infertile group showed significantly (p< 0.001) higher ROS levels (331.55+58.97 cpm) /106 spermatozoa compared to fertile controls (4.2+1.21 cpm) / 106 spermatozoa. mtDNA sequencing revealed that 66% of the infertile group harboured one or more nucleotide changes in the gene ATPase6 & 8, Cytb, ND3, ND4 and ND5 in the mitochondrial genome compared to control men inspite some common nucleotide changes (A750G, A4769G) in both the groups. Conclusions: Approximately 80% of the infertile men showed significantly elevated ROS levels in the semen. Out of these approximately 60% of the infertile men with elevated ROS levels showed significantly higher nucleotide changes in sperm mtDNA when compared to the controls. Therefore, higher frequency of mtDNA nucleotide changes may be responsible for elevated ROS levels that might further increase the incidence of nucleotide changes. These adverse phenomena may also have an impact on DNA integrity that may lead to impaired fertility. Therefore, screening mtDNA mutations in infertile men with severe oxidative stress may help in the better management for infertility treatment.

## 1643/T/Poster Board #192

**Comparative Genomic Hybridization Microarray (aCGH) to Identify Unrecognized Structural Chromosomal Defects Associated with Human Male Infertility.** *C. Jorgez[1], L. Lipshultz[1], D. Lamb[1,2].* 1) Dept Urology, Baylor College of Medicine, Houston, TX; 2) Dept Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX.

OBJECTIVE: Known genetic factors account for approximately 15% of male infertility. Numerical and structural chromosome abnormalities, such as Klinefelter syndrome and microdeletion of the Y-chromosome represent the most commonly known and tested genetic causes. Nevertheless, more than 50% of male infertility may have a genetic cause. Thousands of genes are likely needed for male fertility, yet there are few diagnostic tests available. Our goal is to identify the molecular basis of male infertility. We tested the hypothesis that structural chromosome defects too small to be seen on karyotype are present in infertile men. We searched for regions of chromosome duplication or deletion to identify gene dosage abnormalities associated with non-obstructive azoospermia (NOA). MATERIALS AND METHODS: Fertile and NOA men with normal karyotypes were analyzed by aCGH using 720K NimbleGen arrays (Roche). Analysis was performed with Nexus Copy Number software (BioDiscovery).These high-resolution arrays span most of the genome, allowing detection of submicroscopic chromosome defects and permits definition of the break-points of each duplication or deletion. Each suspected gain or loss was then validated by FISH and QPCR. RESULTS: Chromosome duplications or deletions in nine distinct regions (1q32, 7p14.3, 9q32, 11q13, 14q22, 14q32, 17q12, 19p13, 20q11) that lacked copy number variants were identified in 88% of NOA men. These regions encode genes highly expressed in testis. Other duplicated or deleted chromosomal regions were also found. No duplications or deletions were found on chromosomes 4 or 18. Analysis of several men with known Y-microdeletions in the AZFa, b, and/or c regions showed that the deletions in Yq were more complex then expected based upon the results of a multiplex PCR based assay. In addition, gains in Xp22 and Yp were observed with 2 men presenting Y-chromosome microdeletion together with a duplication of SHOX. CONCLUSIONS: Definition of the genetic cause of male infertility is important for both diagnosis and treatment, because today use of the assisted reproductive technologies allow many otherwise infertile men to become fathers. Nevertheless, these technologies may also allow transmission of unknown defects to their progeny. Consequently, the identification of genetic factors is important for appropriate management of the infertile couple. Array technology identifies unrecognized clinically relevant genetic factors causing male infertility.

## 1644/T/Poster Board #193

**Is Apoptosis a potential marker for sperm quality?** *M.S. Juchniuk de Vozzi[1], C.S. Pereira[1], S.A. Santos[1], I. Pola[1], P.A. Vozzi[1], M.A.C. Vasconcelos[2], A.C.J.S. Rosa-e-Silva[2], L. Martelli[1].* 1) Deparment of Genetics, School of Medicine of Ribeirão Preto, University o, Ribeirão Preto, São Paulo, Brazil; 2) Department of Gynecology and Obstetrics, School of Medicine of Ribeirão Preto, University of São Paulo, Brazil.

Apoptosis is a cell death program involved in different steps of spermatogenesis, first at puberty, at the beginning of spermatogenesis, then in adult testicles by controlling normal spermatogenesis. Many studies have shown that apoptosis deregulation in the germinal cells resulted in male infertility. In addition, apoptosis detection in ejaculated spermatozoa arouses a growing interest in research as a reliable marker of spermatozoon quality. Our objective was to compare the apoptosis markers in sperm of infertile patients to the control group samples. All the infertile patients (n=18) presented oligozoospermia and normal somatic karyotype. The control group was characterized by normal spermogram, normal karyotype and proved fertility. The presence of apoptotic sperm was detected by using the recombinant annexin V conjugated to fluorescein (FICT annexin V) and propidium iodide (PI) nucleic acid binding dye. Both semen analysis and the apoptosis test were performed at the same time. The analysis of 500 cells from the control group has shown a mean value of 83,88 % of live cells, 4.5% of apoptotic cells and 11.47 % of necrotic sperms. The oligozoospermic group presented 75.015% of live cells, 12.19 % of apoptotic sperm and 12.29% necrotic sperm in the sample. Both rates of apoptosis and necrosis showed significant difference between the groups (0.03056 and 0.04207). Necrosis was the preferencial via of cell death in the control group while there was no difference between the frequencies of necrosis (0.1229) and apoptosis (0.1219) in the infertile group. Our findings have shown a significant difference in the apoptosis rates between the control group with proven fertility and the infertile group. We suggest that the apoptosis can be a candidate semen quality marker.

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## 1645/T/Poster Board #194

**Genetic causes of male infertility in India.** *A. Khattri[1], N. Gupta[2], B. Chakravarty[2], M. Deenadayal[3], L. Singh[1], K. Thangaraj[1].* 1) Centre for Cellular and Molecular Biology, Hyderabad, Andhra Pradesh, India; 2) Institute of Reproductive Medicine, Salt lake, Kolkata, India; 3) Infertility Institute and Research Centre, Hyderabad, India.

During spermiogenesis chromatin of the sperm becomes highly compact due to sequential replacement of somatic histones by transition nuclear proteins (*TNP*), which are then replaced by protamines (*PRM*). Improper replacement of these proteins can cause sperm DNA damage leading to male infertility. Calmodulin dependent protein kinase 4 (*CAMK4*) phosphorylates the protamines, which is responsible for interaction of protamines with chromatin. Therefore, we screened *CAMK4*, *TNP1* & *2* and *PRM1*, *2* & *3* genes, which are involved in the above functions, in Indian men which includes 300 infertile and 289 fertile men. We have observed a total of 78 polymorphic sites in these genes. Although the frequency of many SNPs was low, 9 mutations in *CAMK4* gene and 15 in *PRM* genes and 3 in TNP genes were observed exclusively in infertile men and 2 SNPs g.-25:C>T in *PRM3* showed significant difference between infertile and fertile men. The g.-25:C>T substitution is present in the upstream of the *PRM3* gene and may be affecting the expression of *PRM3* gene. This novel predicted *PRM3* gene was confirmed for its transcription and full-length transcript was isolated and found to have testis specific expression. We further predicted that many infertile men-specific mutations could cause either loss or appearance of potential binding sites for various splicing factors. The SNPs of *PRM1* and *PRM2* genes were found to be in LD, but not in *PRM3*. Haplotype analysis revealed that the haplotype PRM-H6 and TNP-H3 conferred significantly increased risk for infertility. We addressed for first time the association SNPs and haplotypes of *CAMK4*, *TNP* and *PRM* genes with male infertility in ethnically matched Indian men. Further analysis of these genes on other populations is required to validate them as a candidate for infertility in males. In another aspect of our study we are looking for the genes those are up/down regulated in testis of infertile vs fertile men using Affymetrix microarrays for expression analysis. Currently we are analyzing and validating microarray results using Real Time PCR. These results will be presented during the conference.

## 1646/T/Poster Board #195

**Familial Frameshift SRY Mutation Inherited from a Mosaic Father: new insights into Testicular Dysgenesis Syndrome.** *C. Le Caignec[1], B. Isidor[1], C. Capito[2], F. Paris[5], S. Baron[3], N. Corradini[4], B. Cabaret[4], M.D. Leclair[2], M. Giraud[1], D. Martin-Coignard[6], A. David[1], C. Sultan[5].* 1) CHU Nantes, Service de Génétique Médicale, Nantes, France; 2) CHU Nantes, Service de Chirurgie Infantile, Nantes, France; 3) CHU Nantes, Clinique Médiale Pédiatrique, Nantes, France; 4) CHU Nantes, Service d'Oncologie Pédiatrique, Nantes, France; 5) Service d'Hormonologie, Hôpital Lapeyronie, CHU Montpellier, Montpellier, France; 6) Unité de Génétique Clinique, Centre Hospitalier du Mans, Le Mans, France.

Context: The SRY gene encodes a transcription factor responsible for initiating testis differentiation. Mutations in SRY almost always result in XY sex-reversal with pure gonadal dysgenesis and an increased risk of gonadal tumour. Most of these mutations are de novo affecting only one individual in a family. Only a small subset of mutations is shared between a phenotypically normal father and one or more of his affected children. Incomplete penetrance and somatic mosaicism are two hypotheses that may explain a normal phenotype in a father carrying a SRY mutation. Patients-Results: We describe a family with two sisters with XY sex-reversal and pure gonadal dysgenesis and a phenotypically normal brother. A novel constitutional frameshift SRY mutation was identified in both sisters and was absent in the brother. The single base-pair deletion (c.71delA) led to a premature stop-codon in position 60 of the protein removing entirely the HMG domain and the DNA-binding domain of SRY. The father of the three children presented with hypospadias, cryptorchidism, testicular seminoma and oligoasthenozoospermia, an association termed testicular dysgenesis syndrome (TDS), and the SRY mutation in a mosaic state in the peripheral blood and the tumour. Conclusions: This observation of somatic and germinal mosaicism for a SRY mutation may explain the variable penetrance in some familial gonadal dysgenesis. Importantly, the present report is the first one describing the association of SRY mutation in a male with TDS. This suggests that mutations in a sex-determining gene may contribute to the pathogenesis of TDS.

## 1647/T/Poster Board #196

**A role for methylation of the hMLH1 promoter in loss of hMLH1 expression in patients with non-obstructive azoospermia.** *F. Sun, H. Tian, J. Lian.* Sch Life Sci, Univ Sci Tech China, Hefei, China.

Decreased numbers of meiotic recombination hMLH1 foci have been demonstrated in infertile men. However, little is known about the mRNA and protein expression of hMLH1 in patients with non-obstructive azoospermia (NOA). This study is aim to identify the expression level of hMLH1 and its methylation status in the testes of normal controls and of patients with NOA. The expression level of hMLH1 mRNA and the localization of hMLH1 protein in the testes of 3 controls and 3 patients with NOA were performed by the reverse transcription-PCR and immunohistochemistry, respectively. Methylation specific-PCR was used to study whether the promoter regions of hMLH1 were methylated. The genome DNAs of both normal and NOA patients' testes were modified by bisulfate and the CpG islands (region B or region C) of hMLH1 promoter were amplified with unmethylation or methylation primers. The pathological types of NOA patients were subdivided as sertoli cell only syndrome (SCOS), hypospermatogenesis and germ cell arrest. The levels of hMLH1 mRNA were decreased in testes of patients with germ cell arrest and SCOS compared to that in normal controls, however, no obviously decreased levels of hMLH1 mRNA were found in patients with hypospermatogenesis. Immunohistochemical results indicated that hMLH1 proteins were exclusively expressed in primary spermatocyte. The expression levels of hMLH1 were also found to be significantly reduced in patients with germ cell arrest. Complete absence of hMLH1 expression was observed in SCOS, since these testes have no any meiotic cells. Similar to the expression levels of hMLH1 mRNA in patients with hypospermatogenesis, no significant alteration in hMLH1 levels in these patients. Aberrant promoter methylation of hMLH1 was detectable in these cases. Defective hMLH1 expression was identified in patients with NOA, which was strongly related to their testicular pathological types. Methylation of the hMLH1 promoter may be a mechanism for loss of hMLH1 expression in male infertility.

## 1648/T/Poster Board #197

**Single nucleotide polymorphisms and a novel mutation (311C→A) in sperm nuclear proteins gene of Indian infertile men.** *S. Venkatesh[1], M. Kumar[1], M. Tanwar[1], MB. Shamsi[1], R. Kumar[2], NP. Gupta[2], R. Dada[1].* 1) Anatomy, Lab Molec Reproduction & Gen, New Delhi, India; 2) Department of Urology, AIIMS, New Delhi, India.

Aim: Sperm testicular proteins, the protamines and transitional nuclear proteins play a crucial role in sperm nuclear condensation. Mutation in any of the corresponding genes or aberrant post translation modification may lead to abnormal sperm chromatin condensation leading to male infertility. Therefore the study was aimed to screen nucleotide changes in protamine (PRM 1 & 2) and transitional nuclear protein (TNP 1 & 2) genes in Indian oligozoospermic men. Methods: The study included 30 oligozoospermic men and 20 fertile controls. Semen analysis was performed according to WHO (1999) guidelines. Sperms were separated from leukocytes, epithelial cells and debris by density gradient method. Sperm DNA was isolated by phenol-chloroform method. PRM and TNP genes were amplified, and purified by guanidinium thiocyanate method. The final products were sequenced by standard PCR-DNA sequencing protocol. The nucleotides were numbered from the first nucleotide of the corresponding gene. Results: Sperm count (10.52 ± 4.20 Vs 92.32 ± 23.44), and progressive sperm motility (28.50 ± 12.25 Vs 62.5± 21.5) were significantly compromised in oligozoospermic men compared to controls. In total, 6 nucleotide changes were observed in the study out of which, 139C→A in PRM1gene was found to be common in patients and controls, but 1083C→A in PRM2 gene was found to be present at higher frequency in patients than controls. However, a novel mutation in PRM 2 gene (311C→A) changing amino acid threonine to asparagines was observed in one patient (sperm count-17million/ml) with 50% abnormal head morphology and 15% forward motility. However, we found a SNP 839 T→C in TNP1 gene in 4% of the patients but not in controls. TNP2 mutation analysis showed a SNP 991C→T in TNP2 gene in 30% of the controls but not in the patients.. Conclusion: The frequency of SNPs observed in PRM genes was similar in both oligozoospermic and controls except a novel mutation (311C→A) in the exon of PRM2 gene. This mutation might be associated with the abnormal sperm parameters, by altering the PRM2 protein conformation that could lead to infertility. This is the first of kind of study in PRM and TNP genes in Indian infertile population. Higher frequency of SNPs and rare novel mutation in Indian infertile population could be the reason for impaired fertility in this population. Further larger number of studies screening for these variants and its correlation with assisted reproductive outcome is warranted.

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## 1649/T/Poster Board #198

**Cystic Fibrosis screening in Italian infertile couples.** *A. Saluto[1], A. Murro[1], A. Celeste[1], A. Marongiu[1], G. Del Noce[2], G. Voglino[1].* 1) Laboratory of Molecular Genetics, PROMEA S.p.A, Turin, Italy; 2) Division of Andrology, PROMEA S.p.A, Turin, Italy.

Cystic Fibrosis (CF) is the most common autosomal recessive genetic disorder primarily affecting Caucasian populations caused by mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) gene, with an incidence of about 1/2500 live births and a prevalence of 1/28. CFTR gene testing is currently performed in couples undergoing Assisted Reproduction Techniques (ART), because of the high prevalence of healthy carriers in the population and the pathogenic relationship with Congenital Bilateral Absence of Vas Deferens (CBAVD). CF has a complex phenotype with manifestations in many organs but involvement of the respiratory tract is the dominant clinical feature. Thus far, more than 1600 mutations in the CFTR gene have been identified; the most common mutation is ΔF508, whose prevalence varies among populations of different geographical and ethnic backgrounds, accounting approximately for the 70% of the mutations in European patients. Our aim was to evaluate the frequency of CFTR mutations in infertile couples referred to our center for Assisted Reproductive Techniques (ART) (either IVF-ICSI or IUI). We analysed 3632 infertile couples in the last six years (2003-2008); molecular analysis of the CFTR gene was performed on genomic DNA extracted from peripheral blood lymphocytes by using OLA assay (Cystic Fibrosis Genotyping Assay, Abbott Laboratories, Germany). The 3.5% (254/7264) of the tested patients showed the presence of a CFTR gene mutation corresponding to a prevalence of 1/28; 171/254 mutations (67%) were ΔF508 in agreement with previous studies for the Italian population (Riccaboni et al. 2008). In one patient we identified two mutations, N1303K and R347H in different CFTR alleles; he was 38 years old and the cause of infertility was CBAVD. This patient had a history of recurrent bronchitis and was recovered for three months for chronic obstructive bronchopathy with no indications for CF. The combinations of CBAVD and chronic sinopulmonary disease is a confirm that some patients with bilateral absence of the vas deferens may have a mild form of Cystic Fibrosis; moreover, some patients with a mild form of CF could be undiagnosticated (Gilljam et al. 2004).

## 1650/T/Poster Board #199

**PREIMPLANATATION GENETIC SCREENING (PGS) FOR ANEUPLOIDY IN 143 COUPLES UNDERGOING DONOR EGG *IN VITRO* FERTILIZATION (IVF) CYCLES REDUCES THE MISCARRIAGE FROM 10% TO 2.7%.** *R. Pen[1], A. Benner[1], J. Liebermann[2], P. Browne[3], W.G. Kearns[1].* 1) Shady Grove Center for Preimplantation Genetics, 15001 Shady Grove Rd., Suite 220,Rockville, MD 20850; 2) Fertility Centers of Illinois, 900 N. Kingsbury, River Walk 6 Chicago, IL 60610; 3) Shady Grove Fertility RSC, 15001 Shady Grove Rd., Suite 400, Rockville, MD 20850.

We determined the prevalence of aneuploidy, the miscarriage rate and the number of healthy deliveries in couples undergoing donor egg IVF cycles.

One-hundred forty-three couples underwent donor egg IVF-PGS due to poor outcomes from prior fertility therapy. Laser-assisted embryo biopsy was performed on day-3 and PGS was done on 1869 cleaving embryos from 143 initiated cycles. The mean donor age was 26.5 years (21- 31). Multi-color fluorescence *in situ*hybridization was used to determine aneuploidy for chromosomes 13, 14, 15, 16, 17, 18, 21, 22, X and Y. Hybridization, stringency washes and fluorescent microscopy was performed. Clinical outcomes (aneuploidy, embryo transfer, clinical pregnancies and delivery rates) of these cycles were determined. Clinical pregnancy was defined by ultrasound identification of an intrauterine gestational sac and fetal heart beat.

All 143 women had an embryo transfer. Four percent (75/1869) of the embryos were not diagnosed due to poor embryo quality and cell lysis. Forty-eight percent (861/1794) of the embryos were aneuploid for at least 1 of the 10 chromosomes tested. Seventy-three percent (681/933) of the cytogenetically normal embryos developed into a blastocyst. The clinical pregnancy rate was 77% (110/143) per patient and 77% (110/143) per embryo transfer. The miscarriage rate was 2.7% (3/110). The delivery rate was 65% (93/143) not including fourteen additional viable gestations beyond 15 weeks. There were no misdiagnoses nor identified mosaic embryos.

This aneuploidy screening data from donor egg cycles provides insight into the presence of aneuploidy in a low risk population. Nearly 50% of donor egg embryos were aneuploid. The pregnancy rates were similar in these patients to those undergoing donor egg IVF without PGS. However, PGS lowered the expected miscarriage rate observed in our donor oocyte IVF cycles from 10 % to 2.7%. This suggests that PGS in this patient group successfully screens and selects for embryos most likely to achieve a successful outcome, the delivery of a healthy baby free of a genetic disease.

## 1651/T/Poster Board #200

**Gene location matters: polar body analysis has limited informativity in centromeric genes.** *G. Altarescu, T. Eldar-Geva, B. Brooks, E. Haran-Zylber, I. Varshaver, E.J. Margalioth, E. Levy-Lahad, P. Renbaum.* Zohar PGD Lab & IVF Unit, Shaare Zedek Medical Ctr, Jerusalem, Israel.

Objective: To assess the degree of heterozygosity and percent of allele drop out (ADO) of polar body 1 (PB1) analyzed during Preimplantation Genetic Diagnosis (PGD) for monogenic diseases. Introduction: PGD is performed by biopsy of blastomeres or polar bodies (PBs) utilizing mutation detection and multiple polymorphic marker analysis to minimize misdiagnoses. Because only maternal alleles are analyzed in PBs, the chances of finding informative markers are higher than in blastomere analysis. An additional advantage is the sequential analysis of PB1 and PB2: when PB1s are heterozygous, this significantly decreases the degree of misdiagnosis due to ADO. However, when PB1 are homozygous, ADO can never be ruled out and additional blastomere biopsy may be required. Methods: Retrospective analysis of all PB-PGD cycles performed in our unit from 2004 till present for monogenic disorders and determination of the frequency of heterozygosity of PB1 and ADO for each gene tested.Results: Twenty seven diseases were tested by PB PGD in 68 families, a total of 1157 PB1 were diagnosed. The PB1 heterozygosity frequency was 6 - 100% and ADO rates were 4 -23%.We found that all genes we tested which were located within 5 Mb of the centromere (GJB2 and 6, NF1, RET and SMAX1, 236 PB1s) showed very low heterozygous PB1 rates (6 to 12%), whereas any genes located greater than 10Mb from the centromere showed an average of 80% heterozygosity (sd= 11%). ADO rates ranged from 4% for the SMAX1 locus to 23% for the EMD locus and were not related to the position of the gene on the chromosome. Conclusion: Genes residing close to the centromere show a low frequency of heterozygosity of PB1 and therefore PB PGD should not be considered for these disorders. The ADO rates were similar with the previous reported data.

## 1652/T/Poster Board #201

**PREIMPLANTATION GENETIC DIAGNOSIS FOR CRYPTIC TRANSLOCATION CARRIER COUPLES.** *E. AKCAY[1], E. TUTAR[1], M. GULTOMRUK[1], L. ERKAN[1], B. BALABAN[2], B. URMAN[2], N. ERCELEN[1].* 1) Genetics & Genomic Sciences Center, American Hospital, Istanbul, TURKEY; 2) American Hospital, Women' s Health Center, Istanbul, TURKEY.

To present the preimplantation genetic diagnosis results of reciprocal and robertsonian translocations that were analyzed in 26 ICSI/PGD cycles for 20 couples. Cytogenetic analysis was performed on cultured lymphocytes derived from peripheral bloods of couples. Two telomeric probes with two or one centromeric probes for the chromosomes involved in the translocation were analyzed on metaphase spreads of translocation carrier individual. Each blastomere biopsied from third day embryo analyzed with these telomeric and centromeric probes. They were also analyzed for common aneuploidies with PGT probe [13, 18, 21, X, Y (Vysis, Inc.)]. Preimplantation genetic diagnosis was performed for 26 translocation carrier cycles. Average maternal age was 32.4 years. 233 oocytes were retrieved, 189 of them were in M2 and 165 2PN (87.3%) embryos were observed. Average of 1.7 normal embryos were transferred in 17 cycles. 153 embryos were analyzed and only 20.3% (31/153) were normal or balanced. 16.3% (14/86) of embryos were analyzed normal or balanced in reciprocal translocations, while 25.4% (17/67) of embryos were analyzed normal or balanced in robertsonian translocations. Clinical pregnancy per embryo transfer (ET) was 20% (2/10) and 42.9% (3/7) in reciprocal and robertsonian translocations, respectively. 5 clinical pregnancies were resulted. 5 babies (3 singleton and 1 twin) were delivered, 1 singleton pregnancy still going on. Individuals that carry chromosomal translocations can produce chromosomally unbalanced gametes and they are at an increased risk for chromosomally abnormal fetus (1). Preimplantation genetic diagnosis should be recommended for poor prognosis translocation carrier couples to reduce number of pregnancy losses and to have successful pregnancy. In the literature, a significant decrease is observed in spontaneous abortions in translocation heterozygotes, from 81% before PGD to 13% after PGD [Munne et al., 2000]. There is no clinical abortion case in our transferred cycles. In conclusion, PGD significantly decreased losses and increased the number of viable pregnancies(P<0.001)(2). REFERENCES: 1. Munne S., Analysis of chromosome segregation during preimplantation genetic diagnosis in both male and female translocation heterozygotes, Cytogenet Genome Res.2005;111(3-4):305-9 2. Escudero T, Estop A, Fischer J, Munne S, Preimplantation genetic diagnosis for complex chromosome rearrangements, Am J Med Genet A. 2008 Jul 1;146A(13):1662-9.

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## 1653/T/Poster Board #202

**PGD On 3,270 Embryos From 343 Cycles Due To Parental Reciprocal Translocation, Robertsonian Translocations Or Pericentric Inversions.** *A. Benner, R. Pen, A. Siegel, A. Kittai, W.G. Kearns.* Shady Grove Center for Preimplantation Genetics, Rockville, MD.

Carrier couples with a structural chromosomal abnormality are at great risk of producing genetically unbalanced gametes. Therefore, these couples are at high risk of producing genetically unbalanced progeny. This study of 343 PGD cycles from referring *in vitro* fertilization (IVF) clinics was performed to determine embryo structural chromosome imbalances due to parental structural chromosome rearrangements.

Two-hundred twenty-eight patients underwent 343 IVF cycles and PGD for genetic imbalances due to their structural chromosome abnormalities. Laser-assisted embryo biopsy was performed on 3,270 day-3 embryos. Multi-color fluorescence *in situ* hybridization (FISH) using telomere and/or genomic loci DNA probes was used to determine embryonic genetic balances of the chromosome(s) involved in the parental structural aberrations. Parental structural chromosome rearrangements included reciprocal translocations, Robertsonian translocations and pericentric inversions. Clinical pregnancy (CP) was defined by ultrasound identification of an intrauterine gestational sac and fetal heart beat. Of all cycles, 29.2% (100/343) had no transfer because all tested embryos were either 1) genetically unbalanced or 2) genetically balanced but found to be morphologically abnormal in development and quality on the day of transfer. Just over 7.6% of the embryos had no diagnosis because of poor embryo quality and cell lysis. The total CP for 218/228 patients for all structural chromosome aberrations was 37.6% (82/218); 41.2% (42/102) for reciprocal translocations, a 34.7% (33/95) CP rate for Robertsonian translocations and 33.3% (7/21) for pericentric inversions. The overall biochemical pregnancy rate was 8.7% (19/218) per patient and the miscarriage rate was 8.7% (19/218) per patient. We've had 42 deliveries with an additional 19 viable ongoing clinical gestations (most are beyond > 12 weeks). If all of these ongoing pregnancies continue to healthy deliveries, our overall delivery rate will be 28% (61/218). The remaining 10 patient transfers are awaiting hCG results.

This data shows that PGD for structural chromosome abnormalities is a viable option for carrier couples of a structural aberration to have a healthy child free of a genetic disorder.

## 1654/T/Poster Board #203

**Polar body analysis with Array-CGH in a clinical trial.** *T. Buchholz[1], M. Klehr-Martinelli[1], B. Seifert[2], M. Bals-Pratsch[2].* 1) Gyn-Gen-Lehel, Center for Polar Body Diagnosis, Munich, Germany; 2) Center for Reproductive Medicine, Regensburg, Germany.

Chromosomal aneuploidies are the major cause of pregnancy loss or implantation failure. Therefore many efforts are in progress to establish assays for the detection of aneuploidies in preimplantation genetic diagnosis (PGD). In the past years, we were able to gain much experience with polar body (pb) analysis by fluorescence in situ hybridisation (FISH). Both aneuploidy testing and translocation analysis are well established in our lab. The main disadvantage of this technique is that only a maximum of 10 chromosomes can be analysed. In order to analyse all 23 chromosomes, we are using an Array-CGH procedure. To get sufficient DNA from the sample, each pb is amplified by whole genome amplification. The array carries 600 bac clones covering the whole genome to detect gain or loss of chromosomes. To test the feasibility in a clinical setting with the restriction of the German embryo protection law, we started a clinical trial, including 10 patients (mean age 37,4 years). Following a standard IVF protocol (2.-4. cycle) oocytes retrieval was carried out in all 10 patients (mean no of MII oocytes 11,6). Both pbs of max 5 oocytes (in two cases only of 2 or 3 oocytes) from each patient were extracted and amplified. All array analyses were successfully carried out, leading to an average of 42% euploid oocytes. Following the embryo transfer of a maximum of 2 day-3-embryos, 3 clinical pregnancies could be achieved in 7 patients; further 3 patients are still awaiting pregnancy testing. The study is ongoing and so is the follow up of the patients. Due to our preliminary results, we conclude that further studies, especially to confirm the benefit in regard to clinical pregnancy and baby take home rates for particular subgroups of patients, need to be carried out.

## 1655/T/Poster Board #204

**OUTCOMES OF PREIMPLANTATION GENETIC DIAGNOSIS FOR SINGLE GENE DISORDERS.** *N. ERCELEN[1], H. COMERT[1], L. ERKAN[1], O. ILBAY[1], B. YAZAR[1], R. MERCAN[2], A. ISIKLAR[2], B. BALABAN[2], B. URMAN[2].* 1) Genetics & Genomic Sciences Center, American Hospital, Istanbul, TURKEY; 2) IVF Center, American Hospital, Istanbul, TURKEY.

OBJECTIVE: To present the results of Preimplantation Genetic Diagnosis (PGD) studies for the identification of a causative gene mutations together with/without Human Leucocyte Antigen (HLA) typing in several single gene disorders. METHODS: Preimplantation HLA matching was only applied on parents requesting treatment of their affected children. Following standart IVF protocol, single embryonic cells were removed from 3rd-day embryo following oocyte pick-up. Negative control for each blastomere was also prepared to control the contamination risk. Multiplex semi-nested PCR by using specific primers and polymorphic markers specific for each disease was applied for the mutation analysis. To detect and avoid misdiagnosis due to allele drop out (ADO), a haplotype analysis for father, mother and affected child was performed for each family before the preimplantation HLA typing. HLA genes from blastomeres were tested together with short tandem repeats (STRs) in the HLA regions by using multiplex semi-nested PCR system. RESULTS: 281 embryos were analyzed for different single gene disorders in 38 PGD cycles performed for 27 couples.7,4 embryos were analysed per cycle. 23,5% of total embryo analysed (66/281) were selected and transferred. Average of 1,7 embryos were selected for each cycle. 105 embryos from 15 cycles of 11 couples were analyzed for specific gene mutation and HLA compatibility. 17 unaffected embryos which were HLA matched to their affected siblings were selected and transferred. Two clinical pregnancies obtained from the PGD for HLA typing resulted in 3 healthy live-births who are potential donors of stem cells for their affected siblings. One was twin and the other was singleton. Interestingly, each one of twins was stem cell donors for each of their thalassemia major sisters. Few months ago, cord blood sample of one of twins was used for transplantation of his HLA compatible affected sister. Totally, obtained 9 clinical pregnancies (clinical pregnancy rate/ ET cycle: 32%) resulted in 10 healthy live-births: 5 of them were singleton, one was twin and one was triplet. CONCLUSION: These outcome data showed that due to its accuracy and efficiency, application of single-cell PCR based DNA analysis is a useful tool for the PGD of monogenic disorders. In addition, HLA typing during PGD not only offers production of unaffected offspring , but also possible treatment of affected siblings.

## 1656/T/Poster Board #205

**Distinguishing balanced from normal embryos in preimplantation genetic diagnosis (PGD) for chromosomal rearrangements.** *A. Kuliev, J. Cieslak - Janzen, Z. Zlatopolsky, I. Kirillova, Y. Ilkevitch, Y. Verlinsky.* Reprod Gen Inst, Chicago, IL.

Preimplantation genetic diagnosis (PGD) is the only hope for carriers of balanced chromosomal translocations for having an unaffected child, without facing risk for repeated spontaneous abortions. It is presently performed by interphase FISH analysis, which cannot distinguish the normal from balanced embryos, and also fails detecting small inversions and insertions, for which no locus specific probes are commercially available. To overcome this limitation, we introduced different methods for conversion of interphase nucleus into metaphase. One of them involves the fusion of biopsied blastomeres with mouse zygotes, which despite being practical is labor intensive and may raise ethical concerns. The other is based on the chemical induction, involving the exposure of selected single blastomeres to caffeine. We performed PGD in 474 cases of balanced rearrangements, overall, 227 of which were done by conversion, including 94 by chemically method. This resulted in transfer of 112 normal embryos in 69 cycles, 34 pregnancies and 5 spontaneous abortions. The overall number of predicted unbalanced embryos (73%) was similar in conversion and interphase methods, with a total of 581 balanced or normal embryos transferred in 354 cycles, resulting in 130 clinical pregnancies (38%), and birth of 104 unaffected children, with no misdiagnosis. Overall, only 21 pregnancies resulted in spontaneous abortions (16%), suggesting at least a four-fold reduction of spontaneous abortion rate, compared to non-PGD couples.

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## 1657/T/Poster Board #206

**Use of microarray CGH for Preimplantation Genetic Diagnosis.** *S. Munne, C. Gutierrez-Mateo, JF. Sanchez-Garcia, S. Tormasi, R. Prates, N. Goodall, T. Escudero, P. Colls.* Reprogenetics, Livington, NJ.

Introduction - Preimplantation Genetic diagnosis (PGD) to screen against chromosomally abnormal embryos has been extensively applied using FISH with 9-12 probes. Recently, CGH has been used for PGD to screen all chromosome abnormalities in a single cell. However, CGH is time consuming and is not compatible with embryo biopsy on day 3 of development and day 5 transfer, thus CGH is usually done on embryos on day 5 of development(blastocysts) and then the embryo is frozen until results are obtained. Unfortunatelly, embryo freezing may imperil embryo survival. Here we present the first clinical results using array CGH, which allows day 3 biopsy of a single cell, obtain results in 24 hours, and to transfer by day 5 without the need of embryo freezing. Materials and Methods- Single day 3 embryos were biopsied, single blastomeres amplified, fluorescently labeled and hybridized onto BlueGnome 24sure BAC arrays. The normal embryos were replaced to women undergoing IVF. The abnormall embryos were fixed and analyzed by 12 probe FISH and reanalyzed if necessary in subsequent hybridizations for other chromosomes found to be abnormal by aCGH. Preliminary results have shown that full chromosome abnormalities were reliably diagnosed with a 6% error rate, but not so structural abnormalities. Thus, only full chromsome abnormalities were taken into account. Results- Five cycles of IVF and PGD were performed in women with a history of recurrent miscarriage. Thirty five embryos were biopsied, of which 3 did not produced aCGH results (9%). Twenty one embryos were classified abnormal by aCGH (65%); 13 had single or double aneuploidy events and 8 were chaotic. Eleven embryos were classified as normal and ten were replaced, the other having arrested in culture. The majority of abnormal embryos (19) were fixed and analyzed by FISH. The abnormalties detected by aCGH were confirmed by FISH in 95% of them. The 9 and 12 FISH probe would have classified 76%; and 81%, respectively, of embryos as abnormal. Conclusions- aCGH seems to detect 19% more abnormal embryos than FISH with 12 probes and with a 5% error for full chromosome abnormalities. We consider the test to be ready for clinical application of PGD, and now it remains to see if this technique will be supperior to FISH in improving pregnancy rates and reducing miscarriages.

## 1658/T/Poster Board #207

**Trophectoderm Biopsy, preimplantation aneuploid screening (PGS) and a fresh embryo transfer within an in vitro fertilization cycle (IVF) is a viable alternative to day-3 blastomere biopsy and PGS.** *A. Siegel[1], R. Pen[1], A. Benner[1], J. Liebermann[2], M. Katz-Jaffe[3], W. Schoolcraft[3], W.G. Kearns[1].* 1) Shady Grove Center for Preimplantation Genetics, 15001 Shady Grove Rd, Ste. 220, Rockville, MD 20850; 2) Fertility Center of Illinois, 900 N. Kingsbury, River Walk 6, Chicago, IL 60610; 3) Colorado Center for Reproductive Medicine, 10290 RidgeGate Circle, Lone Tree, CO 80124.

We determined whether trophectoderm biopsy and PGS for 10-chromosomes is a viable alternative to day-3 blastomere biopsy and preimplantation genetic screening.

Participating patients either had ≥ 3 frozen blastocysts or good quality day-4 or day-5 growing blastocysts in culture. All patients had an indication for PGS, primarily > 2 prior miscarriages. Biopsied trophectoderm cells (mean = 5) were fixed using a modified Carnoy's method and FISH was performed for chromosomes 13, 14, 15, 16, 17, 18, 21, 22, X and Y. All PGS results were complete within 24-hours of biopsy and only embryos normal for all tested cells and chromosomes were recommended for transfer.

One-hundred fourteen blastocysts were biopsied from 33 patients. Of the frozen blastocysts, 78% survived the thaw and 67% re-expanded. All trophectoderm cells from each embryo were successfully diagnosed. Forty-one percent (47/114) of the blastocysts were PGS normal. Of the 33 patients, 3 had no transfer due to the presence of an abnormal cell(s). Of the patients undergoing embryo transfer, there was a 70% (21/30) clinical pregnancy rate (CP) as defined by a fetal heartbeat and gestational sac. The biochemical pregnancy rate was 3% (1/30) and there were no miscarriages.

This study shows the feasibility and efficacy of doing a blastocyst biopsy and PGS with transfer within 24-hours of biopsy. The outstanding clinical pregnancy rate, low biochemical pregnancy rate and no miscarriages strongly suggests that blastocyst biopsy and PGS may be a less-invasive and more beneficial option to day-3 blastomere biopsy and PGS.

## 1659/T/Poster Board #208

**RESULTS OF 373 ICSI/PGD CYCLES IN POOR PROGNOSIS PATIENTS.** *E. TUTAR[1], M. GULTOMRUK[1], L. ERKAN[1], B. URMAN[2], N. ERCELEN[1].* 1) Genetics & Genomic Sciences Center, American Hospital, Istanbul, TURKEY; 2) Women's Health Center, American Hospital, Istanbul, TURKEY.

Objective: To present results of 373 ICSI/PGD cycles in poor prognosis patients with one of indications of advanced maternal age (AMA), recurrent implantation failure (RIF), recurrent spontaneous abortions (RSA), severe male factor (SMF) or translocation carrier (TRL). Materials and Methods: Aneuploidies: Embryos biopsied on day 3 were fixed on slide(s), hybridized with PB probe (Vysis,Inc.) and analyzed for chromosomes 13, 16, 18, 21, 22 at the first round. They were also hybridized with 4-Color CUSTOM Probe (Vysis, Inc.) and analyzed for chromosomes 15, 17, X and Y at the second round. Translocations: Metaphases derived from peripheral bloods of carrier individuals were analyzed for chromosomes involved in translocation. Blastomeres biopsied from day 3 embryos were analyzed for chromosomes involved in translocation and for common aneuploidies (13, 18, 21, X, Y). Euploid or normal (balanced) embryos were transferred on day 5. Prenatal diagnosis was recommended in case of pregnancy. Results: 2087 embryos were analyzed in 373 ICSI/PGD cycles. 1955 (93.7%) of them were diagnosed as normal (35.8%) or abnormal (64.2%). Average maternal age was 36.1. 5.6 embryos were analyzed and 1.7 embryos were transferred for each cycle from total 456 (23.3%) transferred ones. Clinical pregnancy was documented in 72 of 274 transferred cycles which was yielded 26.3% clinical pregnancy/ET rate. Implantation rate was observed as 18.3%. 64 healthy babies were delivered and there are 6 ongoing pregnancies. Most frequently observed aneuploidy was monosomies (44.7%) which were followed by trisomies (23.1%), polyploidies (19.7%) and nullisomies (12.5%). Discussion: Most common reason for early pregnancy loss is aneuploidy in oocytes or embryos which increases with maternal age and may be also increased in chromosomally normal couples with recurrent early pregnancy loss or repeated unsuccessful IVF cycles although morphologically high quality embryos were transferred. Therefore, PGD has been recommended for use in conjunction with IVF to select and transfer only chromosomally normal embryos and thereby to improve the clinical outcome (1). References: 1. Preimplantation genetic testing: a Practice Committee opinion, Fertil Steril_ 2008;90:S136-43. 2008 by American Society for Reproductive Medicine.

## 1660/T/Poster Board #209

**Genetic Problems of Assisted Reproductive Technology (ART) in Japan.** *N. Takeshita, T. Sekine, T. Taniguchi, A. Oji, M. Saigusa, H. Hayashi, Y. Mastue, A. So, Y. Katagiri, M. Morita.* Obstetrics & Gynecology, Toho Univ Sch Med, Tokyo, Japan.

The first birth of a child by in vitro fertilization (IVF) in 1978 was a landmark event not only in the field of medicine but also for the general public. On the other hand, some alarming issues including the genetic problems associated with reproduction technologies have also been reported. A total of 700 centers currently provide reproductive healthcare services in Japan, where one out of 60 babies is now born by ART procedures. Against this background, a long-term follow-up for newborns is indispensable from the viewpoint of genetic issues. When taking the propagation of variable genes to the next generation into account, one of the possible genetic problems of concern in such babies is microdeletion in the azoospermic factor (AZF) region of the long arm of the Y-chromosome, which may be transmitted through ICSI. The AZF region has been reported in many articles to be a complex structure known as the palindrome. Attention should also be paid to numerical and structural changes of the sex chromosome and autosome. Since around 2001, the existence of an important relationship has been reported between ART and epigenetic diseases such as changes in genome imprinting. The reprogramming of genetic expression may occur in the gametes and embryos in respective stages. According to previous reports, ART may also be related to such processes of reprogramming. In addition, a preimplantation genetic diagnosis, which has already been approved for certain indications in Japan, is now increasingly being carried out in Western countries. Aneuploidy screening for chromosomes is also now performed more frequently than the earlier genetic diagnosis.

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## 1661/T/Poster Board #210

**The 3-dimensional distribution of human chromosomes in sperm.** *T. Liehr[1], F. Hunstig[1], S. Bhatt[1,2,3,4], F. Pellestor[2,3,5], K. Mrasek[1], A. Weise[1], I. Simonyan[6], R. Aroutiounian[7], M. Manvelyan[1,6,7].* 1) Jena University Hospital, Institute of Human Genetics and Anthropology, Jena, Germany; 2) INSERM U847, Montpellier, France; 3) University of Montpellier I, Montpellier, France; 4) Baylor College of Medicine, Houston, Texas, USA; 5) Department of Reproduction Biology, CHU Montpellier, Montpellier, France; 6) Research Centre of Maternal and Child Health Protection, Yerevan, Armenia; 7) Department of Genetic and Laboratory of Cytogenetics, State University, Yerevan, Armenia.

Nuclear architecture studies in human sperm are sparse. By now performed ones were practically all done on flattened interphase nuclei. Thus, studies close at the in vivo state of sperm, i.e. on three-dimensionally conserved interphase cells, are lacking by now. Only the position of 14 chromosomes in human sperm was studied, yet. Here for the first time a combination of multicolor banding (MCB) and three-dimensional analysis of interphase cells was used to characterize the position and orientation of all human chromosomes in sperm cells of a healthy donor. We could show that the interphase nuclei of human sperm are organized in a non-random way, driven by the gene density and chromosome size. Overall, here we present the first comprehensive results on the nuclear architecture of normal human sperm. Future studies in this tissue type, e.g. also in male patients with unexplained fertility problems, may characterize yet unknown mechanisms of infertility. Supported in parts by DAAD (A0704616/Ref326), Evangelische Studienwerk e.V. Villigst, Ernst-Abbe-Stiftung, IZKF Jena and DFG (LI 820/9-1, 436 ARM 17/11/06, 436 RUS 17/88/06, LI 820/11-1, LI 820/13-1, LI 820/15-1, LI 820/21-1).

## 1662/T/Poster Board #211

**Identification of Copy Number Variants (CNV) Associated with Recurrent Pregnancy Loss.** *E. Rajcan-Separovic[1], D. Diego-Alvarez[2], W. Robinson[2], Y. Qiao[1,2], C. Harvard[1], C. Fawcett[3], C. Tyson[3], M. Stephenson[4].* 1) Dept Pathology (Cytogenetics), Child & Family Research Inst, Vancouver, BC, Canada; 2) Dept Medical Genetics, Child & Family Research Inst, Vancouver, BC, Canada; 3) Cytogenetic Lab, Royal Columbian Hospital, New Westminster, BC, Canada; 4) Dept Obstetrics and Gynecology, University of Chicago, Chicago, IL.

Recurrent early pregnancy loss (RPL), defined as two or more miscarriages of less than 10 weeks of gestation, affects 3-5% of couples trying to establish a family. In 40% of couples with RPL, no factor is identified, despite extensive evaluation. We initiated an array CGH study of miscarriages from couples with RPL to assess for submicroscopic chromosomal changes, termed copy number variants (CNVs), in chromosomally normal diploid (46,XX or 46,XY) miscarriages. Array CGH was performed on extracted DNA from the diploid miscarriages and on DNA from peripheral blood from both partners, to determine whether CNVs, if present, were de novo or inherited from one of the partners. Extensive demographics and obstetrical histories were obtained from the RPL couples, as well as embryopathology results from the miscarriages, to correlate the CNV results with clinical findings. Twenty diploid miscarriages from 16 couples with a history of RPL have been studied to date. Eleven unique, previously unreported CNVs were detected in 8 of the miscarriages and all were inherited from one of the partners. Five unique CNVs were detected in one of the partners but these CNVs were not found in the miscarriage DNA. The most intriguing candidate miscarriage CNVs found to date are: a) CNVs containing genes maternally imprinted in the placenta (inherited from female partners), b) CNVs occurring on the X chromosome (inherited from female partners and c) CNVs with a putative role in miscarriage (inherited from either partner). Copy number alterations of two candidate miscarriage genes maternally imprinted in the placenta, CTNNA3 (alpha T catenin) and TIMP2 (Tissue Inhibitor of Metalloproteinases 2), were screened for in a cohort of 200 women with RPL and 63 fertile controls (women giving birth after the age of 37 and with no history of infertility) using a Quantitative Multiplex Fluorescent PCR of Short Fragments (QMPSF) assay. Copy number alteration of CTNNA3 was identified in 2/200 of the RPL women and 0/63 fertile controls. Our preliminary results suggest that alterations of genes maternally imprinted in the placenta, such as CTNNA3, may be responsible for recurrent pregnancy loss in a small percentage of couples presenting with RPL. Array CGH appears to be a useful technology to identify novel biomarkers associated with recurrent pregnancy loss.

## 1663/T/Poster Board #212

**Molecular Study of Internal Apoptotic Pathway BAX and BCL2 Genes and Mitochondrial Genome in Idiopathic Repeated Pregnancy Loss.** *S. Seyedhassani[1,2], M. Houshmand[2], S. Kalantar[1], G. Modabber[2], A. Aflatoonian[1].* 1) Medical Genetics Dept, Res/Clinical Ctr Infertility, Yazd, Iran; 2) National institute for genetic engineering and biotechnology, Tehran, Iran.

Introduction: About 1 in 300 couples involve with Repeated Pregnancy Loss ( RPL ) and the main part of them remains unknown. Apoptosis plays a role in early human development and embryonic loss.The aberrant expression of apoptotic related genes is seen in RPL. It seems internal apoptotic pathway and mitochondria as a main core of it, have important role in fertilization and proliferation of the cells. Bax is an important nuclear gene in mitochondrial pathway of apoptosis.This protein forms a heterodimer with BCL2, and functions as an apoptotic activator. Material and methods: 335 consecutive cases were studied. Genetic counseling, clinical, paraclinical, and cytogenetic studies were done for each couple. We analyzed the familial pedigree of them and then screened the idiopathic cases. In total 96 females who were suffered from idiopathic RPL. 1-Four multiplex PCR are done on each sample for detection of mitochondrial deletions. 2-Mitochondrial D-loop part consisting of the hyper variable regions is analyzed by PCR-sequencing method. 3-Bax gene is evaluated by PCR-sequencing method for promoter region and all seven exons. 4-Bcl2 gene is evaluated by PCR-sequencing method for promoter region and PCR-SSCP for the exons. Results: 1-No mitochondrial deletions were found in 96 DNA samples. 2-D-loop region was evaluated by direct sequencing and we found 166 different variations in our study population. Among them, 95 variations were seen in RPL cases , 28 in control samples , and also 43 in both of them . 3-Change of A to G in promoter region of Bax gene was seen at nt. -55 in 93 females (96.87%) . Discussion: 1-Some of these nucleotide alterations might be involved in repeated pregnancy loss and could be included in a panel of molecular biomarkers for susceptibility in pregnancy loss and even failure of in-vitro fertilization. 2-A high rate of mutation in mitochondrial DNA in the D loop was found in samples from patients with RPL relative to healthy controls . 3-In seven SNPs that were found in case and control groups, we found significant difference between groups ($P<0.05$) (T16126C, T16189C, C16223T, C16294T, T16311C, T16362C, T16519C) . From the RPL group mutations, 15 SNPs were significant and four mutations was novel (A503G, A335G , T217C , C114ins.) 4-We believe that mutation in Bax gene will lead to early apoptosis.

## 1664/T/Poster Board #213

***NAT2* haplotypes modify the effects of smoking, alcohol, and caffeine on fertility.** *K. Taylor[1], L. Murray[1], C. Small[1], M. Wilson[2], W. Tang[2], M. Bouzyk[2], M. Marcus[1].* 1) Dept Epidemiology, Emory Univ, Atlanta, GA; 2) Emory Biomarker Service Center, Atlanta, GA.

Purpose. The enzyme N-acetyltransferase 2 (*NAT2*) is responsible for metabolizing and detoxifying xenobiotics such as caffeine, tobacco smoke, pesticides, and prescription drugs. Common polymorphisms in the *NAT2* gene determine haplotypes that have slow or fast acetylator phenotypes and follow distinct metabolic pathways. The slow haplotypes have been associated with increased risk of bladder cancer, hepatocellular carcinoma, endometriosis, and other conditions. We investigated whether *NAT2* haplotypes affected time to pregnancy or modified the effects of xenobiotic exposures on time to pregnancy. Methods. We conducted a prospective cohort study investigating fertility in a population of 470 women office workers ages 20-41 who were at risk for pregnancy. Fertility was measured by counting the menstrual cycles until a pregnancy occurred (time to pregnancy). Information on exposures and covariates was collected in an intake interview and in daily diaries. Urine samples were collected and served as the source of DNA. Three *NAT2* polymorphisms (rs1799929, rs1799930, and rs1208) were genotyped in 319 women using the Beckman SNPStream system. Discrete survival analysis was used to determine whether *NAT2* haplotypes modified any effects of alcohol, smoking, or caffeine on time to pregnancy. Results. The 319 women were each followed for an average of 8 menstrual cycles, resulting in 124 pregnancies. 161 women carried two copies of the slow haplotype and were determined to be slow acetylators. Increasing levels of alcohol, smoking, and caffeine were all associated with increased time to pregnancy in a dose-response manner. The most fertile women were nonsmokers who consumed less than three alcoholic drinks per week and less than 200 mg of coffee per day. There was no main effect of *NAT2* haplotype or genotypes on time to pregnancy. Interaction was observed between the *NAT2* haplotype and smoking, alcohol, and caffeine; slow acetylators were more susceptible to the effects of all three exposures. These associations and interactions remained after adjustment for potential confounders. Conclusion. When studying the effects of xenobiotics on human health, it may be of scientific importance to incorporate genetic information about relevant metabolic enzymes. This is of particular importance when estimating the effects of these exposures among those with varying levels of genetic susceptibility.

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## 1665/T/Poster Board #214

**Comparison of Self-Contained Gene Set Methods for Gene Expression Studies.** *B. Fridley, G. Jenkins, J. Biernacka.* Hlth Sci Res, Mayo Clinic, Rochester, MN.

Gene set methods incorporate prior biological knowledge into statistical analyses and aid researchers in the interpretation of the results. Over the past few years, multiple approaches for gene set analysis have been proposed for expression and SNP data. The various methods can be divided into two types: competitive and self-contained. Benefits of the self-contained methods are that they can be used for genome-wide, candidate gene, or pathway studies; and these tests are more powerful then the competitive methods. We investigated numerous self-contained methods that can be used for both continuous and discrete phenotypes. To assess the power and type I error rate for the approaches, an extensive simulation study was completed in which the scenarios varied according to: correlation between genes within a pathway, number of genes in a pathway, number of associated genes, effect sizes, and the sample size. The following methods were assessed: tail strength (TS), principal component analysis (PCA) using either 80% of the explained variation threshold, first PC or top 5 PC, a global model, Kolmogorov-Smirnov (KS) test, Fisher's method based on asymptotic and empirical distribution. Results from simulations in which genes were independent showed that the KS and TS methods perform the worst for most scenarios, while the PCA using 80% variation threshold and Fisher's methods preformed the best for the majority of scenarios.

## 1666/T/Poster Board #215

**ParaHaplo: A Program Package for Haplotype-based Whole-genome Association Study using Parallel Computing.** *K. Misawa, N. Kamatani.* RIKEN, Minato-ku, Japan.

Since more than one million of single nucleotide polymorphisms (SNPs) are analyzed in genome-wide association study (GWAS), multiple comparisons are problematic. To cope with multiple-comparison problems in GWAS, haplotype-based algorithms were developed to correct for the multiple comparisons at multiple SNP loci in linkage disequilibrium. The permutation test can also control problems inherent in multiple testing; however, the calculation of exact probability and the execution of permutation tests are both time-consuming. Faster methods for calculating exact probabilities and executing permutation tests are needed. We developed a set of computer programs for the parallel computation of accurate P values in haplotype-based GWAS. Our program, ParaHaplo, is targeted to workstation clusters using the Intel Message Passing Interface (MPI). We compared the performance of our algorithm with that of the regular permutation test on CHB and JPT of HapMap. ParaHaplo can detect smaller difference between two populations than SNP-based GWAS. We also found that parallel-computing technique made ParaHaplo 100-fold faster than non-parallel version of the program. The executable binaries and program sources are available at http://sourceforge.jp/projects/parallelgwas/?_sl=1.

## 1667/T/Poster Board #216

**Penalized estimation of haplotype frequencies from sibs.** *K. Zhang.* Dept Biostatistics, Univ Alabama at Birmingham, Birmingham, AL.

Haplotype inference plays an important role in association studies because haplotype based analysis can provide additional power for gene mapping but haplotypes of diploid individuals cannot easily be acquired by experiments. Therefore, many EM based methods have been developed to infer haplotypes and estimate their frequencies from unrelated individuals as well as general pedigrees. One drawback of these methods is that many rare haplotypes with low explanatory power can be included, especially in the presence of missing data. This problem becomes more severe when haplotypes are estimated from sibs because the completely missing genotypes of parents must be included in the analysis in order to take the relationship between sibs into account. To discourage the conclusion of rare haplotypes with low explanatory power, we propose a penalized method for haplotype inference. Specifically, a linear penalty is imposed to haplotypes with low frequency and the penalty levels off for haplotypes with frequency greater than a prespecified threshold. Then the penalized likelihood is used to infer haplotypes and estimate their frequencies by a general minorize-maximize (MM) algorithm. The partition-ligation technique is also implemented to handle large number of markers. We evaluate its performance and compare it with several available methods for haplotype inference from sibs through extensive simulations. Our results indicate that our method outperforms other available methods in most situations.

## 1668/T/Poster Board #217

**Use of Multiple Controls in a Genome-wide Association Study.** *A. Kuchiba[1], H. Sakamoto[1], S. Ohnami[1], A. Saito[2], S. Chiku[3], H. Totsuka[4], T. Yoshida[1].* 1) Genetics Division, National Cancer Center Research Institute, Tokyo, Japan; 2) Statistical Genetics Analysis Division, StaGen Co., Ltd., Tokyo, Japan; 3) Science Solutions Division, Mizuho Information and Research Institute, Inc., Tokyo, Japan; 4) Bioinfomatics Group, Research and Development Center, Solution Division 4, Hitachi Government and Public Corporation System Engineering Ltd., Tokyo, Japan.

Genome wide association studies (GWAS) have been widely adopted as a potentially powerful approach for common complex diseases. Most GWAS feature case-control designs, in which issues related to optimal and appropriate selection of control samples often arise. In case-control studies, researchers often find an opportunity to include more than one control series. This seems to be the case especially for GWAS, in response to the increasing demand for a large, high-power collaborative study by sharing data and/or samples. Some GWAS simply pool and use a "combined control" derived from different sources primarily to enhance the power. However, these association studies can have biased estimators caused by combining multiple sources. Another approach is to demonstrate a consistent association over multiple case-control studies between a single common case and several control populations; the observed associations then appear more robust, and the power may be also improved, as compared to a single case-single control comparison. However, the amount of information extracted will be overestimated, and the summary estimates may not be appropriate, because there is a correlation in the analyses due to the shared case population. Here we describe a method of generalized least squares to take the correlation into account. The method also considers between-control heterogeneity by a random effects model. Statistical performance of this screening approach will be compared with the simple "combined control" approach by simulation studies. An application of the proposed method will be illustrated using our GWAS data on the hospital-based gastric cancer cases (originated from various parts of Japan), one control population from a metropolitan area and the other from a single rural town. Although the basic of an association study is a nested case-control design, our approach will aid a proper and efficient use of precious control resources to prioritize the SNP-disease associations for the next stage of investigation.

## 1669/T/Poster Board #218

**Mediating effects of smoking and chronic obstructive airway disease on the relationship between the CHRNA5-A3 genetic locus and lung cancer risk.** *J. Wang, M.R. Spitz, C.I. Amos, A.V. Wilkinson, X. Wu, S. Shete.* Dept Epidemiology, UT MD Anderson Cancer Ctr, Houston, TX.

Recent genome-wide association (GWA) studies of lung cancer have shown that the CHRNA5-A3 region on chromosome 15q24-25.1 is strongly associated with an increased risk of lung cancer. However, different etiological explanations for the association between genetic variants and lung cancer have been provided. We applied rigorous statistical approaches, including mediation analysis and Bayesian network techniques, to examine the mediating effect of smoking behavior and self-reported physician-diagnosed emphysema on the relationship between the CHRNA5-A3 region genetic variant rs1051730 and the risk of lung cancer. Our results showed that rs1051730 is a causal factor for lung cancer, and it is associated with lung cancer through smoking behavior and COPD. We also showed that COPD is an intermediate phenotype that explains most of the effect of smoking behavior on lung cancer. Our results also suggested that smoking behavior is a mediator of the relationship between rs1051730 and COPD. Finally, we found that environmental tobacco smoke (ETS) is not a mediator for the relationship between rs1051730 and lung cancer among never smokers.

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## 1670/T/Poster Board #219

**A Two-Stage Genome-wide Association Study of Young-Onset Hypertension in Han Chinese Population of Taiwan.** *KM. Chiang[1,9], HC. Yang[2], YJ. Liang[2], CM. Chung[1], HY. Ho[3], CT. Ting[3], TH. Lin[4], SH. Sheu[4], WC. Tsai[5], JH. Chen[5], HB. Leu[6], WH. Yin[7], TY. Chiu[8], C. Fann[1], JY. Wu[1], SJ. Lin[6], YT. Chen[1], JW. Chen[6], WH. Pan[1].* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Institute of Statistical Science, Academia Sinica, Taipei, Taiwan; 3) Taichung Veterans General Hospital, Taichung, Taiwan; 4) Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan; 5) National Cheng Kung University Hospital, Tainan, Taiwan; 6) National Yang-Ming University School of Medicine and Taipei Veterans General Hospital, Taipei, Taiwan; 7) Cheng Hsin Rehabilitation Medical Center, Taipei, Taiwan; 8) Min Sheng General Hospital, Taoyuan, Taiwan; 9) Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan.

Hypertension is a common and complex disorder. To identify its susceptibility genes may contribute to genetic screening and treatment for hypertension. Although many large scale genome-wide association studies have been performed, only a few studies have successfully identified the loci that are related to the hypertension, not to mention the scanty Asian studies. Young-onset hypertension (YOH) may be a more feasible target disorder to investigate than the late-onset one due to its stronger genetic component. We performed a two-stage genome-wide association study to map YOH susceptibility genes. In the first stage, we analyzed 400 YOH cases and 400 age and gender matched controls with BMI adjusted in all the analyses. Illumina HumanHap550 Genotyping BeadChip was used for genotyping. Two different analyses, exact conditional logistic regression with p-value combination tests combined a truncated product p-value procedure and a sliding-window procedure and with the genome-wide haplotype association tests combined haplotype trend regression and a sliding-window procedure, were used to identify potential SNPs which are validated in the second-stage by an independent set of 600 cases and 600 age and gender matched controls. In the conditional logistic regression analyses, genotype information was either treated as nominal (AA, AB and BB) or ordinal (1, 2 and 3) and all of the results were adjusted by the false discovery rate (FDR). Finally, there were 20 and 24 SNPs with strong association signals (the ranges of the $-\log_{10}$FDR are 5.16 to 7.57 and 6.18 to 10.35) selected by the p-value combination analyses with a window size of 7 and 9, respectively. A total of 36 genes were implicated and some of these genes have been related to the hypertension and cation channel or metabolism. In the haplotype analyses, four loci were selected for its strong association signals ($-\log10p > 5$) which flank 11 genes. Some of these genes are related to the angiogenesis. All of these SNPs are currently being validated with the additional 600 case-control pairs.

## 1671/T/Poster Board #220

**Nonsynonymous single nucleotide polymorphisms of essential hypertension in Korean.** *K. Hong[1], H. Jin[1], J. Lim[1], Y.S. Cho[2], M.J. Go[2], J. Jung[3], J. Lee[3], J. Choi[4], C. Shin[5], S. Lee[6], H.K. Park[1], B. Oh[1].* 1) Biomedical Engineering, Kyung Hee University, Seoul, Seoul, Korea; 2) Center for Genome Science, National Institute of Health, Seoul, Korea; 3) DNA Link, Inc., Seoul, Korea; 4) EQUISPHARM co., Ltd, Gyeonggi-do, Korea; 5) Department of Internal Medicine, Korea University Ansan Hospital, Gyeonggi-do, Korea; 6) Department of Internal Medicine, Kyung Hee University East-West Neo Medical Center, Seoul, Korea.

The 95 percent of hypertension, not knowing the obvious cause, is known as essential hypertension which is considered polygenic, resulting from accumulation and combination of a number of genetic and environmental risks. At present, no causative variation of essential hypertension has been reported. To identify causative variations influencing on blood pressure and hypertension, we investigated the association using 1,180 SNPs that cause amino acid change (nonsynonymous SNPs; nsSNPs) in Korean Association REsource consortium (KARE) from the Affymetrix 5.0 SNP array platform. The genotypes of 1,180 nsSNPs are tested for the association with systolic blood pressure and hypertensive status. Subjects were taken from two community-based independent cohorts, Ansung (n = 4,183) and Ansan (n = 4,659), South Korea. Five SNPs (rs16835244, rs2286672, rs6265, rs17237198, and rs7312017) were significantly associated with systolic blood pressure and replicated the associations in both cohorts. Among the five SNPs, two SNPs (rs16835244 and rs2286672) were maintained the association with hypertension risks. The SNP rs16835244 replaces 288th Ala of arginine decarboxylase by Ser and the SNP rs2286672 replaces 172nd Arg of phospholipase D2 gene by Cys. The multiple alignments of peptide sequences reveal the evolutionary conservation of the amino acid residues among orthologs from vertebrates. The ADC gene governs the metabolic pathway of L-arginine into agmatine. Agmatine, the decarboxylation product of arginine and known as a putative neurotransmitter, has been known to be involved in the control of vascular smooth muscle tone. The PLD2 also regulates the endocytosis of angiotensin II receptor, type 1 (AGTR1), and the impaired PLD2 regulation may play a role in the pathogenesis of hypertension. In this study, we applied a different strategy and identified candidate nsSNPs for the blood pressure as well as hypertension in two independent cohorts. Even though these finding are remained to be verified by other populations, the genetic variants will be helpful to guide the study of the underlying mechanism the regulation of the blood pressure as well as the development of the new therapeutic medicines.

## 1672/T/Poster Board #221

**Detecting causal genetic effects for survival.** *P.J. Lipman[1], J.D. Muehlschlegel[2], S.C. Body[2], C. Lange[1].* 1) Department of Biostatistics, Harvard School of Public Health, Boston, MA; 2) Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

In this communication, we present a new statistical approach to detect causal genetic effects on survival after surgery in the presence of genetic associations that might influence survival as well and thereby could confound the results. Using causal inference methodology, the proposed method is based on standard statistical methodology and can be implemented straightforwardly. Using simulation studies, the theoretical properties of the approach are verified and the power is assessed for realistic scenarios. The practical relevance of the approach is illustrated by an application to survival after cardiac surgery.

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## 1673/T/Poster Board #222

**Rapid exact likelihood-based quantitative trait association analysis in large pedigrees.** *T.D. Dyer, V.P. Diego, J.W. Kent Jr., H.H.H. Göring, J. Blangero.* Dept Genetics, SW Foundation Biomed Res, San Antonio, TX.

Quantitative trait association analysis in large pedigrees is a formidable computational task due to the necessity of taking the non-independence among relatives into account. With the growing awareness that rare sequence variants may be important in human quantitative variation, association study designs involving large pedigrees will increase in frequency due to the greater chance of observing multiple copies of rare variants amongst related individuals. Therefore, it is important to have statistical genetic test procedures that utilize all available information for extracting evidence regarding genetic association. Optimal testing for marker/phenotype association involves the exact calculation of the likelihood ratio statistic which requires the repeated inversion of potentially large matrices. In a genome-wide association context, such computation may be prohibitive for those without access to super-computing clusters. Several widely-used approximations attempt to reduce this computational burden but invariably lead to test statistics exhibiting reduced power. In this study, we provide a new approach for speeding up exact likelihood calculations for association analysis. For quantitative traits, analysis of genetic association involves joint modeling of a measured genotype fixed effect and residual polygenic random effects. The new method uses eigenstructure techniques to dramatically simplify the complex matrix manipulations that are required in full likelihood calculation. Following a spectral decomposition of the kinship matrix, the resultant orthogonal matrix of eigenvectors and their eigenvalues can be used to transform the original non-independent phenotypic data into uncorrelated data. A linear up-dating of this transformation that is a function of the current value of the estimated residual heritability is all that is required to reduce the multivariate likelihood function to a function of univariate likelihoods. For genome-wide association analysis, this procedure need only be performed once and the resulting transformation stored. The achieved reduction in computational burden is sufficient that researchers with large pedigrees need no longer resort to inefficient approximate methods. This approach also leads to an analytical method to rapidly calculate power functions for association in arbitrarily large complex pedigrees. These procedures are being added to our general statistical genetic computer package, SOLAR.

## 1674/T/Poster Board #223

**Using imputation of classical HLA alleles to fine map disease association signals within the MHC.** *S. Leslie, L. Moutsianas, A. Dilthey, P. Donnelly, G. McVean.* Department of Statistics, University of Oxford, 1 South Parks Road, Oxford, OX1 3TG, United Kingdom.

Genetic variation at classical HLA alleles is a crucial determinant of transplant success and susceptibility to a large number of infectious and autoimmune diseases. Large-scale studies involving classical type I and type II HLA alleles are currently limited by the cost of allele typing technologies. Recently we developed a statistical method using SNP variation within the MHC region to predict alleles at key class I (HLA-A, HLA-B and HLA-C) and class II (HLA-DRB1, HLA-DQA1, HLA-DQB1) loci. This uses a population genetic approach, combined with a database of individuals with known HLA and SNP alleles within the MHC region, to predict HLA alleles for individuals for which only SNP information is known. The method promises to facilitate large-scale experiments, including disease-association studies and vaccine trials, where detailed information about HLA type is valuable. To date investigation of the method has been limited to small databases (approximately 60 - 240 individuals) and previous studies have shown that increasing the database size results in a significant improvement in the accuracy of the method. We show results for a study using a database of over 2,000 individuals of European heritage with classical HLA typing for both Class I and Class II HLA alleles. We particularly consider the effect of using imputations on the power to detect associations in GWASs and the ability to fine map associations to particular HLA alleles or groups of alleles, and also to combinations across several HLA loci. We apply our method to detecting associations between HLA alleles and disease phenotypes and show results on the efficacy of using HLA allele imputation in such a setting. We also provide software to apply the method to SNP data derived from major SNP typing platforms.

## 1675/T/Poster Board #224

**On the genome-wide analysis of copy number variants in family-based designs: Methods for combining family-based and population based information for testing dichotomous or quantitative traits, or completely ascertained samples.** *A. Murphy[1,2,3], A. Rogers[1,2], J. Chu[1,2], B.A. Raby[1,2,3], C. Lange[2,3,4].* 1) Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Center for Genomic Medicine, Brigham and Women's Hospital, Boston, MA; 4) Department of Biostatistics, Harvard School of Public Health, Boston, MA.

We propose a new approach for genome-wide association testing association between copy number variants (CNVs) and clinical phenotypes in family-based designs. Our new methodology integrates the population level and family level data available in family-based designs into a more powerful association test, which can be used to test either quantitative or dichotomous phenotypes, and if control CNV data are available from a public repository, can also be used in completely ascertained samples. We extend the framework developed by Won et al. (submitted) and Lasky-Su et al. (submitted) for testing association between SNPs and quantitative traits or affection status in completely ascertained samples, respectively, to the CNV probe intensity data, using the FBAT-CNV statistic introduced by Ionita-Laza et al. (2008). Like the methodology for testing SNP associations, our approach increases power of the FBAT statistic, while preserving the robustness of the test against population admixture and stratification. We show, via simulation studies, that our methodology boosts the power of the FBAT statistic to levels comparable to that of population-based designs. Using genotype intensities, we illustrate the practical relevance of our approach in a genome-wide association study of lung function in a population of child asthmatics.

## 1676/T/Poster Board #225

**Evaluation of robust statistical methods for small, real-world pharmacogenetic studies.** *N. Bing, L. Li, L. Budde, G. Kazeem, M. Nelson.* Statistical Genetics, GlaxoSmithKline, Durham, NC.

Pharmacogenetic research conducted during drug development is generally limited to data collected in clinical trials. As a result, sample sizes are often small and continuous endpoints of interest can be highly skewed, which presents several challenges for hypothesis testing. We have evaluated penalized maximum likelihood logistic regression (PMLLR) and permutation testing as robust methods for analysis of binary and continuous endpoints, respectively. Binary endpoints with small sample sizes can result in small or zero cell counts for many genetic variants. Statistical methods based on large sample assumptions, such as traditional logistic regression, are unreliable in this situation. Although Fisher's exact test is appropriate for small sample sizes, it does not allow for covariates. We found that PMLLR provides a robust test of genetic association when covariates are required and compares favorably with Fisher's exact test when analyzing datasets with very small sample size or highly polymorphic variants, such as HLA. Inflation of type I error is an obvious concern when fitting models with the skewed continuous endpoints. Although logarithm transformation and/or censoring are commonly applied in practice, they are often insufficient to remove such inflation, particularly when dealing with small sample sizes. Permutation testing with an ordinary least squares model, where the correlation between endpoint, covariates and genotype is preserved, provides the specific tests of interest and controls the type I error rate. Suitable transformation of the endpoint combined with permutation has greater statistical power than permutation tests using the untransformed endpoint. In general, for genetic association hypothesis testing, we recommend PMLLR for case control analyses, if covariates are needed in the model; and permutation test for quantitative trait analyses, if endpoint is skewed. The applicability of such robust statistical methods has been demonstrated in several of our real-world pharmacogenetic studies with reliable results.

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## 1677/T/Poster Board #226

**Identification of Association between disease and multiple markers within a candidate region via sparse partial least squares regression.** *H. Chun[1], D. Ballard[2], J. Cho[3,4], H. Zhao[1,3].* 1) Department of Epidemiology and Public Health, Yale University, New Haven, CT; 2) Program in Computational Biology and Bioinformatics, Yale University, New Haven, CT; 3) Department of Genetics, Yale University, New Haven, CT, USA; 4) IBD Center, Division of Gastroenterology, Department of Medicine, Yale University, New Haven, CT, USA.

Although genome wide association studies have led to the identifications of hundreds of genes underlying dozens of traits in recent years, most published studies have primarily used single marker-based analysis. Intuitively, more information will be utilized when multiple markers are jointly analyzed. As a result, many methods have been proposed in the literature for association analysis between traits and multiple markers. Among these methods, simulation and real data analyses have shown that it is often more effective to reduce the dimensionality of the markers in a region through principal components analysis of all the markers first, and then perform association analysis between traits and the first several principal components. However, one major limitation of this approach is that the principal components are derived purely from marker genotypes, without consideration of their relevance to traits. Furthermore, these components are constructed as linear combinations of all the markers even when only a limited number are potentially relevant to traits. In this presentation, we propose to use sparse partial least squares regression to derive the components that are linear combinations of only relevant markers. This approach is able to use information from both traits and marker genotypes. Extensive simulation and real data analyses on a Crohn's disease data set suggest the superiority of this approach over all existing methods.

## 1678/T/Poster Board #227

**Effect estimates in a 2-stage design: from genome-wide association to sequencing.** *L. Faye[1 2], S.B. Bull[1 2], L. Sun[1 3].* 1) Dalla Lana School of Public Health, Univ Toronto, Toronto, ON, Canada; 2) Prosserman Centre for Studies of Complex Traits in Human Populations, Samuel Lunenfeld Research Institute, Mount Sinai Hospital; 3) Department of Statistics, University of Toronto, Toronto, ON.

Genome-wide association studies (GWAS) of complex diseases and quantitative traits aim to detect regions of genetic association for further intensive study; however, tag SNPs may not entirely capture the variation at the underlying causal loci. Advancements in sequencing technology have made targeted sequencing in a region of interest a feasible way to localize a potential causal variant. A 2-stage design can limit costs by sequencing (Stage II) a subset of the GWAS (Stage I) samples in region(s) identified via significant tag SNPs. In this work we explore the consequences of 2-stage designs for the bias and variance of the genetic effect estimate (e.g. odds ratio) in both the GWA and sequencing stages. Selection bias in genetic effect estimation arises because the same sample is used for both gene discovery and effect estimation. This phenomenon is also known as the Winner's Curse and its effect in linkage analyses and in case-control association was demonstrated by Goring et al (2001) and Garner (2007), respectively. In the 2-stage design, the correlation (rho) between the tag SNP and causal variant, and the proportion (pi) of Stage I samples used for Stage II sequencing add complexities to the understanding of the Winner's Curse. We quantify the effect of the Winner's curse in the 2-stage design through rho and pi. We develop analytical expressions for the bias and variance of the effect estimates at the tag and causal SNPs, and evaluate them numerically for various 2-stage study designs. Besides genotyping cost, the effect of pi is mainly on the variance of the estimate, and if pi is too low, estimates in stage II may not be reliable due to large variance. When the causal SNP is rare, variance inflation is exacerbated by low minor allele frequency. The effect of rho, however, is less straightforward. In comparison to the genetic effect of a true causal variant, the effect estimate at a tag SNP is attenuated if rho is low (Dudbridge 2008). On one hand, as rho increases, selection at the tag SNP exerts greater influence on the estimate at the causal SNP, increasing bias. On the other hand, power increases as rho increases, which decreases the effect of selection. The balance between these two trends determines the degree of bias at the causal SNP estimate. Understanding the contributions of these factors is informative for proper study design as well as for analysis and interpretation of sequencing data.

## 1679/T/Poster Board #228

**Comparing imputation accuracy using local and HapMap haplotype reference panels in a candidate gene study of iron phenotypes.** *L. Turkovic[1], C.C. Constantine[1], J.B. Carlin[1,2,3], K.J. Allen[2,3], L.C. Gurrin[1].* 1) Centre for MEGA Epidemiology, University of Melbourne, Melbourne, Victoria, Australia; 2) Murdoch Children's Research Institute, Melbourne, Victoria, Australia; 3) Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia.

Imputation of genotypes offers the opportunity to explore disease associations at untyped loci by exploiting the correlation implied by linkage disequilibrium between SNP genotypes observed in an external reference dataset (e.g. HapMap), where only some of these SNPs have been typed in the study samples. A related scenario is the availability of additional genotyping of a sub-sample of the participants (perhaps via re-sequencing), which can then be used as a reference dataset for imputing genotypes for SNPs that were typed on a subset of participants. We examine one such scenario in the HealthIron (HI) study, a sub-study of an Australian cohort of 31192 people of northern European descent. A random sample stratified by HFE was genotyped for 476 SNPs in 44 genes of iron metabolism. Samples from about 20% of participants (n=188) were subjected to further genotyping with re-sequencing of variants in exonic regions of some of these candidate genes. We compared the accuracy of genotype imputation for those participants without re-sequencing data using two different haplotype reference panels: one based on SNPs from HapMap (Phase II) and another using re-sequencing data from the HI study. Imputation was performed by using IMPUTE (Marchini, Nat Gen 07). Imputation accuracy was assessed by leaving out each SNP in turn, imputing genotypes for that SNP and comparing to the observed genotype, then calculating percentage of matches between predicted and observed. Three different reference panels were used: 1) HapMap SNPs 2) HI SNPs restricted to those available in HapMap 3) full HI data. For SNPs in the CYBRD1 gene (ferric reductase), scenarios 1) to 3) used 17, 17 and 26 SNPs respectively to build the imputation model. For SNPs where the probability of a correct call exceeded 0.9, 86%, 92% and 98% of imputed genotypes were called, with concordances of 97%, 99% and 99%. Improved concordance and less uncertainty in genotype calling was apparent for genes with larger number of SNPs. One such example is TF gene (transferrin), using 25, 25 and 43 SNPs respectively and calling threshold of 0.9: 92%, 93% and 94% of imputed genotypes were called and 97%, 99% and 99% of those were correct. Use of a local reference panel out-performed HapMap even when local data were restricted to SNPs appearing in HapMap only. The decreasing cost of sequencing makes establishing a local reference set feasible, and might improve the precision of estimated measures of association.

## 1680/T/Poster Board #229

**Incorporating Covariates into Multipoint Linkage Disequilibrium Mapping in the Case-Parent Design.** *Y.F. Chiu[1], H.Y. Kao[1], K.Y. Liang[2], W.H. Pan[3].* 1) Biostatistics and Bioinformatics, National Hlth Res Inst, Zhunan, Miaoli, Taiwan; 2) Department of Biostatistics, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, USA; 3) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

Most complex diseases are manifested by the joint effects of genes, environmental factors, and their interactions. Incorporating environmental factors and interactions into association mapping can help localize the locus of the disease. The case-parent design, which involves affected offspring and both of their parents, permits testing and estimating offspring- and maternally-mediated genetic effects. As a result, researchers often adopt this design to assess gene-environment interactions, and particularly for early onset diseases with rare disease alleles. The present study develops a robust multipoint fine-mapping approach to incorporate covariates into the association mapping of case-parent designs. Incorporating quantitative or qualitative covariates into this fine mapping through parametric and non-parametric modeling makes it possible to assess or account for main covariate effects and gene-covariate interaction effects while localizing the disease locus. Simulation results indicate that the efficiency in estimating the disease locus increases considerably when incorporating a covariate associated with the disease. This is especially true when the genetic effect of the disease locus is small. The proposed approach was applied to a young-onset hypertension data sample. The relative efficiency of estimating the locus of young-onset hypertension increases 110-fold after incorporating triglyceride (TG) into the association mapping while localizing the disease variant in the lipoprotein lipase (LPL) gene in the non-parametric model. By incorporating the information of a SNP into the fine-mapping, the proposed method further assesses the gen-gene interactions between the SNP and the disease locus using the parametric model. Results suggest that with the incorporation of covariates, the proposed method can not only improve efficiency in estimating disease loci, but also elucidate the etiology of a complex disease.

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## 1681/T/Poster Board #230

**Adjustment for multiple, correlated tests in pathway analysis of sex steroid hormone levels in pre- and postmenopausal women from the Breast and Prostate Cancer Cohort Consortium (BPC3).** *L. Beckmann[1], A. Huesing[1], V.W. Setiawan[2], R. Ziegler[3], S. Hankinson[4], R. Kaaks[1], BPC3 consortium.* 1) Dept Clinical Epidemiology, German Cancer Research Ctr, Heidelberg, Germany; 2) Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles; 3) Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD; 4) Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, and Department of Epidemiology, Harvard School of Public Health, Boston, MA.

Background: In this study, we present a new approach to control the family-wise error rate in association analysis of traits with SNPs in multiple genes within a pathway, which requires careful adjustment for the number of tests as well as the correlation between the SNPs due to linkage disequilibrium (LD).

Data: The approach is evaluated in an analysis of the association of 700 SNPs in 36 genes in the sex steroid pathway in a pooled sample from the European Prospective Investigation into Cancer and Nutrition (EPIC) and the Nurses' Health study (NHS). Levels of the androgens dehydroepiandrosterone (DHEAS), androstenedione (Δ4), and testosterone (TESTO), as well as the estrogens estrone (E1) and estradiol (E2), and sex-hormone binding globulin (SHBG) were measured in blood samples of women who subsequently developed breast cancer and in matched control subjects (n=3,852).

Methods: We performed linear regression on each SNP for four models: recessive (rec), dominant (dom), codominant (cod) and log-additive (add), and defined $p_{min}=\min(p_{rec}, p_{dom}, p_{cod}, p_{add})$, the minimum p-value. For all SNPs within a gene, 1,000 permutations were used to adjust for multiple testing (number of SNPs and the four tests), and correlation due to LD using a step-down-min-p-algorithm. The adjusted p-values were Bonferroni-corrected by the number of genes. The minimal adjusted p-value among the SNPs within a gene was considered as the global p-value of this gene.

Results: Significant signals for association were found for pre- and post-menopausal women between SHBG levels and SNPs in the *SHBG* gene ($p<10^{-3}$). In postmenopausal women, we found globally significant results for association of E1 and E2 with SNPs in the gene encoding aromatase *CYP19* ($p<10^{-3}$), and in the follicle stimulating hormone receptor gene *FSHR* ($p<10^{-3}$). SNPs in the estrogen receptor gene *ESR1* were also found to be associated with estrone ($p<10^{-3}$). For DHEAS, we found significant associations with SNPs in *FSHR* ($p<10^{-3}$), and a SNP in the aldo-keto reductase gene *AKR1C3* ($p<10^{-3}$). Our results confirm previous findings regarding genes *SHBG* and *CYP19* while the associations with *FSHR* and *AKR3C1* provide new insights. The proposed procedure to control the family-wise error rate offers a practical approach to adjust for multiplicity in genetic pathway analyses.

## 1682/T/Poster Board #231

**Detection of foeto-maternal genetic effects: an evaluation of methods.** *M. Bourgey[1], J. Healy[1], M-H. Roy-Gagnon[1,2], D. Sinnett[1,3].* 1) Hemotologie-Oncologie, CHU Sainte-Justine, Montreal, PQ, Canada; 2) Department of Social and Preventive Medicine, Faculty of Medicine, University of Montreal, Montreal, PQ, Canada; 3) Department of Pediatrics, Faculty of Medicine, University of Montreal, Montreal, PQ, Canada.

Multifactorial diseases with early onset likely include complex genetic components involving both the affected individual's inherited genotype and parentally mediated mechanisms. The mother plays a crucial role not only as genetic parent but also as foetal environment. Hence, a maternal genotype may damage a foetus through its effect on the intrauterine milieu regardless of whether the genotype is present in the foetus. Distinguishing between maternal and foetal genetic influences is thus essential in studying the genetic basis of early onset disorders. In this study, we use simulation experiments to evaluate the performance of two approaches to test for foetal and maternal genetic effects: Cordell et al.'s stepwise conditional logistic regression (CLR) approach, which uses triads (affected offspring and their parents), and Weinberg et al.'s log-linear, likelihood-based hybrid design (HD). In addition to triads, the latter includes unrelated control individuals and their parents. Recruiting parents of controls can be very difficult and increases genotyping costs. Thus, we also investigate a log-linear, likelihood-based approach that does not include parents of controls (HD-NPC). We use an ongoing study of an early-onset complex disease (Acute Lymphoblastic Leukemia) to guide our simulations. We investigate the type I error and power of the different approaches to detect the genetic effects of the child, the mother, and the foeto-maternal interaction. Since the main drawback of not having the parents of the controls is the need to assume mating symmetry, we also assess the impact of mating asymmetry on the performance of the different methods. We simulate varying levels of mating asymmetry based on a statistic that we developed to measure mating asymmetry. In practice, when choosing study designs and analytical approaches, investigators need to compromise between accuracy and power and feasibility of the design in terms of sample ascertainment and costs. Our results provide useful information to guide investigators studying foeto-maternal genetic effects.

## 1683/T/Poster Board #232

**SNPs as epidemiological tools in Mendelian Randomization studies of the role of biomarkers in disease.** *T. Frayling[1], J. Perry[1], The. DIAGRAM1 consortium[10], G. Paulisso[2], T. Sparso[3], C. Langenberg[4], H. Grallert[5], C. Palmer[6], A. Morris[6], V. Steinthorsdottir[7], A. Jackson[8], M. Boehnke[8], T. Hansen[3], O. Pedersen[3], T. Illig[5], N. Wareham[4], I. Barroso[9], M. McCarthy[10], L. Ferrucci[11], M. Weedon[1].* 1) Genetics of Complex Traits, Peninsula Medical School, University of Exeter, UK; 2) Department of Geriatric Medicine and Metabolic Diseases, Second University of Naples, Naples, Italy; 3) Institute of Biomedicine, University of Copenhagen, Denmark; 4) Medical Research Council (MRC) Epidemiology Unit, Addenbrooke's Hospital, Cambridge, UK; 5) Helmholtz Zentrum Muenchen. German Research Center for Environmental Health Institute of Epidemiology, Germany; 6) Ninewells Hospital and Medical School, University of Dundee, Scotland, UK; 7) Decode genetics, Iceland; 8) Centre for Statistical Genetics, Ann Arbor, Michigan, USA; 9) Metabolic Disease Group, Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 10) Oxford Centre for Diabetes, Endocrinology and Metabolism, Headington, Oxford, UK; 11) Gerontology Research Center, National Institute on Aging, Baltimore, Maryland, United States of America.

Many circulating biomarkers are associated with metabolic diseases such as type 2 diabetes but it is not known if altered levels of biomarkers causally influence diseases, or are the result of confounding or secondary to disease. Recent GWAS studies have identified common SNPs near the IL18, SHBG and BCMO1 genes that are robustly associated with circulating levels of IL18, SHBG and Beta-Carotene respectively. We aimed to use the principle of Mendelian Randomization to test the hypothesis that gene variants known to alter circulating levels of biomarkers increase the risk of type 2 diabetes, to the extent expected given the correlations between gene variants and biomarker levels and biomarker levels and Type 2 diabetes. We used data from 1200 members of the InCHIANTI study to estimate the correlation between biomarkers and type 2 diabetes. We next used type 2 diabetes case control studies consisting of 4107 cases and 5187 controls from the DIAGRAM consortium, and, where there was initial evidence of association (p<0.01), a total of 20,394 cases and 28,470 controls. A 1 standard deviation (SD) increase in IL18, Beta-Carotene and SHBG levels was associated with type 2 diabetes odds ratios of 1.65 (1.36-2.02), 0.68 (0.56-0.82) and 0.73 (0.59-0.89), respectively. The IL18 (rs2250417), BCMO1 (rs6564851) and SHBG (rs1799941) variants were associated with 0.28, 0.27 and 0.21 per-allele SD increases in IL18, Beta-carotene and SHBG levels respectively. Observed odds ratios for IL18 and BCMO1 variants with type 2 diabetes were 1.00 (95%CI:0.99-1.03), P=0.39) and 0.98 (0.93-1.04) P=0.57) respectively, which were each less strong than the expected effects of 1.15 (1.09-1.21) and 0.90 (0.85 - 0.95). In contrast, using 20,394 cases and 28,470 controls, the observed odds ratio for the SHBG variant and type 2 diabetes was 0.93 (0.91-0.96) P=0.000001, which was consistent with the expected effect. In conclusion, Mendelian randomization studies provide preliminary evidence that raised SHBG levels causally reduce the risk of type 2 diabetes, but that raised levels of Beta-Carotene and IL18 do not. Mendelian randomization represents another way in which GWAS-findings can contribute to improved biological insights of disease.

## 1684/T/Poster Board #233

**Avoiding the high Bonferroni penalty in genome-wide association studies.** *X. Gao[1], L.C. Becker[2], D.M. Becker[2], J.D. Starmer[3], M.A. Province[1].* 1) Div Statistical Genomics, Washington Univ, St Louis, MO; 2) Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 3) Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC.

A major challenge in genome-wide association studies (GWASs) is to derive the multiple testing threshold when hypothesis tests are conducted using a large number of single nucleotide polymorphisms. Permutation tests are considered the gold standard in multiple testing adjustment in genetic association studies. However, it is computationally intensive, especially for GWASs, and can be impractical if a large number of random shuffles are used to ensure accuracy. Many researchers have developed approximation algorithms to relieve the computing burden imposed by permutation. One particularly attractive alternative to permutation is to calculate the effective number of independent tests, Meff, which has been shown to be promising in genetic association studies. In this study, we compare recently developed Meff methods and validate them by the permutation test with 10,000 random shuffles using two real GWAS data sets: an Illumina 1M BeadChip and an Affymetrix GeneChip® Human Mapping 500K Array Set. Our results show that the simpleM method produces the best approximation of the permutation threshold, and it does so in the shortest amount of time. We also show that Meff is indeed valid on a genome-wide scale in these data sets based on statistical theory and significance tests. The significance thresholds derived can provide practical guidelines for other studies using similar population samples and genotyping platforms.

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## 1685/T/Poster Board #234

**Finding Unique Filter Sets in PLATO: Preparation for Efficient Interaction Analysis.** *B. Grady, S. Dudek, M. Ritchie.* CHGR, Vanderbilt Univ, Nashville, TN.

In the current era of genetic research, genome wide association studies (GWAS) are frequently used in an attempt to identify common genetic variants that predispose to risk for disease. Although the genotyping technology used to implement GWAS has progressed rapidly, the analysis methodologies for detecting associations has lagged behind. In particular, the methods to detect interactions between multiple variants in GWAS datasets have not been well developed. It is possible that the estimated heritability of many common diseases could best be explained by interactions between multiple susceptibility loci. The Platform for the Analysis, Translation, and Organization of large-scale data (PLATO) is a filter-based method which brings together many analytical methods in an effort to solve this problem. It allows a user to filter a dataset down to a subset of the original genetic variants before running subsequent methods, which may be especially useful for interaction analysis. In order to streamline PLATO for the efficient analysis of data, we wanted to determine which of 24 analytical filters were producing partially or fully redundant results. Using a kappa score to identify agreement between filters, we were able to group the methods into 4 meta-groups. We then tested the MAX statistic as put forth by Sladek et al. [Sladek et al., Nature 445:881-85 (2007)]. In a simulation study, one filter from each of the four meta-groups were selected and then run on 100 datasets containing effects on the order of an odds ratio of 1.5 as additive, dominant and recessive single-locus effects and a total of 1000 SNPs. They were also run on 1000 datasets containing no effect to determine the Type 1 error rate of the approach. To find the MAX statistic, the four filters were run on each SNP in each dataset and the smallest p-value among the four results was taken. Subsequently, one thousand permutations were performed to build a null distribution to compare the initial result against. In this way, the power of the MAX statistic was determined for detecting each of the three effects. The Type 1 error and false positive rates were also examined. The results show that the PLATO approach with the MAX statistic has higher power to find all types of effects and a lower false positive rate than any of the individual filters alone. In the future we will extend the concept of PLATO with the MAX statistic to interaction analyses.

## 1686/T/Poster Board #235

**How to Select the Covariates for a Propensity Score Approach to Correction for Population Stratification Bias?** *H.-Y. Huang, L.-Y. Wang, W.-C. Lee.* Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan.

The case-control association study has gained in popularity for mapping disease-susceptibility gene(s) of complex human diseases. However, the study is prone to population stratification (PS) bias, which is an issue if both the allele frequencies and the disease prevalences vary across population strata. A genomic propensity score (GPS) approach (Zhao et al, 2009) had been proposed to correct for the PS bias that considered both genetic (null markers) and non-genetic factors (confounders such as age, sex, gender, exposure status of smoking, drinking, and race). In general, the non-genetic factors, say, the environmental exposures, include (a) risk factors, which are associated with the disease under study and (b) null exposures, which have no effect on the disease under study. These environmental exposures can be measured and defined clearly and easily. In this paper, we propose a propensity score approach to correct for PS bias that focuses on the “null exposures”. We compare our method with GPS. Also, the evaluation of the number of null exposures used in the propensity score approach and the characteristics of the null markers are studied in this paper. We conduct simulation studies to evaluate the performance of this method. Our results show that even if the propensity score method considers only a group of null exposures that have no effect on the disease, the PS bias can still be well adjusted for and be consistently corrected. The more null exposures used in the estimation of propensity scores, the greater the bias reduction can be. We also find that in certain situations, adjusting for the risk factors in a genetic association study can increase the magnitude of PS bias instead of decreasing it. In summary, by adjusting for the propensity scores based on the null exposures, our method provides a convenient and robust tool for obtaining almost unbiased estimates of population-based genetic association studies.

## 1687/T/Poster Board #236

**Admixture Mapping Provides Evidence of Association of loci on chromosome 1 with obesity.** *S.J. Kang[1], J.L. Butler[2], B. Tayo[3], G. Lettre[4], C.W.K. Chiang[2], R. Hackett[5], C. Guiducci[5], T. Nguyen[5], T. Feng[1], A. Adeyemo[6], C. Rotimi[6], A. Luke[3], H. Lyon[2], J. Hirschhorn[2,5], R. Cooper[3], X. Zhu[1].* 1) Dept Epidemiology & Biostat, Case Western Reserve Univ, Cleveland, OH, usa; 2) Children’s Hospital Boston and Harvard Medical School, MA, USA; 3) Loyola University Chicago Stritch School of Medicine, Chicago, IL, USA; 4) Montreal Heart Institute, Universite de Montreal, Montreal, QC, Canada; 5) Broad Institute of Harvard and MIT, Cambridge, MA, USA; 6) NIH Intramural Center for Genomics and Health Disparities, Bethesda, MD, USA.

Obesity is a heritable trait and it has been shown that African Ancestry is positively correlated with BMI. To identify genetic variants associated with obesity, we performed admixture mapping analysis with an obesity related trait-BMI using Affymetrix 6.0 platform in unrelated 247 high and 456 low BMI African Americans from Maywood, IL. We first identified 2606 highly ancestry informative SNPs (AIMs) from the SNPs that passed QC. Using the ancestry estimated at each AIM by the software ADMIXPROGRAM, we performed case-only and case-control admixture mapping analyses. We observed two loci with case-control Z-score >3.0 on chromosome 1 (rs12067308, Zcase-control=3.28) and chromosome 2 (rs14230, Zcase-control=3.14). The corresponding local maximum case-only Z-scores on chromosome 1 is 1.89 at rs12067308, and 1.18 at rs14230. We then restricted the analysis on a 18 Mb region based on 1 unit drop of case-control Z-score on chromosome 1 and tested the association of SNPs genotyped on Affymetrix 6.0. Further we only tested the SNPs with allele frequency difference >0.2 between HapMap CEU and YRI data. Among such 1850 SNPs, we observed 92 SNPs with p-value<0.05. We further performed association analysis for the 92 SNPs in 1188 unrelated Nigerian samples. We observed 2 SNPs (rs12065422 and rs782224) with p-values <0.05 and their risk directions are consistent with that in Maywood cohort. SNP rs12065422 was further genotyped in two independent African-American cohorts with sample sizes 756 and 494 respectively. Although not significant in each of the two cohorts, the meta-analysis of combining the 4 cohorts resulted in a p-value of 0.00029 for rs12065422. In summary, we did not identify any regions with very strong admixture signals, and within the region with the best signal, no SNPs were more strongly associated than expected by chance. It remains possible that the SNP with the best evidence for association, rs12065422, could be contributing to an admixture signal, but follow-up studies would be required to determine whether this SNP is associated with obesity.

## 1688/T/Poster Board #237

**On genome-wide association studies in family-based design: An integrative analysis approach combing ascertained family-samples with unselected controls.** *J. Lasky-Su[1,2,3], B. Himes[1,3], S. Weiss[1,2,3], C. Lange[1,2,3,4].* 1) Dept Med, Brigham & Womens Hosp, Boston, MA; 2) Center for Genomic Medicine, Brigham and Women’s Hospital, Boston, MA; 3) Harvard Medical School, Boston, MA; 4) Harvard School of Public Health, Boston, MA.

Large numbers of control individuals with genome-wide genotype data are now commonly available through various public and private databases. These controls individuals are often used in case-control genome-wide association studies (GWAS) to increase the overall statistical power. Often these controls are “unselected” for the disease of interest, meaning that the individuals have the disease of interest at the rate that is prevalent in the population. Although outside controls are commonly being incorporated into case-control GWAS, they have not yet been incorporated into a family-based setting. In this communication, we propose an approach to integrate unselected controls in the genome-wide analysis of an ascertained family-based sample. This analysis approach follows three steps. First, we perform a family-based association test. Second, we use the between family information in conjunction with the genotypes from properly matched controls individuals in a Cochran-Armitage (C-A) trend test. The p-values from the C-A trend test are then calculated by rank-ordering the individual test statistics in order to minimize the effects of population stratification. Third, we generate a combined p-value using the p-values from the first two steps. The proposed method fully utilizes the information in the controls to increase the power of the overall approach, while maintaining the original robustness of the family-based analysis against confounding. In the presence of population substructure, population-based adjustment methods can be incorporated in the approach to further increase the statistical power and limit population stratification. Simulation studies were used to assess the achievable power levels and compare them with other analysis approaches. We found that this analysis approach is substantially better than the standard family-based analysis and achieves power levels that are considerably higher than those of case-control designs with unselected controls. We illustrate the approach by an application to a genome-wide association analysis of asthmatic parent offspring trios and publically available control individuals.

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## 1689/T/Poster Board #238

**A Phylogenetic Approach for Correction of Population Stratification in Genetic Association Studies.** *M. Li[1], L.-S. Wang[2].* 1) Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Department of Pathology and Penn Center for Bioinformatics, University of Pennsylvania School of Medicine, Philadelphia, PA.

The rapid development of genotyping technology and extensive cataloguing of SNPs across the human genome have made genetic association studies the mainstream for gene mapping of complex human diseases. For many diseases, the most practical approach is the population-based design with unrelated individuals. Although having the advantages of easier sample collection and greater power than family-based designs, unrecognized population stratification in the study sample can lead to both false-positive and false-negative findings, and might obscure the true association signals if not appropriately corrected. To solve this problem, we develop a novel method -- PHYLOSTRAT, which corrects for population stratification by using phylogeny constructed from SNP genotypes and principal coordinates from multidimensional scaling analysis. This hybrid approach efficiently captures both discrete and continuous population structures. By extensive simulations and the analysis of a synthetic genome-wide association dataset from the Human Genome Diversity Project, we show that our method outperforms several existing population stratification correction methods, including the genomic control approach and the EIGENSTRAT approach, in terms of controlling type I error rate and requiring fewer number of random SNPs for inference of population structure. Given the flexible nature and the hierarchical characteristic of phylogenetic trees, we anticipate that our method will well capture complex population structures in human populations, and thus provides a useful tool for genetic association studies.

## 1690/T/Poster Board #239

**Search of genes associated to skin test against Leishmania antigens.** *L.C. Pereira, L.M. Garrido, A.P. Fioretti, R.G.M. Ferreira, C.E.M. Kawamata, F.A.B. Santos, L.M.A. Camargo, H. Krieger.* University of São Paulo, São Paulo, Brazil.

Leishmania is a protozoan that may cause a tropical disease endemic at the Amazonian region, affecting about 2,000 individuals every year, with a mortality ratio close to 10%. In Brazil there are two forms of leishmania: cutaneous and visceral, both transmitted by the insect of the subfamily Phlebotominae. The test of Montenegro is an immunological technique of delayed skin hypersensitivity with 86% to 100% of specificity. It is performed by inoculating a small amount of leishmania antigen in the arm and after 48 hours it is possible to observe a papule. This papule is measured and if the papule size is equal or larger than 5mm the individual is taken as positive to leishmania infection. A sample composed of 60 individuals from Monte Negro (10° 15' S, 63° 18' W), located at the Amazonian region in the state of Rondonia was ascertained in order to verify the association of some genetic markers and Montenegro phenotypes leishmania susceptibility. Five Taqman (Applied Biosystems) SNP genotyping probes were chosen based on available literature informative. A case control test, showed that no significant association could be detected with all SNP (ds1084617 (P= 0.050); the same happened with ds1084293 (P=0.893); ds11693991 (P= 0.963); ds1747360 (P=0.714) and ds1659795 (P=0.769)). Although SNP ds1084617 showed a border line $X^2$ value, it should be tested in larger samples in order to clarify its effect (CAPES, CNPQ).

## 1691/T/Poster Board #240

**Evaluating the relationship between imputation error and statistical power in genetic association studies.** *L. Huang[1], C. Wang[1], N.A. Rosenberg[1,2].* 1) Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI; 2) Department of Human Genetics, University of Michigan, Ann Arbor, MI.

Genotype imputation methods have afforded genome-wide association (GWA) studies significantly increased power to detect trait-associated genetic variants in high-density SNP genotype data, especially in populations that are genetically similar to populations in the International HapMap Project. The success of imputation-based GWAs can be partly attributed to the dramatic increase in the number of markers that can be tested for association compared to earlier tag SNP approaches. However, imputed genotypes are seldom known with certainty, and errors in these imputed genotypes - even at low rates - might substantially reduce the statistical power of an imputation-based association test. Using genome-wide SNP data (>500,000 markers) from 29 populations, we determined the relationship between genotype imputation error rates and the sample size inflation required to achieve statistical power at an imputed marker equal to that obtained if genotypes at that marker were known with certainty. For markers with common alleles (minor allele frequency > 0.1), imputation error rates of ~2-5% led to considerable inflation in the required sample size (~15-40%). In some African populations that are genetically distant from the HapMap populations, higher imputation error rates of ~8-10% led to a sample size increase as high as ~50-100%. In general, for markers with a minor allele frequency of 0.3, each 1% increase in the imputation error rate was associated with at least a ~7% increase in the required sample size. Our results will inform the use of imputation both in current SNP-typing studies and in forthcoming sequence-based studies, and they suggest that power and sample size calculations should account for a potentially considerable loss of power from even modest levels of imputation error.

## 1692/T/Poster Board #241

**A Novel Association Test for Analysis of Admixed Populations.** *X. Wang[1], X. Zhu[2], C. Li[3], M. Li[1].* 1) Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA; 2) Department of Epidemiology & Biostatistics, Case Western Reserve University; 3) Department of Biostatistics, Vanderbilt University.

Admixed populations offer a unique opportunity for mapping complex human diseases that have large disease allele frequency differences between the ancestral populations. However, association analysis in such populations is challenging because population stratification caused by local ancestry differences between cases and controls may lead to spurious association. This problem can be exacerbated in genome-wide association studies, which typically involve large numbers of subjects. It is thus urgent to develop statistical methods for association analysis of admixed populations. Here we develop a novel association test that simultaneously utilizes information provided by SNP marker genotypes and ancestry informative markers (AIMs). Our method is based on a retrospective likelihood framework which models the conditional distribution of a test SNP given disease status of a study subject and genotypes of AIMs surrounding the test SNP. The parameters involved in the likelihood include allele frequency and penetrances at the test SNP for each of the ancestral populations. This retrospective likelihood allows us to explicitly model local ancestry differences between study subjects and thus eliminates the effect of population stratification at the test SNP. To estimate local ancestry, we use ANCESTRYMAP. We then test for association by a likelihood ratio test. To evaluate the performance of our method, we conducted simulations and compared with the simple allelic and genotypic association tests, and the multi-dimensional scaling (MDS) approach which adjusts for global ancestry defined by principal components obtained from AIMs. Our results indicate that both our method and the MDS approach yield correct type I error rates when there is population stratification at the test SNP. Since our method adjusts for the local ancestry while the MDS approach considers the global ancestry, we will conduct further investigations to evaluate performance of these two approaches when the local ancestry is different from the global ancestry. We note that compared to other approaches, our method is flexible. With the proposed likelihood framework, we can easily extend our method to the analysis of family data and quantitative traits. We believe that this novel method will provide a useful tool for the analysis of admixed populations.

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## 1693/T/Poster Board #242

**Univariate/Multivariate Genome-Wide Association Scans Using Data from Families and Unrelated Samples.** *L. Zhang[1,2], Y-F. Pei[1,2], J. Li[2], C.J. Papasian[2], H-W. Deng[1,2,3].* 1) The Key Laboratory of Biomedical Information Engineering, Ministry of Education, Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, P. R. China; 2) Basic Medical Sci, UMKC Sch Medicine, Kansas city, MO; 3) Center of System Biomedical Sciences, Shanghai University of Science and Technology, Shanghai 200093, P. R. China.

As genome-wide association studies (GWASes) are becoming more popular, two approaches, among others, could be considered to improve statistical power for identifying genes contributing subtle to moderate effects to human diseases. The first approach is to increase sample size, which could be achieved by combining both unrelated and familial subjects together. The second approach is to jointly analyze multiple correlated traits. In this study, by extending generalized estimating equations (GEEs), we propose a simple approach for performing univariate or multivariate association tests for the combined data of unrelated samples and nuclear families. In particular, we adjust for population stratification by integrating principal component analysis and transmission disequilibrium test strategies. In addition, the proposed method allows for multiple siblings as well as missing parental information. Simulation studies show that the proposed test has improved power compared to two popular methods, EIGENSTRAT and FBAT, by analyzing the combined data, while correcting for population stratification. In addition, joint analysis of bivariate traits has improved power over univariate analysis when pleiotropic effects are present. Application to the Genetic Analysis Workshop 16 (GAW16) data sets attests to the feasibility and applicability of the proposed method.

## 1694/T/Poster Board #243

**Fast Model-Based Estimation of Ancestry in Unrelated Individuals.** *D.H. Alexander[1], J. Novembre[2], K. Lange[3].* 1) Department of Biomathematics, UCLA, Los Angeles, CA; 2) Department of Ecology and Evolutionary Biology, UCLA, Los Angeles, CA; 3) Departments of Biomathematics, Human Genetics, and Statistics, UCLA, Los Angeles, CA.

Population stratification has long been recognized as a potential confounding factor in genetic association studies. Estimated ancestries, derived from multi-locus genotype data, can be used as covariates to correct for population stratification. One popular technique for estimation of ancestry is the model-based approach embodied by the widely-applied program STRUCTURE. Another approach, implemented in the program EIGENSTRAT, relies on principal component analysis rather than model-based estimation and does not directly deliver admixture fractions. eigenstrat has gained in popularity in part due to its remarkable speed in comparison to STRUCTURE. We present a new algorithm and a program, ADMIXTURE, for model-based estimation of ancestry in unrelated individuals. ADMIXTURE adopts the likelihood model embedded in STRUCTURE. However, ADMIXTURE runs considerably faster, solving problems in minutes that take STRUCTURE hours. In many of our experiments we have found that ADMIXTURE is almost as fast as EIGENSTRAT. The runtime improvements of ADMIXTURE rely on a fast block-relaxation scheme using sequential quadratic programming for block updates, coupled with a novel quasi-Newton acceleration of convergence. Our algorithm also runs faster and with greater accuracy than the Expectation-Maximization (EM) algorithm incorporated in the program FRAPPE. Our simulations also show that ADMIXTURE's frequentist estimates of the underlying admixture coefficients and ancestral allele frequencies are as accurate as STRUCTURE's Bayesian estimates. On real world datasets, ADMIXTURE's estimates are directly comparable to those from STRUCTURE and EIGENSTRAT. Taken together, our results show that (a) admixture's computational speed opens up the possibility of using a much larger set of markers in model-based ancestry estimation, and (b) its estimates are suitable for use in correcting for population stratification in association studies.

## 1695/T/Poster Board #244

**A new Family-based Association Test for multiple tightly linked markers.** *Y. Dai, J. Dong, R. Jiang.* Mathematical Science, Michigan Technological University , houghton, MI.

We proposed a new multi-marker test for a family-based association study of a genome-wide scan and candidate region detection. Multi-marker tests usually work better to detect an underlying genetic variant over a genomic region than the single test, especially for the detection of complex diseases, since multi-marker tests consider the joint information over the whole region. In this report, the novel multi-marker family-based association test is based on the genotype scores after wavelet smoothing which can be used to extract the maximum common variation from the multiple tightly linked markers. When multiple markers are correlated because of linkage disequilibrium (LD), we might expect that identification of the common variation would capture more of the genetic signal encoded in this region. The new Family-based Association Test regards the multi-marker genotypes in the specified region as a signal. After wavelet transformation, the signal would be transformed into the time-frequency space which is used to describe the signal in a more efficient way, since wavelet transform naturally deals with noisy signals like Genetic data. After using an empirical Bayesian thresholding with adaptability to the sparse and dense signals based on the spatial ordering of SNPs, the genetic signal over multi-markers could be compressed into the mortified genotype scores after wavelet smoothing. The new test is formed based on the phenotype and the adjusted genotypes to detect the association between the phenotype and these SNPs. Using wavelet smoothing can suppress noise automatically under different LD patterns and enable a significant amplification of the genetic variation, while it keeps the genetic information contained in the spatial ordering of SNPs. The new multi-marker family-based association test can be applied to a wide range for both qualitative and quantitative traits. It would be a potentially powerful method for family-based genetic study of multiple tightly linked markers and can also provide an alterative tool for the detection of underlying causative genetics variants. In the simulation study, we examine the type-I error and compare the power with other FBAT tests under different LD patterns. It has the correct type-I error rate when applied to data with population stratification and is more powerful than single-marker family-based test followed by Bonferroni correction. The proposed test is constantly superior when the parent traits are missing.

## 1696/T/Poster Board #245

**Assessing False Discovery Rate in Large-Scale Family Based Association Testing.** *W. Wang[1], I. Chang[1,2], L. Chuang[3], T. Quertermous[4], C.A. Hsiung[1].* 1) Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Miaoli, Taiwan; 2) National Institute of Cancer Research, National Health Research Institutes, Zhunan, Miaoli, Taiwan; 3) Department of Internal Medicine, National Taiwan University, Taipei, Taiwan; 4) Division of Cardiovascular Medicine, Falk Cardiovascular Research Center, Stanford University, CA, USA.

We present a model-free approach to conditional false discovery rate (cFDR) for a large-scale simultaneous family based association tests (FBATs). When association between a set of markers in a candidate gene and a group of phenotypes is studied by FBATs, we indicate that a *joint* null hypothesis distribution for these FBATs can be obtained by the fundamental statistical method of conditioning on sufficient statistics for the null hypothesis. Based on the *joint* null distribution of the class of FBATs obtained, we use its conditional expected number of tail counts, given the central count, under the null hypothesis to define the cFDR. This conditional approach to false discovery rate takes advantage of the correlation between the FBATs. The usefulness of this approach is illustrated by providing false discovery rates to a genetic study on the association between *PTPN1* and a group of blood pressure related phenotypes, for which several significant findings were reported in testing the null hypothesis that the marker loci and the trait loci are unlinked.

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## 1697/T/Poster Board #246

**Combining next generation sequencing and genotype imputation to identify rare risk variants in common complex disorders.** *S. Zöllner[1,2,3], M. Zawistowski[1,3].* 1) Dept Biostatistics, Univ Michigan, Ann Arbor, MI; 2) Dept Psychiatry, Univ Michigan, Ann Arbor, MI; 3) Center for statistical Genetics, Univ Michigan, Ann Arbor, MI.

Results from genome wide association studies (GWAS) illustrate that common variants are insufficient to explain the heritability of most common traits. Rarer variants may be major contributors to common complex diseases and can now be evaluated using emerging next generation sequencing methods. Typically, statistical power is likely insufficient to evaluate rare causal variants individually; instead genes are assessed for an excess of rare variants among cases. Here, we introduce a novel method for identifying genes where the distribution of rare variants differs between cases and controls. The basic algorithm consists of three steps: First we select all rare polymorphisms and apply bioinformatics tools to infer functionality. As resequencing studies discover large numbers of rare variants, we assign weights to each variant to focus on variation that is likely to be functional. Second, we add the weighted minor allele frequency of all rare polymorphisms independently in cases and controls and thus generate a statistic summarizing the excess of rare variation in each group. Third, we assess significance by permutation, generating one p-value for every gene studied. Using simulated data, we identify the most powerful weighting scheme under several genetic models and show that our method is substantially more powerful than the commonly used private allele method. A major advantage of our method is that it can be applied even when only a subset of the data is sequenced and the rest is genotyped for a dense marker panel, for example, when candidate genes identified in a GWAS are subsequently sequenced in a subset of individuals from that study. By imputation, the rare variants that are detected by resequencing can be evaluated in the entire sample. As most imputation methods perform poorly for rare variants, we optimize an existing method (MACH). Based on this improved rare variant imputation, we show that including imputed genotypes increases the power of our test considerably. Finally, we illustrate how our method can be applied to existing GWAS data, by imputing rare variants genotyped in HAPMAP or the 1000 Genomes samples, and show results from two re-analyzed GWAS.

## 1698/T/Poster Board #247

**Regression multi-marker tests for gene-based genetic association analysis.** *S. Bull[1,2], Y. Yoo[1], L. Sun[2,3].* 1) Samuel Lunenfeld Res Inst, Mount Sinai Hospital, Toronto, ON; 2) Dalla Lana School of Public Health, Univ Toronto, Toronto, ON; 3) Dept of Statistics, Univ Toronto, Toronto, ON, Canada.

Background: Treating a gene as the unit of analysis may reduce the multiple testing burden and capture the effect of susceptibility genes more efficiently. The best analytic approach however depends on the underlying genetic model which is generally unknown; e.g. whether there is a single causal locus or multiple common or rare causal variants within a gene. Regression analysis of multiple SNPs can reveal individual SNP associations as well as provide global evaluation.

Methods: We examine tests based on a weighted linear combination of parameter estimates (LC-B) or test statistics (LC-Z) in multi-SNP regression analysis. The test statistics have central and non-central 1 df chi-square distributions under the null and alternative respectively, and the weights depend on the regression coefficient covariance matrix and SNP correlation. However, because the LC tests lose power if the SNP effects have opposite direction, we propose multi-bin tests (MLC-B and MLC-Z) which formulate the linear combination within each bin constructed by SNPs in close linkage disequilibrium ($r^2$>0.5). We compare these methods with other multi-marker tests: the multi-df generalized Wald test, and the 1 df minimum P test adjusted for multiple testing according to test statistic correlations. Numerical and simulation studies were based on HapMap genotype/haplotype data combined with specification of alternative genetic models involving multiple causal loci for a quantitative trait. We assigned the quantitative trait given the haplotype configuration at the causal loci, assuming a normal trait distribution. Using observed multi-SNP LD , we varied the selection of SNP markers included in the regression and observed the effect of the relationship between tag and causal SNPs. Power was calculated using the joint genotype distribution and the expected value of regression coefficients and covariance matrix. Type I error and power were verified empirically..

Results and Conclusions: The multi-marker LC tests were generally more powerful than the minimum P test in the case of multiple causal loci. Depending on the relationship between causal loci and the tag SNPs used in the analysis, LC test power can exceed Wald test power especially when many SNPs are included in the regression. The multi-bin LC tests were more robust to the problem of opposite SNP effects and generally slightly more powerful than the Wald test with fewer df.

## 1699/T/Poster Board #248

**A generalized family-based association test for dichotomous traits.** *W.M. Chen[1,2], A. Manichaikul[3], S.S. Rich[1].* 1) Center for Pubic Health Genomics, Univ Virginia, Charlottesville, VA; 2) Dept of Public Health Sciences, Division of Biostatistics and Epidemiology, Univ Virginia, Charlottesville, VA; 3) Depart of Biomedical Engineering, Univ Virginia, Charlottesville, VA.

Recent advances in genotyping technology make it possible to utilize large scale association analysis for disease gene mapping. Powerful and robust family-based association methods are crucial for successful gene mapping. We propose a family-based association method, the Generalized Disequilibrium Test (GDT), in which the genotype differences of all phenotypically discordant relative pairs are utilized to assess association within a family. The improvement of GDT over existing methods is three fold: 1) Information beyond first-degree relatives is incorporated efficiently, yielding substantial gains in power compared to existing tests; 2) The GDT statistic is implemented using a robust technique that does not rely on large sample theory, resulting in further power gains, especially at high levels of significance; and 3) Covariates and weights based on family size are incorporated. Advantages of GDT over existing methods are demonstrated by extensive computer simulations and by application to recently published large scale genome-wide linkage data from the Type 1 Diabetes Genetic Consortium (T1DGC). In our simulations, the GDT consistently outperforms other tests (such as PDT and FBAT) for a common disease and frequently outperforms other tests for a rare disease; the power improvement is > 13% in 6 out of 8 extended pedigree scenarios. All of the six strongest associations identified by the GDT have been reported by other studies, while only three or four of these associations can be identified by existing methods (including PDT, FBAT, TDT, 1-TDT, GEE, and MQLS). For the T1D association at gene *UBASH3A*, the GDT resulted in genome-wide significance ($P = 4.3\times10^{-6}$), much stronger than the published significance ($P = 10^{-4}$). The GDT is implemented in freely available software.

## 1700/T/Poster Board #249

**Assessing gene-set enrichment in genome-wide association studies.** *L.S. Chen[1], U. Peters[1,3], C.M. Hutter[1], J.D. Potter[1,3], L. Hsu[1,2].* 1) Public Health Science, Fred Hutchinson Cancer Center, Seattle, WA; 2) Biostatistics, University of Washington, Seattle, WA; 3) Epidemiology, University of Washington, Seattle, WA.

Insight into genetic susceptibilities underlying complex diseases can be enhanced by considering functionally related set of genes simultaneously. We develop an algorithm called "Gene-set Ridge regression in ASsociation Studies (GRASS)", using a novel form of regularized logistic regression which is tailored to gene-set association analysis. Specifically, it selects concerted signals from all of the genes and jointly assesses their associations with disease risk. We show that this algorithm performs well in situations with a large number of predictors compared to sample size. We apply GRASS to a colon cancer genome-wide association study and identify TGF-beta signaling, p53 signaling and the colorectal-cancer pathway as the top statistically significant enriched pathways.

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## 1701/T/Poster Board #250

**Internal consistency of ancestry proportion estimates and genetic background variables.** *J. Divers[1], D.T. Redden[2], A. Amit[2], T.M. Beasley[2], R.J. Carroll[3], C.D. Langefeld[1], D.B. Allison[2].* 1) Dept Biostatistical Sciences, Section on Statistical Genetics and Bioinformatics, Wake Forest University Health Sciences, Winston-Salem, NC 27103; 2) Department of Biostatistics, Section on Statistical Genetics, University of Alabama at Birmingham, Birmingham, Alabama 35216; 3) Department of Statistics, Texas A&M University, College Station, TX 77843.

Population stratification and admixture are concerns in genetic association studies. Failure to adequately control for them in genetic association tests may lead to inflated type I error rates or loss of power. Structured association tests (SAT), where individual ancestry proportion estimates computed using ancestry informative markers (AIMs) or a measure genetic background computed using principal component analysis (PCA) are used as covariates to control for confounding, are widely applied in current genetic association studies. We have showed that the SAT approach can be cast in the general linear model framework, and as such implicitly assumes that the predictors are measured without error. However, independently of the approach chosen to estimate the underlying confounding effect, the resulting estimate should be seen as imperfect measurement of the true underlying confounding factor. Ignoring the measurement error inherent to these estimates may limit the ability to control for the underlying confounding factors. Measurement error correction methods offer a way to overcome this problem but require an a priori estimate of the measurement error variance. We have shown how Cronbach's alpha, a measure of internal consistency typically used for measuring the reliability of items that compose a scale, can be applied to provide an upper bound of this variance. Specifically, we showed that one can compute 22 estimates of individual ancestry proportions, one for each chromosome, and combine them to obtain a global measure of ancestry, and more importantly an estimate of the measurement error variance. We now show how this approach can be extended to account for the fact that chromosomes on the human genome do not have the same length and that longer chromosomes are likely to be more informative than shorter ones since they are likely to host more AIMs. We also show how the variance-covariance matrix for the measurement error component can be estimated when the admixed population result from mating between more than two ancestral populations, or when PCA is used to provide a measure of genetic background as is usually the case for the genome-wide association studies. Finally, we used simulation studies to validate the proposed methods.

## 1702/T/Poster Board #251

**Genome-wide association study identified novel susceptibility loci for Adiponectin in a population-based cohort of African Americans.** *A. Doumatey, A. Adeyemo, J. Zhou, G. Chen, D. Shriner, H. Huang, C. Rotimi.* Center for Research on Genomics and Global Health , NHGRI,National Institutes of Health, Bethesda, MD.

Adiponectin is exclusively produced by adipose tissue and has anti-diabetic and anti-atherosclerotic properties. Circulating adiponectin is decreased in obesity and type 2 diabetes. In vitro studies have reported that adiponectin production is regulated by other factors such as cytokines including tumor factor necrosis alpha (TNF-a), Interleukin-6 and glucocorticoids. In vivo studies of adiponectin regulation and adiponectin related pathways are not only few but lack a global and systematic approach. Thus, the physiological regulation of adiponectin remains unclear. In this study, we used the genome-wide association (GWA) strategy to systematically find gene network that may be associated with circulating adiponectin in a population-based cohort of African Americans enrolled from the Washington, DC metropolitan areas. We used the Affymetrix 6.0 fixed SNPs array to genotype a total of 898 unrelated African Americans. After quality filters, the analysis focuses on experimentally determined 806,047 autosomal SNPs. Plasma adiponectin was measured in all genotyped individuals using an immunoassay method (ELISA) from R&D systems. All the analyses were controlled for age, sex, BMI and ancestry proportion. We found significant associations with plasma adiponectin and multiple genetic variants including SNPs rs1982453 in the contactin-5 (CNTN5) gene, p=8.9x10-13, rs12279202 in importin-7 (IPO7) gene, p=3.01x10-10; rs11659639 near the Melanocortin 4 receptor (MC4R) gene, p=4.24x10-8; rs2238776 in the T-box 1 (TBX1) gene p=8.4x10-9; and rs13201744 near F13A1 gene=1.65x10-8; In addition, we observed significant associations with a cluster of 5 SNPs (rs16838686, rs6728303, rs7575199, rs12617342, rs16838663) in the THSD7B gene with p-values ranging from 4x10-6-8.7x10-7. In summary, we observed significant associations in two genes (IPO7, MC4R) that have been previously linked to obesity in multiple human studies. Also, we observed several novel associations that conceptually fall in pathways that are consistent with our knowledge of adiponectin functions and regulation pathways.

## 1703/T/Poster Board #252

**DNA-pooling enables cost-efficient genome wide association studies.** *M.A. Earp, M. Rahmani, A.R. Brooks-Wilson.* Genome Sciences Centre, University of British Columbia, Vancouver, British Columbia, Canada.

Genome-wide association studies (GWAS) using high-density microarrays are being used to map loci contributing to complex traits in human populations. The cost of individually genotyping all of the samples necessary for these studies limits the use of this approach. DNA pooling can be used in the GWAS design to dramatically reduce genotyping cost. Individual samples are physically pooled to create a composite DNA sample that is run on the genotyping array of choice. For each loci on the array, a pool allele frequency is calculated. The allele frequency difference between case and control DNA pools is used to nominate a small subset of loci for individual genotyping and association analysis. We have carried out a simulation study to determine how to design a maximally powered DNA-pool-based GWAS given practical limitations such as available samples and cost. The CaTS power calculator was used to calculate power values for individual and pool-based GWAS given different sample sizes and replicate arrays (Skol et al, 2006). For the pool-based GWAS simulations, the effective sample size, relative to the individual genotyping design, was calculated based on an empirical estimate of the error associated with pooling (MacGregor et al, 2008). In this simulation, given 600 samples per case and control, 4 arrays extracted 76% of the available information from the samples, with a 150-fold reduction in the number of arrays. The minimum genotype relative risk (GRR) detectable at 80% power was 1.65 for individual genotyping and 1.73 for 4 replicate arrays on a single DNA pool. A similar pool-based-GWAS using 4 arrays on 1000 cases/controls would have 94% power to detect a GRR = 1.65, and at 80% power to detect a GRR of 1.47. An inverse exponential relationship exists between the number of replicate arrays used and the information extracted from the available samples. As sample size decreases, fewer replicate arrays are needed to extract a given percentage of information from a pooled DNA sample. For example, 2, 13, and 35 replicate arrays would be needed to extract 90% of the information from the 200, 600, and 1000 cases and controls. In conclusion, by increasing sample size, DNA-pool-based GWA studies can remain highly powered but with dramatic reductions in cost (up to 100 fold). For 600-2000 cases and controls, increasing the number of replicate arrays beyond 15 does not yield significant gains in power. A higher density array could be considered to increase power.

## 1704/T/Poster Board #253

**Prioritizing "Multi-hit" Genes for Replication in GWAS for Specific Language Impairment (SLI).** *P.D. Evans[1], E. Gamazon[1], K. Kelsey[2], J.B. Tomblin[2], D.L. Nicolae[1], N.J. Cox[1].* 1) Genetic Medicine, The University of Chicago, Chicago, IL; 2) Communication Sciences and Disorders, Iowa City, IA.

The SCAN database (SNP and Copy number ANnotation; http://www.scandb.org) provides physical and functional annotation for SNPs and CNVs from public databases, pairwise and multilocus linkage disequilibrium (LD), as well as an indication of whether a SNP is a significant predictor of transcript levels for one or more genes (assessed in LCLs from CEU and YRI trios using the Affymetrix Exon 1.0 ST Array). Our research shows that SNPs reproducibly associated with complex human traits are also more likely to predict transcript levels for one or more genes. Thus, we have developed a "multi-hit tool" for use with the SCAN database that utilizes the physical, LD and transcript expression level information. We first annotated each SNP to a set of genes based on physical location and LD. A gene has physical/LD annotation contains the given SNP or contains a variant that is in LD with that SNP ($r2 > 0.5$); thus, a SNP may have none or several genes in its physical/LD annotation set. A gene has expression annotation to a SNP if the SNP is a predictor of transcript levels for that gene (at a user-specified p-value threshold). The multi-hit tool identifies all genes that are implicated by at least two independent SNPs ($r2 < 0.3$) in the input SNP list. We applied the multi-hit tool to results from a GWAS on specific language impairment, a highly heritable disorder characterized by deficits in language development in a child despite normal overall intelligence. Multi-hit gene lists were then created for 2 quantitative and 1 dichotomous phenotypes using associated SNPs with a p-value of 10-4 or better and a threshold for expression of 10-4. We assessed the significance of the resulting multi-hit gene lists by tallying the length of the multi-hit gene lists created with the multi-hit tool from 1,000 SNP sets created randomly from SNPs with the same minor allele frequency (within .05 bins) and included on the Affymetrix 6.0 platform used for the actual SLI GWAS. All 3 of the SLI phenotypes had significantly more genes multiply implicated than would be expected given the number of input SNPs and their allele frequency distribution. Among the genes most strongly implicated were: AGXT2L2, which has been implicated in autism, ATOH8, which has been shown to have a role in CNS development, KLF16, which plays a role in dopamine regulation, and NAB2, which plays a role in myelination.

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## 1705/T/Poster Board #254

**Comprehensive haplotype association tests for genome wide data of myopia in Chinese schoolchildren.** *Q. Fan[1], Y-J. Li[2,3], LK. Goh[4], M. Seielstad[5], TL. Young[2,4], SM. Saw[1,6].* 1) Department of Epidemiology and Public Health, National University of Singapore, Singapore, Singapore; 2) Center for Human Genetics, Duke University Medical Center, Durham, NC; 3) Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC; 4) Duke University-National University of Singapore Graduate Medical School, Singapore; 5) Genome Institute of Singapore, Singapore; 6) Singapore Eye Research Institute, Singapore National Eye Center, Singapore.

Context: The Singapore Cohort study Of the Risk factors for Myopia (SCORM) is a 10-year longitudinal study of refractive error change in school children aged 7 to 9 years. A total of 1116 DNA samples from SCORM Chinese participants were genotyped using the Illumina HumanHap 550 and 550 Duo Beadarrays. Genome wide single locus association analyses were completed. Objective: The goal of current study was to evaluate the haplotype association results using haplotype association score approaches and two haplotype sharing analytical tests. Methods: In this study, multiple haplotype approaches were performed on chromosomal regions where top four SNPs derived from single locus association tests. Multiple refractive error phenotypes were evaluated, such as spherical equivalent, myopia (SE < -0.50 D), and high-grade myopia (SE < -6.00D). We analyzed all SNPs for each chromosome, as well as tag SNPs. Haplotype tests were conducted for sliding windows of various sizes (2-5 markers) and step size of one SNP for each chromosome. Suggestive evidence of association was declared if the p-value from the haplotypes global test is <10e-5 and smaller than any single-locus p values. Univariate p-values of significant regions were examined for all makers. The analysis were carried out using PLINK and software R. The tag SNPs were extracted from whole genotyped SNPs by using Haploview. Results: Genotypes of 929 subjects with right eye SE data from the 4th annual study visit were analyzed (mean SE=-2.03D, SD=2.27). We identified 11,982 (chr 11) and 9299 (chr 13) tag SNPs. For chromosome 11, 7 haplotype regions on chr 11 were identified. The smallest haplotype global p value is 3.7x10e-15, which mapped across two gene regions. A cluster of significant haplotypes mapped to MYP 7 with a global p value of 9.1x10e-6 and two significant haplotypes (CAA and CAG). In addition, one tagSNP within this haplotype also showed single locus association (p= 1.0x10e-5). For chromosome 13, there were three regions with smaller global p values < 10e-10. A significant 3 tag SNP cluster was noted in a gene region involved in regulation of cell proliferation and cell motility and this association is mainly attributable to the haplotype GAG. Conclusions: These results demonstrate that haplotype association can identify myopia susceptibility loci, which were not necessarily discovered by GWAS single-locus approach.

## 1706/T/Poster Board #255

**Two-locus Analysis for Genome-wide Association Studies.** *S. Fang, Q. Sha.* Mathematical Sciences Dept, Michigan Technological Univers, Houghton, MI.

A genome-wide association (GWA) study is an approach that involves scanning markers across the whole genome to find genetic variations that contribute to a particular disease. The completion of the Human Genome Project in 2003 and the International HapMap Project in 2005 made it possible to find the genetic contributions to diseases. Considerable successes have been reported using this strategy. On the other hand, joint effects of genes play an important role in GWA studies, especially when detecting genes with moderate marginal effect but strong joint effect with other genes. However, to evaluate the joint effects requires heavy computing in GWA studies; and many multiple-marker tests have lower powers due to large degrees of freedom. In this article, we propose a novel two-stage approach, which is promising to analyze GWA studies, to address these problems. In the first stage, all single nucleotide polymorphisms (SNPs) are ranked by a single-marker test which can assess the association of a single SNP and the disease. Then we use a SNP clustering algorithm to group the highly correlated SNPs within a certain physical distance and select a SNP with the smallest p-value as a representative in each cluster. In the second stage, we test the two-locus joint effects based on the representatives with the highest ranking. We use a likelihood ratio test to test each two-locus joint effects under two-locus monotonic models. Comparing with a regular two-stage method, the proposed method not only does reduce the computational burden, it also increases the power by reducing the number of tests. We perform simulation studies to investigate the power and type I error under different scenarios. The results show that our two-stage approach is more powerful and efficient than a single-marker method and a regular two-stage analysis based on the genotypic test.

## 1707/T/Poster Board #256

**A Natural Aggregation Function for Pathway/Network-Based Approaches to GWAS and Gene Expression Analysis.** *D. Heckerman[1], C. Kadie[1], X. Zhang[1], J. Schymick[2], J. Ravits[3], B. Traynor[2], J. Listgarten[1].* 1) Microsoft Res, Los Angeles, CA; 2) Nat. Inst. on Aging, NIH, Bethesda, MD; 3) Benaroya Res. Inst., Seattle WA.

Early genome-wide association studies (GWAS) focused on the association between one or a small number of SNPs and a phenotype. Such studies were likely to miss associations where a large number of SNPs have a mild influence on the phenotype. Thus, following the approach of gene-set enrichment analyses (GSEA) where similar considerations apply, researchers have begun to look for associations based on sets of SNPs corresponding to pre-defined or learned gene sets. A necessary component of such approaches is an aggregation function that quantifies the relationship between a set of SNPs and the phenotype. We present a novel class of functions that naturally capture this relationship and can also be applied to GSEA. The central idea is to quantify the relationship between a set of SNPs and a phenotype by how well those SNPs predict the phenotype. Given SNP and phenotype data from a collection of individuals, we train a probabilistic classification/regression model that predicts the phenotype based on SNPs using data from some of the individuals, and apply this trained model on the remaining data, yielding a probability distribution over the phenotype for each individual. Finally, we test for associations between aspects of this distribution and the actual phenotype observations using standard methods to obtain a p-value for the SNP set. For example, when the phenotype is binary, we can test for an association between the probability of having the phenotype and actually having the phenotype. When the phenotype is continuous, we can test for an association between the mean of the predictive distribution and the outcome. The approach can also be applied to GSEA, where gene expression rather than SNPs are used to predict experimental conditions (or phenotype). We have applied this approach to GWAS with binary phenotype variables using data from KEGG to define SNP sets, 50/50 train/test splits, L1-regularized (lasso) logistic regression for the classification model, and the Mann-Whitney test for association. The approach yields uniformly distributed p-values on null data (obtained by permuting phenotype on real data). When applied to a GWAS data set from the US for the disease ALS, three pathways are significant at the 0.05 level after Bonferroni correction: neuroactive ligand-receptor, complement and coagulation cascades, and axon guidance. On an independent data set from Italy, these pathways show support with p-values of 0.005, 0.02, and 0.0002, respectively.

## 1708/T/Poster Board #257

**Using public control genotype data to increase power and decrease cost of case-control genetic association studies.** *L.A. Ho[1], E.M. Lange[1,2].* 1) Dept. of Biostatistics, Univ North Carolina, Chapel Hill, NC; 2) Dept. of Genetics, Univ North Carolina, Chapel Hill, NC.

Genome-wide association (GWA) studies are a powerful approach for identifying novel genetic risk factors associated with human disease. A GWA study typically requires the inclusion of thousands of samples to have sufficient statistical power to detect single nucleotide polymorphisms (SNPs) that are associated with only modest increases in risk of disease given the heavy burden of a multiple test correction that is necessary to maintain valid statistical tests. The financial costs of performing a GWA study remains prohibitive for many scientific investigators anxious to perform such a study using their own samples. A number of cost remedies have been suggested, including the utilization of two-stage genotyping designs and, most recently, free genotype data on publicly available controls. Herein, we compare the statistical power of association studies using cases and screened controls to studies that are based only on, or additionally include, free public control genotype data. We describe a two-stage replication-based study design, which uses free public control genotype data in the first stage, composed of the complete set of SNPs, and follow-up genotype data on study controls in the second stage, composed of a reduced set of SNPs that demonstrate evidence for association in the first stage. In our two-stage design, the division of cases between the first and second stages is chosen to optimize power. We assess the impact of systematic ancestry differences between study cases and public controls and batch genotype effects, resulting from genotyping cases and public controls at different times and on different platforms, on our proposed two-stage design. We show that the proposed two-stage replication-based design can dramatically increase statistical power and decrease cost of large-scale genetic association studies, over association studies that do not utilize public control data, while preserving many of the advantages inherent when using an epidemiologically matched set of controls. We note that the proposed two-stage is particularly advantageous when the number of study controls is limited.

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## 1709/T/Poster Board #258

**A Fundamentally Different Approach to the Analysis of Genome-Wide Association Studies.** *W.D. Jones, J.S. Parker.* Statistics and Bioinformatics, Expression Analysis, Inc., Durham, NC.

Genome-wide association studies (GWAS) are population studies used to determine genomic regions significantly associated with disease susceptibility or other phenotypic traits. The GWAS analysis process is often laborious and is made unnecessarily difficult by the implicit requirement that all SNPs be genotyped as accurately as possible, often resulting in inaccuracies under various conditions (e.g., in the presence of copy number variants, low MAF, sample processing batch effects). We have developed a method that performs rigorous statistical association of SNPs with binary or continuous outcomes without the genotype call requirement, enabling more SNPs and more samples within a study to be analyzed, thus improving power. The method utilizes an analysis of the matrix decomposition of allelic intensity measurements, coupled with the identification and removal of latent but unwanted data "dimensions" that originate from hidden subpopulation structure and technical artifacts. In summary, by keeping the individual study measurements in their original form (i.e., in the intensity continuum), we are able to more robustly handle latent unwanted data structures within the GWAS dataset. Using this method, we will demonstrate the ability to simultaneously analyze associations of outcomes with SNP polymorphisms and copy number, whereas prior to this method, these analyses were typically performed separately. We will further demonstrate the utility of this method using publicly available GWAS datasets from CGEMS and WTCCC, where we confirm many of the same SNPs found significant in the initial published analyses (Wellcome-Trust Case-Control Consortium, *Nature*, 2007, Yeager et al., *Nature Genetics* 2007, Hunter et al., *Nature Genetics* 2007) while simultaneously finding new SNP associations, including copy number associations.

## 1710/T/Poster Board #259

**Using Maximal Segmental Score to Estimate Region-specific P-values in Genome-wide Association Studies.** *Y.C. Lin[1,2], C.S.J. Fann[3], I.B. Lian[4], C.J. Chang[5].* 1) Institute of Biomedical Informatics, National Yang-Ming University; 2) Bioinformatics Program, Taiwan International Graduate Program, Academia Sinica, Taipei 115, Taiwan; 3) Institute of Biomedical Sciences, Academia Sinica, Taipei 115, Taiwan; 4) Department of Mathematics, National Changhua University of Education, Changhua 500, Taiwan; 5) Graduate Institute of Clinical Medical Sciences, Chang-Gung University, Taoyuan 333, Taiwan.

Background: Genome-wide association testing has become a powerful tool for identifying susceptibility genes underlying common complex diseases. The advent of genotyping technology has greatly increased marker density and the number of association tests performed. Multiple-test correction methods such as Bonferroni and FDR controlling method (FDRc) tend to be conservative and yield low power and various approaches had been proposed to overcome the problem. Aim: We proposed to use the method of maximal segmental score (MSS) to estimate region-specific p-value to locate genomic segments most likely harbor the disease gene via a simulation study. We compared MSS with other tests, eg. longest significance run (LSR) that also consider multiple markers simultaneously. Method: We considered a sequence of p-values obtained by marker-wise tests, eg. family- or population-based association. At the first step, the p-values were transformed into a sequence of scores according to a specific scoring system. Second, MSS was applied to scan the sequence of scores to identify the region with the highest cumulative score, ie. "maximal excursion", and the tail probability was calculated. The segment with a significant high score would suggest the existence of putative disease genes within the region. Simulation: We simulated SNP genotype data of 100 and 200 markers for 1000 trio families with allele frequencies 0.075, 0.1 and 0.15. The intermarker distance was generated from a left-truncated normal distribution with mean 6 kb and standard deviation 10.7kb. The location of the disease gene was assigned to be within the two central markers and the disease haplotype was comprised by 10 markers clustering around the disease gene. An additive disease model was assumed with a 10% penetrance and relative risks 1.5, 1.8 and 2. For comparison, four different MSS scoring systems were considered in transforming the test results. Result: Under the simulation scenario of 100 markers with allele frequency 0.075 and relative risk 1.8, the power was 0.79, 0.37 and 0.065 and false discovery rate was 0.02, 0.02 and 0.28 for MSS, LSR and Bonferroni, respectively. Results for other scenarios showed similar trend. Conclusion: The proposed MSS method has better power and lower false discovery rate comparing to region-c (e.g. LSR) and locus-specific tests (e.g. Bonferroni test, FDRc). The proposed MSS method provides an efficient exploratory tool for the analysis of dense genetic markers.

## 1711/T/Poster Board #260

**Multiple Component Linear Mixed Models to Correct for both Population Structure and Expression Heterogeneity.** *J. Listgarten, C. Kadie, D. Heckerman.* eScience, Microsoft Res, Los Angeles, CA.

The presence of hidden structure is known to be a significant confounding factor in association studies, leading to spurious signal and loss of power when not properly accounted for. Because of the large scale of genome-wide association studies, these factors can often be learned from the data itself and then corrected for, using, for example, Principle-Components based approaches or probabilistic, generative models such as mixed models. Such methods have shown large success in a variety of experimental set-ups. On the one hand, some studies have used these approaches to account for hidden population structure such as race, or family and/or cryptic relatedness, where this structure is defined in the space of SNP data. On the other hand, some studies have used these approaches to account for "expression heterogeneity", that is, hidden structure defined in the space of microarray expression data and arising from batch effects, sample preparation, and environmental factors, for example. In eQTL studies, where one is looking for association between SNPs and expression phenotypes, both types of hidden structure can be present and thus both need to be accounted for. We propose a novel linear mixed model approach that uses two variance components to account for these two confounding factors: (1) an expression-based component, and (2) a SNP-based component. We show that on data containing both of these kinds of structure that our model performs better in terms of calibration of the test statistic (using quantile-quantile plots) and in terms of power of detection (using ROC curves), than models which tackle only one of these two confounding factors. Additionally, because two variance components are used beyond a residual variance component, SVD-based efficiency tricks useful for a single-component model can no longer be applied, and so we have devised alternative methods for efficiently computing the mixed models over large sets of hypotheses.

## 1712/T/Poster Board #261

**Shared controls for genome-wide association studies.** *S. Mukherjee[1,2], O. Kilpivaara[3], J. Simon[5], R. Kurtz[4], S. Olson[5], R.L. Levine[3,4], R.J. Klein[2].* 1) Gerstner Sloan-Kettering Graduate School of Biomedical Sciences, New York, NY; 2) Cancer Biology and Genetics Program, Memorial Sloan-Kettering Cancer Cancer Center , New York, NY; 3) Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Cancer Center , New York, NY; 4) Department of Medicine, Memorial Sloan-Kettering Cancer Cancer Center , New York, NY; 5) Dept of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Cancer Center , New York, NY.

Genome wide association (GWA) studies have been advocated as the method of choice for identifying genetic variants associated with complex diseases. In a classical GWA study design, cases and controls are matched for ethnicity, age, sex, socio-economic background and other environmental factors. Recently, the concept of shared controls for GWA study such as the blood donor cohort in the Wellcome Trust Case Control Consortium (WTCCC), has been shown to be a possible approach. We are interested in investigating the idea of using controls from published GWA studies as shared controls for our datasets. There is a critical need to develop methodologies to match cases with shared controls. Our objective is to determine power using shared controls and to develop an algorithm to allow the use of shared controls in GWA studies. In our investigation, we used controls from WTCCC as shared controls for a study of myeloproliferative neoplasms (MPN). In that study 217 cases were genotyped on the Affymetrix platform. Similarly, 2200 Cancer Genetic Markers of Susceptibility (CGEMS) study controls were used as shared controls for a study of pancreatic cancer. In that study, we had initially genotyped 174 cases and 177 controls on the Illumina platform. After performing quality control and removing ambiguous SNPs (A/T or G/C), a common set of SNPs was used to combine data from different sources. To determine potential population stratification biases that could be introduced by the shared controls, we carried out principal component analysis(PCA) using EIGENSTRAT. Analytical power calculation and simulation studies were performed. The result demonstrates the presence of population substructure in both studies. For MPN GWA study, 113 cases and 2959 control individuals who cluster on the first two principle components were selected for association test. Four SNPs with P-values $<10^{-7}$ including a JAK2 SNP rs10974944 were significantly associated with MPN risk after correcting for residual population stratification and multiple testing. The validation was done in an additional MPN cases to confirm our findings. We did not identify any significant marker associated with pancreatic cancer in the present study. In summary, a JAK2 SNP associated with MPN was identified using shared controls. Shared controls is a promising approach to increase the power of a study. Thus a systematic method to use shared controls will substantially lower time and cost of GWA studies.

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## 1713/T/Poster Board #262

**Estimation of the Null Distribution of Fisher's Exact Test P-values in GWA Studies.** *Y. Okada[1,2], K. Yamamoto[2], R. Yamada[1,3].* 1) Functional Genomics, Human Genome Center, Institute of Medical Science, the University of Tokyo; 2) Department of Allergy and Rheumatology, Graduate School of Medicine, the University of Tokyo; 3) Center for Genomic Medicine, Kyoto University.

The assessment of variable inflation of test statistics is an essential process to avoid the spurious associations when a large number of null hypothesis are tested, as in the case of genome wide association (GWA) studies. Variable inflation can be quantified by comparing the observed distribution of test statistics with their expected distribution under the null hypothesis without the inflation, such as genomic control method for $\chi^2$ test statistics. Because $\chi^2$ test is inaccurate and the use of exact test is recommended, when the contingency tables have low expected values, it is useful to correct variable inflation of exact test statistics (p-values). Although the null distribution of $\chi^2$ test statistics is arithmetically given, it is not the case for exact test p-values. Therefore we planned to estimate the null distribution of exact test p-values for a set of tables to be tested as a preparation step to correct variable inflation of them, as below.

For each table in the table set, we calculated the discrete probability density function (p.d.f.) of p-values that the marginal counts of the table could give, and averaged the discrete p.d.f.s of all the tables in the table set, which was our estimated null distribution of exact test p-values for the the table set. We generated simulation case-control genotype data of SNP markers, in a range of variable inflation (Wright's Fst=0~0.001). For each genotype data, we calculated Fisher's exact test p-values of the allelic 2x2 tables of the SNPs, and then estimated the null distribution of Fisher's exact test p-values.

Observed distributions of Fisher's exact test p-values indicated higher degree of variable inflation, as the value of Fst increased. On the other hand, estimated null distributions of Fisher's exact test p-values did not indicate significant differences among Fst. When no variable inflation was induced (Fst=0), observed distributions of Fisher's exact test p-values followed their estimated null distributions.

Our method estimated the null distribution of Fisher's exact test p-values without variable inflation, from the sets of the contingency tables that included the variable inflation.

## 1714/T/Poster Board #263

**Utilizing known risk genes in GWA case control studies.** *M. Ostensson[1], A. Torinsson Naluai[2], S. Nilsson[1].* 1) Mathematical Statistics, Chalmers University of Technology, Gothenburg, Sweden; 2) Clinical Genetics, Gothenburg University, Gothenburg, Sweden.

When searching for genetic variants in common diseases we typically need to take the strongest environmental risk factors into account in order to increase the statistical power to detect the much weaker genetic effects that are present. For the same reason known genetic risk factors of importance can be utilized to increase the power of detecting weaker genetic effects. As an illustration of this we have reanalyzed the GWA from van Heel et al in 2007 for coeliac disease (CD). In CD some alleles at the HLA class II genes DQA and DQB are more or less mandatory. Together they form the molecules DQ2 or DQ8. While 95% av all CD patients carry these molecules, they are also present in about 25% of the population, so most carriers are healthy. From this observation it becomes clear that a case control study of CD in practice have three groups. Patients, HLA-negative controls (lacking both DQ2 or DQ8) and HLA-positive controls (carrying DQ2 or DQ8). For the frequencies of non-HLA alleles associated with CD we would typically find $p_1>p_2>p_3$ where $p_1$, $p_2$ and $p_3$ are frequencies in patients, HLA-negative controls and HLA-positive controls and where $p_2$ would be the population frequency. The most common statistic when searching for associated SNPs is based on a chisquare test in a 2x2 contigency table of of allele counts. Implicitly this test use the hypotheses $H_0$:$p_1=p_2=p_3$, while $H_1$ is $p_1{}^1p_2=p_3$. Due to the previous reasoning we suggest considering another hypotehsis $H_{1a}$:$p_1 > p_2 > p_3$ or $p_1 < p_2 < p_3$ which can be tested by isotonic regression or rather monotonic regression since we need a two-sided alternative. Another option would be to use an alternative like $H_{1b}$:($p_1 > p_2 > p_3$ or $p_1 < p_2 < p_3$) and $p_2=(p_1+2p_3)/3$ indicating that we assume HLA-negatives to be closer to HLA-postives than to patients. The optimal mixture will of course be model dependent. This can be tested by Cochran-Armitage trend test. Using some of the SNPs that were later confirmed in the extended study by Hunt et al in 2008 we compared the ranks and p-values for markers run and found e.g. rs11938795 and r21738074 moving from ranks 88 and 503 to 26 and 16 respectively, when isotonic regression was used. When a trend test was used the same SNPs changed their significance from $1.7x10^{-4}$ and $1.2x10^{-3}$ to $2.6x10^{-5}$ and $6.0x10^{-5}$ respectively.

## 1715/T/Poster Board #264

**Whole Genome Association Analysis of Height Growth Curves Using Curve-Based Multivariate Distance Matrix Regression.** *R.M. Salem[1], E.N. Smith[2], S.S. Murray[2], M. Shaw[2], S. Srinivasan[3], W. Chen[3], G.S. Berenson[3], E.J. Topol[2], D.T. O'Connor[1], N.J. Schork[2].* 1) Univ California, San Diego, La Jolla, CA; 2) Scripps Genomic Medicine, TSRI, La Jolla, CA; 3) Tulane University, New Orleans, LA.

Height is a classical polygenic trait that has long been of interest to genetics researchers. Adult height is a highly heritable trait that follows a regular growth profile: rapid increase in the first four years, followed by a linear phase and an S-shaped curve from onset of adolescence into early adulthood. Several recent genome wide association (GWA) studies have focused on adult height. These studies identified associations with several Single Nucleotide Polymorphisms (SNPs) many of which have been replicated. However, the proportion of the variability explained by these SNPs is small, with modest effects on adult height. A significant limitation of these studies is that they only assessed adult height (state) and hence ignore the developmental trajectory that leads to adult height. It is arguable that ignoring the 'dynamic' nature of height from birth to adulthood will compromise the understanding of the biological effects of DNA sequence variations. We conducted a GWA study of height that exploits dissimilarity among the longitudinal height profiles of individuals with different genotypes. We introduce a novel non-parametric statistical framework to model and analyze the entire height growth profile, which we term 'Curve-Based Multivariate Distance Matrix Regression' (CMDMR). The proposed framework takes advantage of the subject specific height trajectory, which can be modeled with one of several nonlinear parametric and nonparametric models. This framework is applied to height growth data from the Bogalusa Heart Study. We also compared our GWA study results with those obtained with traditional statistical analyses approaches. We identified 7 novel variants in 6 loci (FAM19A1, FGF20, SCD5, MAP3K7, GLCCI1 and TJP2) associated with height profiles using parametric curves (all p-values <1e-6). Additionally, we assessed n=146 previously reported adult height GWAS SNPs, and replicated associations in the BMP2, CHCHD7-RDHE2, DLEU7, EFEMP1, GNA12, HHIP, Histone class 2A, HMGA2, LCORL, PDGFRA, PITX1, PXMP3- ZFHX4, SOCS2, SPIN1-CCRK, and ZBTB38 loci. The CMDMR approach is flexible and provides insights into the dynamic nature of complex traits.

## 1716/T/Poster Board #265

**Assessment of the use of genetic principal components for combining genetic association studies over ethnicity.** *S.A. Santorico.* Department of Mathematical and Statistical Sciences, University of Colorado, Denver, CO 80217, USA.

With our shared population history, it is expected that some genetic causes of disease will be common to multiple ethnic groups. Given differences in environmental background and exposures, we would also expect some effects to differ in presence as well as magnitude. To date, studies of the genetics of complex disease have typically been conducted separately by ethnicity. This has been done so as to avoid detection of genes that appear to be associated with disease but that rather reflect differences in disease prevalence and differences in the frequency of gene mutations in the ethnic groups. By keeping studies separate, false findings are minimized; however, the power to find genetic effects is lessened compared to being able to use a combined sample over all studies. With the availability of dense SNP genotyping, e.g, 100,000 to 500,000 SNPs genotyped over the genome, there is now the potential to adjust for this confounding. A recent method by Price et al (2006) proposes the use of principal components analysis on the full set of genotypes to construct independent axes of genetic variation. The information from these axes, also called genetic principal components, can be used to correct for differences in a candidate marker's variation across the ancestral populations. In an application to genetic data collected across Europe, Novembre et al (2008) were able to show how the variation over the axes of genetic variation reflected the actual geography over Europe. In particular, the first axis reflected a northwest gradient over Europe whereas the second axis reflected a southwest gradient over Europe. That is, an individual with high values on both axes would be reflective of a person of Russian origin whereas an individual with low values on both axes would be reflective of Spanish or Portuguese heritage.

While these results provide validation that the method is reflecting geographic, and hence to a fair extent ancestral history, the method has not been adequately assessed in its application to genetic studies of association. Here, the method of Price et al (2006) will be assessed by using simulations based on HapMap data. Does correction for ancestry by way of genetic principal components protects against false positive genetic associations? Is there power to detect common genetic associations and power to detect a genetic effect that differs over ethnic groups? These results will provide guidelines for making the most of available data.

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## 1717/T/Poster Board #266

**On the optimal ranking of SNPs from genome-wide case-control scans.** *I. Tachmazidou[1], M. De Iorio[2], F. Dudbridge[1].* 1) Medical Research Council, Biostatistics Unit, Cambridge, United Kingdom; 2) Department of Epidemiology and Public Health, Imperial College, London, United Kingdom.

With the increase in the number of genetic association studies carried out in the hope of discovering single nucleotide polymorphisms (SNPs) involved in the etiology of complex diseases, there is a pressing need for methods that optimally measure statistical significance. We propose to extend the optimal discovery procedure (ODP) of Storey (2007) for statistical significance testing in genome-wide association studies (GWAS). Our aim is to optimally rank the tests according to their significance, so as to uncover as many true positive signals as possible without incurring too many false positives. The advantage of ODP compared to single significance tests is that when testing each SNP it borrows strength across tests by using information from all SNPs typed in the study. In particular, the ODP for a specific SNP involves considering the alternative and null distribution not only of that SNP, but also of the entire dataset for that SNP. In this way, independent SNPs with similar effect sizes will reinforce each other, and therefore tests that capture common signal structure in the dataset are enhanced.

Storey (2007) has shown that the ODP is an optimal multiple-testing procedure in the sense that it maximizes the expected number of true positive results at each fixed level of expected number of false positive results. However, the exact ODP cannot be known in practice, so we propose several estimators based on both prospective and retrospective likelihoods. We wish to examine how much accuracy is lost when using the estimated ODP. Through simulation studies, we investigate the performance of the estimated ODP, p-values from univariate logistic regression and asymptotic Bayes factors (BFs) (Wakefield 2008). Our results indicate that, whereas the exact ODP is never outperformed, the estimated ODP offers advantages over p-values and BFs only in extreme simulation scenarios. However, in realistic scenarios the estimated ODP yields a similar number of true positives compared to p-values and Bfs, although the rankings returned by the various methods were not identical. Therefore, in practice, we expect the ODP to perform similarly to univariate logistic regression and BFs when testing multiple hypotheses, and no method exhibits a clear advantage in power.

## 1718/T/Poster Board #267

**A HMM-based optimal Multiple Testing procedure for GWAS.** *Z. Wei[1], W. Sun[2], K. Wang[3], H. Hakonarson[3,4].* 1) Department of Computer Science, New Jersey Institute of Technology, Newark, NJ; 2) Department of Statistics, North Carolina State University, Raleigh, NC; 3) Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA; 4) Division of Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA.

Genome wide association studies (GWASs) search for correlation between a phenotypic trait, often a disease, and common genetic variants across an entire genome in an unbiased manner. Such studies promise to identify genetic variants with small effects. In a GWAS, it is typical to test hundreds of thousands of markers simultaneously and to then apply Bonferroni correction to control for family-wise error rate. However, Bonferroni correction is overly conservative, often excluding weak valid associations. It has therefore recently been proposed to control the false discovery rate (FDR) by the Benjamini and Hochberg procedure (BH). It has been shown that BH can effectively control the FDR in case-control association studies, improving sensitivity compared to Bonferroni correction. However, with both approaches, as dependency between markers increases the sensitivity of the study decreases. To improve the efficiency of a GWAS, it would be desirable to control the FDR by employing a precise model to capture the dependencies among markers. We propose to use a Hidden Markov Model (HMM) to capture the dependencies among SNPs. We have developed an optimal multiple testing procedure (PLIS) that employs an HMM. We prove analytically that PLIS controls the FDR at the nominal level and is optimal in the sense that it has the best sensitivity among all valid FDR procedures. Simulation results demonstrate that PLIS improves on conventional FDR procedures in detecting disease associated SNPs. We apply PLIS to a GWAS data set generated on the Illumina genotyping platform for 1,008 Type 1 diabetes (T1D) cases and 1,000 controls. At FDR level 0.0001, PLIS identifies a total of 1,841 SNPs, including 1,217 SNPs from the HLA region, as disease associated. These include 7 of the 15 T1D loci confirmed in a recent meta-analysis, while BH identifies only 3 of the 15 at the same FDR level. With respect to negative control, PLIS does not claim as disease-associated two loci that failed to replicate in follow-up studies, namely the gene for collagen type 1 a2 (COL1A2; rs10255021) and rs672797, in the vicinity of latrophilin 2 (LPHN2). By contrast, the GWAS claims significant associations for these loci after Bonferroni correction. In conclusion, the signal-to-noise ratio in a GWAS can be greatly enhanced by incorporating the dependency structure in a multiple testing procedure. Our proposed PLIS procedure suggests a promising direction for large-scale GWASs.

## 1719/T/Poster Board #268

**A universal, robust genome-wide association studies in family-based designs and an application to four genome-wide association studies.** *S. Won, C. Lange.* Harvard School of Public Health, Boston, MA.

For genome-wide association studies in family-based designs, we propose a new, universally applicable approach. The new test statistic exploits all available information about the association, while, by virtue of its design, it maintains the same robustness against population admixture as traditional family-based approaches that are based exclusively on the within-family information. The approach is suitable for the analysis of almost any trait type, e.g. binary, continuous, time-to-onset, multivariate, etc, and combinations of those. We use simulation studies to verify all theoretically derived properties of the approach, estimate its power and compare it with other standard approaches. We illustrate the practical implications of the new analysis method by an application to a lung-function phenotype, forced expiratory volume in one second (FEV1) in 4 genome-wide association studies.

## 1720/T/Poster Board #269

**Identify less frequent variants with large effect sizes.** *C. Xing.* McDermott Ctr, Univ Texas SW Medical Ctr, Dallas, TX.

The genome-wide association study (GWAS) becomes the prevalent study design in human genetic mapping. However, its success so far is constrained to identifying common variants with modest effect sizes, which explain a small fraction of heritability of complex traits. Conventional methods to identify association in GWAS are to prioritize signals purely by p-values and select those meeting the genome-wide significance level, and thus they favor detecting common variants. Here we attempt to improve the power of GWAS by detecting association of low frequent variants with effect sizes comparable to or even larger than that of common variants. We first investigated the allelic distribution of SNPs from the Affymetrix Genome-Wide Human SNP Array 6.0 in the Atherosclerosis Risk in Communities (ARIC) Study population, which implied that a considerable amount of information on less frequent variants lies in the commercial arrays. Further, we conducted a genome-wide screen for plasma fasting glucose levels using a false discovery rate control procedure accounting for allele frequency, and another genome-wide screen for stature using a regularized statistic in the European American (EA) population of the ARIC study. Both screens identified associations for less frequent variants that were missed by conventional methods. The associations were also positive in the African American (AA) population of the ARIC study, and we replicated the finding in both EA and AA populations of the Dallas Heart Study. This study reveals that there is a cache of less frequent variants in the commercial arrays, and this group of variants can be valuable if proper analytical approaches are used in GWAS.

## 1721/T/Poster Board #270

**Statistical aspects of shared controls in genome-wide association studies.** *D. Zaykin.* Dept Biostatistics, NIEHS/NIH, Res Triangle Park, NC.

I investigate the issue of correlation between results of GWAS due to usage of shared controls, a design that was employed by the 2007 Wellcome Trust Case Control Consortium study. For the commonly used trend test statistic, I find that the correlation between chi-square statistics for two diseases depends on the ratio of the control to the case sample sizes, but not on the allele frequency. I use this result to obtain chances of observing a small association p-value for a disease, given that a small P-value for another disease was observed. Further, I derive a P-value correction that accounts for correlation caused by the usage of shared controls. The method is surprisingly precise in approximating and correcting the actual distribution of P-values obtained with a case-control test. This approach appears to be useful for study designs that share common controls, especially regarding diseases that may imply common, genetically mediated etiology.

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## 1722/T/Poster Board #271

**A nonparametric analysis for multiple cases and shared control study.** *X. Zhan, J. Kang, X. Xia.* Biostatistics Dept, University of Michigan, Ann Arbor, MI.

In multiple disease case control genome-wide association (GWA) studies, it is common to sample shared control group and different case groups from each disease. In such a study design, classical methods are applicable for separately analyzing each disease. However, when jointly analyzing more than one disease, these methods might produce biased results, because the underlying mechanisms of multiple diseases are usually correlated. In this paper, we develop a novel p-value function based nonparametric method to detect the strongest association signals in the whole genome for multiple diseases. Also, we propose a new multiple comparisons for p-value functions to control the overall Type-I error taking the advantages of compound symmetric correlation structure of test statistics. Simulation studies show that our method reduces bias and has better performance than analyzing diseases separately. For Welcome Trust Case Control Consortium (WTCCC) study where 3000 shared controls and 7 common diseases with 1000 cases for each disease, our method lowers statistical threshold of the GWA study and thus it has potential to detect other signals while not falsely ignoring known genetic signal.

## 1723/T/Poster Board #272

**A comparison of single and multi-SNP methods to summarize genetic variation at candidate loci.** *A.E. Hendricks[1], R.H. Myers[2], K.L. Lunetta[1].* 1) Department of Biostatistics, Boston University School of Public Health, Boston, MA; 2) Department of Neurology, Boston University School of Medicine, Boston, MA.

Recently, Genome Wide Association Studies have provided an abundance of data. Despite the wealth of genetic information, most of the genetic heritability for complex diseases is still not explained. Willer et al. (2009) found that the eight most highly associated genetic loci explain only 0.84% of the variation in BMI despite estimates indicating that 40-70% of the variation in BMI is due to genetic factors. Thus, to explain the remaining genetic heritability many researchers are moving beyond single marker analysis to more complex analyses that model the relationship between the disease and two or more genes such as gene-gene interaction, gene network analysis, and gene-set enrichment. A shared obstacle for these methods is determining how to represent or summarize the genetic information within each gene or region that is used in the analysis. Common techniques include using the SNP with the lowest p-value, haplotype(s), or principal components (PC) analysis. Here we also present a simple joint summary method that uses stepwise selection to select the jointly significant SNPs within a gene region and then create a per-individual summary measure by summing the number of risk alleles per SNP weighted by the SNP's regression estimate from the joint model. We use these four methods to summarize the gene region SNCA located on chromosome 4q22.1, which has been implicated in Parkinson's Disease (PD), and evaluate their significance using a permutation procedure. Using a sample of 1783 PD cases and controls in the SNCA gene region, we find the top SNP (rs356229) near the 3' end of SNCA. We also find a 3 SNP haplotype spread out throughout the gene region, and after performing stepwise selection, we retain 9 SNPs in the joint model. For the PC analysis we choose the third PC, PC3, as the PC summary measure as it is most significantly related to cases status. We find some overlap in the SNPs chosen for each summary method. Most notably, the top single SNP appears in the 3 SNP haplotype as well as in the 9 SNP joint model and is in the top 10 most highly weighted SNPs in PC3. Thus, not surprisingly, we see moderate pair-wise correlation between the four summary methods (range: 0.38-0.69) each of which is highly significant (all p-values < 2.2e-16). In addition, we compare the type-I-error and power of each method using simulated data that retain the LD structure of the SNCA region.

## 1724/T/Poster Board #273

**The SEESEQ method: Semiparametric Estimating Equations for SEQuence data.** *D.D. Kinnamon, E.R. Martin.* Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL.

**Purpose:** Association analysis with sequence data is challenging due to the large number of both common and rare variants and the high rate of missing genotypes. We have developed a statistical method that improves on variant-by-variant analysis by combining information on numerous sequence variants into a small set of parameters without knowledge of allelic function.

**Methods and Results:** We assumed variant genotypes (minor allele heterozygotes or homozygotes) are present at a rate in the population conditional on local sequence position scaled to [0,1] and affection status. The natural log of this rate can be related to position by a linear model representing a complex nonlinear function with a small number of parameters. By fully interacting model terms with affection status, case-control differences in the population variant distributions can be estimated as differences in log variant rates and a joint Wald $\chi^2$ test constructed. We derived estimating equations for these model parameters and determined their theoretical properties in the presence of linkage disequilibrium (LD) and missing genotypes. To verify our approach, a population of 10,000 sequences of 10,000 base pairs with LD was generated under a standard coalescent model. Under a null disease model, 1,000 1:1 case-control samples with 10% missing genotypes were drawn with replacement from this population and SEESEQ applied to each.

If the log variant rate model is correctly specified, parameter estimates from SEESEQ are consistent and asymptotically normal with a closed-form covariance matrix, even with LD and missing data. In samples of 200 or 500 total subjects, the mean parameter estimate from the constant log variant rate model nearly equaled the log of the population mean variant rate. Mean estimates of case-control differences in constant, linear, and 3-knot natural cubic spline log variant rate models were all near zero, and the corresponding Wald $\chi^2$ tests had actual Type I errors close to the nominal level for $\alpha = 0.01$ and 0.05.

**Conclusion:** SEESEQ yields consistent estimates and controls Type I error, even with LD and missing data. Because it does not require multiple testing corrections or knowledge of allelic function to determine how to combine variants, it is expected to provide a powerful alternative to existing approaches for rare and common variant analysis.

## 1725/T/Poster Board #274

**Bayesian multivariate phenotype modeling for genome-wide association studies.** *J. Marchini[1], J. Pereira-Gale[1], M. McCarthy[2].* 1) Dept Statistics, Oxford Univ, Oxford, United Kingdom; 2) Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM), Churchill Hospital, Old Road Headington, Oxford, OX3 7LJ, UK.

The majority of genome-wide association studies have been carried out using a single binary or single quantitative trait as the phenotype of interest. For many diseases several potential phenotypes may be available so it is natural to ask the question of how best to test for association in the presence of multiple phenotypes. We consider the case where there are two phenotypes : one case-control phenotype and one quantitative phenotype. We have developed a joint probit and linear regression model for this situation that explicitly models the residual error between the two phenotypes. We work in a Bayesian framework and use a flexible prior structure on the association parameters and correlation parameter. Currently we use a computationally efficient Importance Sampling scheme to estimate the marginal likelihood under both a null and alternative model in order to calculate a Bayes Factor for association and posterior estimates of the parameters of the model. On simulated data, across a variety of different combinations of effect sizes for both traits and various levels of residual correlation we observe a boost in power up to 10% over a marginal analysis of each trait or when combining the marginal analysis in simple ways. One interesting observation is that for fixed levels of association with each trait the power of the joint analysis increases as the residual correlation becomes more negative. We have also applied the method to real data at two SNPs (rs1121980 and rs9939609) at the FTO locus in a total of 3314 samples with Type 2 Diabetes (T2D) disease status and Body Mass Index (BMI) as the case-control and quantitative phenotype respectively. Marginal analysis of the T2D and BMI phenotypes produces $\log_{10}$ Bayes Factors of 2.60 and 5.22 at rs1121980 and 2.97 and 5.13 at rs9939609 respectively. Our joint model produces $\log_{10}$ Bayes Factors of 5.99 and 6.09 at the two SNPs. The prior structure used on the association parameters was the same for the marginal and joint models and we used a uniform prior on the residual correlation between the traits. This analysis further acts to illustrate that a joint analysis of distinct but related phenotypes can provide a boost in the signal of association. We have also investigated the use of the Bayesian Linear Model for multiple quantitative phenotypes and compared to marginal analysis methods we find similar boosts in power.

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## 1726/T/Poster Board #275

**A unified framework for testing multiple phenotypes for association with genetic variants.** *M. Stephens.* Statistics & Human Genetics, Univ Chicago, Chicago, IL.

In many ongoing genome-wide association studies, multiple related phenotypes are available for testing for association with genetic variants. In most cases, however, these related phenotypes are analysed independently from one another. For example, several studies have measured multiple lipid-related phenotypes, such as LDL-cholestrol, HDL-cholestrol, and Triglycerides, but in most cases the primary analysis has been a simple univariate scan for each phenotype. This type of univariate analysis fails to make full use of potentially rich phenotypic data. While this observation is in some sense obvious, much less obvious is the right way to go about examining associations with multiple phenotypes. Common existing approaches include the use of methods such as MANOVA, canonical correlations, or Principal Components Analysis, to identify linear combinations of outcome that are associated with genetic variants. However, if such methods give a significant result, these associations are not always easy to interpret. Indeed the usual approach to explaining observed multivariate associations is to revert to univariate tests, which seems far from ideal. In this work we outline an approach to dealing with multiple phenotypes based on Bayesian multivariate regression. The method attempts to identify which subset of phenotypes is associated with a given genotype. In this way it incorporates the null model (no phenotypes associated with genotype); the simple univariate alternative (only one phenotype associated with genotype) and the general alternative (all phenotypes associated with genotype) into a single unified framework. In particular our approach both tests for and explains multivariate associations within a single model, avoiding the need to resort to univariate tests when explaining and interpreting significant multivariate findings. It also has potential application to the joint analysis of ``intermediate" phenotypes with clinical endpoints, and for the analysis of change (eg drug response) phenotypes. We illustrate the approach on examples, and show how, when combined with multiple phenotype data, the method can improve both power and interpretation of association analyses.

## 1727/T/Poster Board #276

**Entropy based marker selection and Mantel statistics in candidate gene analysis of oxidative stress related mechanisms and breast cancer risk in the MARIE study.** *A. Schulz[1], P. Seibold[1], R. Hein[1], D. Flesch-Janys[2], C. Fischer[3], L. Beckmann[1], J. Chang-Claude[1].* 1) Cancer Epidemiology, German Cancer Research Centre (DKFZ), Heidelberg, Germany; 2) Department of Medical Biometry and Epidemiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 3) Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany.

Haplotype analysis is a well established option for testing association in candidate genes. Often, when haplotypes are estimated statistically, an algorithm based on expectation maximisation (EM) is used. There is, however, no gold standard to decide which markers to jointly include in the EM haplotype estimation when the marker set is too large to estimate haplotypes for all markers at once. We combined an entropy based marker selection algorithm (EMS) with Mantel Statistics using haplotype sharing, a test for marker-phenotype association. Preliminary results show that this approach can improve the identification of causal loci in simulated candidate gene scenarios. Here, we apply the new approach to a real data set of a breast cancer case-control study, investigating genes in oxidative stress related mechanisms. The used data consist of 1628 postmenopausal breast cancer cases and 1947 controls matched by year of birth and study region from the German population-based case-control study MARIE. 131 single nucleotide polymorphisms (SNPs) in 20 candidate genes were analyzed. The SNPs were mainly tagging SNPs ($r^2$>0.8) in genes involved in oxidative stress related mechanisms. In the first stage of the new statistical approach, markers in one gene are screened by an entropy-based criterion of multilocus linkage disequilibrium and selected if they increase the linkage disequilibrium. Haplotypes are then estimated locally for the selected markers. In the second stage, haplotype sharing analysis is applied to the resulting haplotype distribution. This approach is compared to pointwise conditional logistic regression (LR). All test statistics are adjusted for potential breast cancer risk factors. LR yielded ten nominally significant variants (p<0.05), mostly in genes coding for members of the thioredoxin system and in the gene for metallothionein 2A (*MT2A*). The EMS Mantel statistics yielded significant results for four variants, two in *TXN2* and two in *MT2A*, corresponding to the four most significant variants of the LR. The most significant one, rs2281082 in *TXN2* for LR , was inversely associated with breast cancer risk ($p_{trend}$=0.002). The odds ratio compared to the wildtype was 0.79 (95% CI: 0.68-0.92) for the heterozygotes, and 0.78 (95% CI: 0.52-1.17) for the common homozygotes. Using EMS Mantel statistics, the most significant was rs1580833 in *MT2A* (p=0.0007).

## 1728/T/Poster Board #277

**Haplotype construction in admixed populations.** *H. Xu, V. George.* Dept Biostatistics, Med Col Georgia, Augusta, GA.

Haplotype-based analysis is an important approach in human genetic studies, especially in the studies of complex diseases. Haplotypes for human can be determined experimentally, which are however, costly and time-consuming. Statistical construction of haplotype from multi-locus genotype data has been shown to be an effectively approach. Current methods do not take account of population structure. Our previous research shows that population structure could affect the performance of these methods. In this study, we propose new methods for haplotype construction in the presence of population structure. One is based on the maximum-likelihood and the other is an Bayesian approach. Simulation results show that the new methods have increased accuracy compared to the current methods ignoring population structure. This could have important implications in haplotype analysis using admixed populations or samples from metropolitan populations where extend of population structure is expected to be high.

## 1729/T/Poster Board #278

**Unraveling HLA Disease Associations from Genome Scan Data.** *J. Farrell.* Genetics Program L320, Boston University Medical, Boston, MA.

Recent genome-wide association studies (GWAS) have been very successful in finding single nucleotide polymorphisms (SNPs) in the Major Histocompatibility Complex (MHC) which are significantly associated with many diseases. These GWA studies detected significant MHC locus associations with auto-immune diseases, infectious diseases, inflammatory diseases, adverse drug reactions and lung cancer. However, the extensive linkage disequilibrium in the region has often made it very difficult to unravel the HLA risk alleles driving many of these significant disease signals. I have developed a novel method to predict the HLA alleles from SNP data to ascertain their role in these MHC-related diseases. This approach precisely predicts 4-digit HLA types for HLA-A, HLA-B, HLA-C, HLA-DRB1 and HLA-DQB1 alleles from SNPs typed in genome wide association studies. The overall precision and true positive rate for the HLA predictions are greater than 96% among the 2000 test cases used for validation. The actual and predicted HLA allele frequencies are highly correlated ($R^2$=1.0). It is now feasible to comprehensively and efficiently examine the role of common HLA alleles in genome scans showing disease association in the MHC region. The underlying 4-digit HLA type associated with many MHC-related diseases can now be unraveled without the expensive and laborious HLA typing of subjects in large-scale genome wide association studies. Using the predicted HLA genotypes, the utility of this approach is demonstrated with the re-analysis of 6 GWA studies for the following MHC-related diseases: ankylosing spondylitis, autoimmune thyroid disease, multiple sclerosis, psoriasis, rheumatoid arthritis and lung cancer.

## 1730/T/Poster Board #279

**A novel method for the joint detection of multiple risk SNPs.** *I. Hallgrímsdóttir[1], A. Albrechtsen[2, 3], T. Korneliussen[3], J. Witte[1, 4], R. Nielsen[5].* 1) Biostatistics & Epidemiology, UCSF, San Francisco, CA; 2) Department of Biostatistics, Copenhagen University, Copenhagen, Denmark; 3) Department of Biology, Copenhagen Universtiy, Copenhagen, Denmark; 4) Institute for Human Genetics, UCSF, San Francisco, California; 5) Department of Integrative Biology and Department of Statistics, UC Berkeley, Berkeley, California.

Genome-wide association studies (GWAS) have identified numerous loci associated with complex traits. However, the cumulative effects of these loci are much smaller than would be expected based on estimates of heritability for all complex traits studied to date, and it is clear that the current approach of performing single SNP tests in GWAS has only identified a small subset of the causative loci. Possible reasons for this include allelic and locus heterogeneity. To identify risk loci under such models we need to consider the combined effects of multiple SNPs. We have developed a new statistical model for joint detection of multiple risk SNPs. We define sets of SNPs, preferrably chosen so that all SNPs in a set are within the same gene, or within genes in the same pathway. For each set we test the null hypothesis that none of the SNPs are associated with the trait, against the alternative that some subset of them are associated with the trait. We work within a mixed model framework. The method's strength lies in testing in a statistically efficient manner; for each set of SNPs we perform only one test, by summing over all possible subsets of risk SNPs. We first consider quantitative trait models and show in a simulation study that the method has high power over a range of different genetic models. For models with 5 or more risk SNPs, each with a small marginal effect our method has >80% power even for a modest sample size of 500 individuals, while a single locus method has very low power. We compare our method to other existing methods and discuss an extension to dicotomous phenotypes.

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## 1731/T/Poster Board #280

**Inferring genotypes using resequencing data for fine mapping disease loci.** *J. Maller[1,2], G. McVean[1,2], P. Donnelly[1,2], 1000 Genomes Project, Wellcome Trust Case Control Consortium.* 1) Department of Statistics, University of Oxford, Oxford, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom.

INFERRING GENOTYPES USING RESEQUENCING DATA FOR FINE MAPPING DISEASE LOCI Julian B. Maller, Gilean McVean, Peter Donnelly, 1000 Genomes Project, Wellcome Trust Case Control Consortium Department of Statistics, University of Oxford, Oxford, UK Wellcome Trust Centre for Human Genetics, Oxford, UK Fine scale association mapping is a primary and increasingly common strategy for following up a whole genome association scan. The new generation of sequencing technologies has enabled the cost-effective deep-resequencing of 10s to 100s of samples, which provides a more complete catalogue of variation in regions of interest prior to fine mapping. Additionally, the 1000 Genomes Project will soon release genome-wide resequencing data for 100s of samples. If this sort of sequence data could be used to accurately infer genotypes at both known and novel SNPs in an original disease-study sample, it has the potential to greatly improve fine mapping efficiency. We attempted to assess both the accuracy of phasing and imputing from resequencing data, as well as the effectiveness of using such imputation to improve fine mapping efficiency. This analysis was performed using fine mapping data across 13 loci, regional resequencing data across 5 loci, and the 1000 Genomes Project pilot data. In the context of fine mapping, a critical metric is how results of association analysis of the imputed data compare to results from real genotypes. Using a Bayesian framework, and given certain assumptions, one can assign each SNP a relative weight of evidence within each fine mapping region. For each of the fine mapping regions, we took the smallest set of SNPs corresponding to 95% of the regional weight of evidence, and looked at what proportion of the regional weight those SNPs correspond to in the imputed results. Results from imputation using our resequencing on average placed 67% of the weight on SNPs from the 95% set from the real data, and imputing using 1000 Genomes data placed 86% on average on those SNPs. While the imputed data varied in accuracy, for common (MAF ≥ .05) SNPs it was sufficiently accurate to confidently exclude SNPs from fine mapping, define subsets of SNPs on which to focus efforts, as well as localise association signal and refine the region of interest.

## 1732/T/Poster Board #281

**Bayesian modelling categorical anti-C1q data in UK SLE families.** *S. Hunnangkul.* Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK.

Systemic lupus erythematosus (SLE) is an autoimmune disorder disease, influenced by genetic and environmental factors.To date the aetiology of this disease is still unclear.Circulating immune complexes are possibly causing organ dysfunction.Kidney disease and SLE are both heritable, thus it is possible that anti-C1q levels was heritable. Up to now, the heritability of anti-C1q has not previously been described in any publications. A total of 3117 anti-C1q measurements data in 829 SLE families with at least one affected offspring in SLE were used in this project, with SLE defined according to the American Rheumatism Association(ARA)revised in 1982. As the anti-C1q data are non-normal and data transformation did not help much to solve the problem of excess zeros and non-normality. The non-normal anti-C1q response can be categorised into 3 groups with 2 cut-points and 4 groups with 3 cut-points using median and the quantiles(p25,p50,p75) as the cut-off values respectively. We propose the proportional odds model with ordered categorical response of anti-C1q in order to understand the relationship of anti-C1q between parents and siblings as well as heritability estimates of anti-C1q. A Bayesian approach with variance component analysis using WinBUGS is used to modelling with family effects. The heritability of anti-C1q is computed with different priors in variance components such as Gamma distribution Ga(2,2) and Half-normal distribution N(I,0.10)(0,), N(I,0.25)(0,). Comparing the proportional odds models between different cut-points; it is obvious that the results from the 2 cut-point models were similar to the 3 cut-point models. In terms of precision, the use of 4 ordered response categories with 3 cut-points fit the model better than the use of 3 ordered response categories with 2 cut-points as the results illustrated small standard errors and small variation in 95% credibility interval. It means the higher number of order response categories, the better model is fit. The 3 cut-point model with the prior Gamma distribution Ga(2,2) and half-normal distribution HN(0.25) provided more accurate results with small variation in heritability estimates. The heritability estimates from both 2 cut-point and 3 cut-point models with different priors suggested 18%-22% of variation in anti-C1q. Heritability estimates were based on the latent variable that we generated anti-C1q with different categories.

## 1733/T/Poster Board #282

**Fine mapping of multiple causal MHC variants using shrinkage regression.** *C. Vignal[1,2], A. Bansal[1], D. Balding[1].* 1) Imperial College, UK; 2) GlaxoSmithKline, UK.

Rheumatoid arthritis (RA) has often been associated with the MHC, most notably with a group of alleles termed the shared epitope (SE) at *HLA-DRB1*. Additional MHC loci may play a role but inference is hampered by the extent of linkage disequilibrium (LD) in the region. The aim of the current study is to identify non-*DRB1* risk loci using a penalised multivariate approach and assess the robustness of the models derived.

Genotype imputation, incorporating HapMap subjects (CEU parents), was used as a means to jointly analyse SNPs from the Wellcome Trust Case Control Consortium (WTCCC) and the GoRA study, a case-control study for RA. The combined GoRA-WTCCC dataset comprised 6575 subjects and about 6600 part-imputed SNPs from the MHC, plus *HLA-DRB1*. We applied a Bayesian-inspired logistic regression approach to all markers jointly, to select SNPs while adjusting for the effect of *HLA-DRB1*. The latter is often modelled simply using SE-status, but we present results to show that co-adjusting for an additive effect of the 0101 allele improves fit. Parameter inference was based on the posterior mode, with non-zero values indicating marker-disease associations. We investigated two prior distributions: the double exponential (DE) prior, corresponding to the LASSO in logistic regression, and the normal-exponential-gamma (NEG) prior, also called the Hyper-Lasso (HLASSO).

After controlling for type-I error, the penalised approaches greatly reduced the number of positive signals compared to single-SNP association tests, suggesting that correlation between predictors might be better-handled. The HLASSO results were sparser but similar to the LASSO results. In both models, the robustness of the retained variables was verified by bootstrapping. Our findings suggest that variable selection using LASSO or HLASSO show a substantial benefit in identifying risk loci in regions of high LD through the derivation of sparse models.

## 1734/T/Poster Board #283

**Multipoint Association Mapping Using Data from Triads and Unrelated Subjects with Incorporation of Covariates.** *C. Lee, Y. Chiu.* Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes, Taiwan, R.O.C.

Case-controls designs are commonly adopted in association studies as the unrelated subjects are easy to collect; however, spurious association is often observed due to population stratification. To avoid spurious association from population stratification, trios (or triads) designs comprising an affected child and two parents have been recommended where the cases and controls are matched in genetic ancestry and are therefore robust to population stratification. The multipoint linkage disequilibrium mapping proposed by Liang et al. (2001) for trios data is robust to the underlying genetic model of a disease, as only one assumption that there is no more than one disease gene in the chromosomal region is required. Additionally, instead of doing hypothesis testing to detect the disease locus in regular association analysis approaches, it provides the estimate for the disease locus and the variance around it through the generalized estimating equation (GEE) method by simultaneously incorporating all the markers' information; therefore, confidence intervals for the disease locus could also be constructed. In practice, case-parents trios data can be used as a follow-up and confirmatory test for the gene-disease association detected by case-control studies; or, additional unrelated controls can be used as supplemental data to case-parents trios studies to improve statistical power for detecting association, in particular, for late-onset diseases. It is important to extend this robust multipoint association mapping with incorporation of covariates to combine data from case-parent trios and unrelated controls in the present study.

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## 1735/T/Poster Board #284

**Joint analysis of nuclear families and unrelated controls: pitfalls and benefits.** *C.A. Markunas, A.E. Ashley-Koch, S. Schmidt.* Center for Human Genetics, Duke University Medical Center, Durham, NC, USA.

Several genetic association methods allow for the joint analysis of families and unrelated controls. Our goal was to evaluate under what situations the addition of unrelated controls to family data is most beneficial and when it might lead to inflated type I error rates. UNPHASED (Dudbridge 2008) was selected to perform both single locus and haplotype analysis on simulated datasets containing the following structures: 1) 500 nuclear families with three offspring (at least 2 affected), 2) 500 nuclear families plus 500 unrelated controls, and 3) 500 unrelated cases and controls. In addition, three linkage disequilibrium (LD) patterns with moderate, low, and no pairwise LD ($r^2$) and different levels of multilocus LD between a disease gene and nearby markers were simulated. As expected, there was a positive relationship between the power of the single locus analysis and the strength of pairwise LD. Under low pairwise LD, haplotype analysis was substantially more powerful than single-locus analysis, and the addition of unrelated controls to the nuclear families provided the greatest benefit. For example, the gain in power for the joint unrelateds and family analysis was 13-16% for the single-locus analysis vs. 27% for the haplotype analysis (RR=2, MAF=0.3, log-additive model). An important assumption of methods which combine nuclear families and unrelated controls is that they represent the same, homogeneous population. Thus, we tested how robust UNPHASED was to disease minor allele frequency (MAF) differences in the families compared to the unrelated controls. Based on our preliminary findings, the type I error rate was drastically inflated in the presence of moderate disease MAF differences; however, the inclusion of an indicator variable in the model was surprisingly effective in maintaining correct type I error rates. In summary, the likelihood-based method implemented in UNPHASED is useful for taking advantage of publicly available control data, especially for low phenotypic misclassification rates. We expect that imputation methods for a joint analysis of families and unrelated controls will further improve the power to detect association in regions of low pairwise LD.

## 1736/T/Poster Board #285

**Functional Mapping of Age-specific Changes in Body Mass Index with Genome-wide Association Studies.** *K. Ahn[1], K. Das[2], S.J. Finch[3], S.S. An[4], D. Mauger[1], V.M. Chinchilli[1, 2], R. Wu[1, 2].* 1) Dept Public Health Sci, Penn State Col Med, Hershey, PA; 2) Dept Statistics, Pennsylvania State University, Hershy, PA; 3) Dept Applied Math and Statistics, Stony Brook University, Stony Brook, NY; 4) Dept Environmental Health Sci, Johns Hopkins University, Baltimore, MD.

A growing body of evidence shows that cardiovascular disease is correlated with the changes in body function and shape. Thus, by investigating age-specific changes in body mass index (BMI), it is possible that the developing timing of cardiovascular disease can be predicted. Here, we develop a new statistical model for functional mapping of genes that control the rate of change of BMI with age through a genome-wide SNP scan. The model is derived within a mixture framework for genetic mapping, in which a nonparametric approach is implemented to model genotype-dependent values of BMI over age and a semiparametric approach implemented to model the covariance structure of longitudinal data. We particularly investigate the covariance structure of longitudinal data measured at subject-specific unevenly-spaced time intervals. The model provides a quantitative platform to test the interplay between genetic actions and the pattern of development. The model is used to analyze a real data set collected in the Framingham project, aimed to determine "factors influencing the development of heart disease." This analysis leads to the identification of several significant SNPs responsible for the developmental trajectories of BMI. The utilization of the model is validated through simulation studies.

## 1737/T/Poster Board #286

**Pathway-based scan analysis of genome-wide association data.** *T. Nishiyama[1,2], K. Takahashi[3], T. Tango[3], H. Kishino[4].* 1) Doctor of Public Health Program in Biostatistics, Japan; 2) Department of Information and Biological Sciences, Graduate School of Natural Sciences, Nagoya City University, Japan; 3) Department of Technology Assessment and Biostatistics, National Institute of Public Health, Japan; 4) Laboratory of Biometry and Bioinformatics, Graduate School of Agriculture and Life Sciences, University of Tokyo, Japan.

Despite great success of genome-wide association studies (GWAS) in identification of genes underlying complex diseases, the current GWAS have focused only on examining significance on a SNP-by-SNP basis. One of the biggest challenges facing GWAS is a lack of sufficient power to detect small effects as significant. To overcome this limitation, gene set analysis (GSA) methods of analyzing SNP data in GWAS have recently been proposed. However, GSA methods of GWAS do not use gene-pathway structure information, represented by gene products as graph nodes and graph edges between these nodes. Instead of focusing only on the SNPs that are grouped as gene-sets, we propose genome-wide association analysis that incorporates pathway structure information. As a proof of concept, we performed GWAS on a set of 52 cases and 52 controls genotyped for almost 11 000 SNPs, based on pathway structure information on KEGG. To detect clustering of significantly associated SNPs on KEGG pathway, scan statistics were applied with a variable window size. We treated the smallest P values as our statistic of interest and determined its overall significance level. We applied this method based on existing statistics used for GSA methods, such as MAXMEAN and Gene Set Enrichment Analysis (GSEA). The results demonstrated that this new method for GWAS was able to identify biologically meaningful sub-pathways associated with the trait examined. The performance of our scan-statistics approach is extensively compared with GSA methods.

## 1738/T/Poster Board #287

**Tiled regression: the use of regression methods in hotspot defined genomic segments to identify independent genetic variants responsible for variation in quantitative traits.** *A.F. Wilson[1], Y. Kim[1], H. Sung[1], J. Cai[1], F.J. McMahon[2], A.J.M. Sorant[1].* 1) Genometrics Section, Inherited Disease Research Branch, NHGRI, NIH, Baltimore, MD; 2) Genetic Basis of Mood and Anxiety Disorders, NIMH, NIH, Bethesda, MD.

The assumptions of independence between observations and between independent variables are a major theoretical underpinning of much of traditional statistics. However, as the density of genetic markers increases, ultimately encompassing genome wide sequence variants, the correlations between markers increases, depending in large part on the linkage disequilibrium (LD) structure in the sample. Rather than focusing on identifying markers that best characterize LD blocks, stepwise regression is used to identify independent markers that are responsible for additive effects on a quantitative phenotype. To address this issue, the genome is divided into segments based on predefined "hotspot" regions (i.e., well defined regions of increased recombination). Let the term "tile" denote both the sequence of DNA between two hotspot regions and the hotspot region itself. A tile may include many markers in one or more LD blocks. In tiled regression, each SNP or sequence variant is assigned to a tile based on chromosome and physical position. A test of the overall multiple regression model, where the regression coefficients for all the genetic variants are set to 0, is performed on the SNPs or sequence variants in each tile in order to determine if any marker in the tile made a significant contribution to the overall regression. Stepwise regression (forward with a backward look) is then used to select the important markers in each tile. If the multi-colinearity between markers within LD blocks is high, stepwise regression can be used from the outset. Thereafter, the significant markers in independent tiles are combined in higher order stepwise regressions. The tiled regression framework can be extended to include qualitative traits with stepwise logistic regression, and to family data that can be represented in a linear regression context with methods such as the Regression of Offspring on Mid-Parent (ROMP) or Generalized Estimating Equations (GEE). The tiled regression method for quantitative traits is illustrated with data from the STAR*D study (Sequenced Treatment Alternatives to Relieve Depression) [Rush et al., 2004]. The method classified 705 SNPs from 68 genes into 197 tiles. Results are similar to those reported by McMahon et al. [2006], with the most significant marker being rs7997012.

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## 1739/T/Poster Board #288

**Evaluation of Mixed-Model Approaches for Control of Population Structure in Human Genetic Association Studies.** *N. Liu[1], H. Zhao[2, 3], D. Allison[1].* 1) Dept Biostatistics, Section on Statistical Genetics, Univ Alabama at Birmingham, Birmingham, AL; 2) Dept of Epidemiology and Public Health, Yale Univ, New Haven, CT; 3) Dept of Genetics, Yale Univ, New Haven, CT.

In genetic studies, associations between genotypes and phenotypes may be confounded by unrecognized population stratification and/or admixture. Studies have shown that even in European populations, which are thought to be relatively homogeneous, population stratification exists and can affect the validity of association studies. A number of methods have been proposed to address this issue in recent years. Among them, the approaches based on mixed-model have several advantages compared to other methods. However, these approaches have not been thoroughly evaluated on large human datasets. The objectives of this study are to (1) evaluate various methods based on mixed-model for genetic association mapping using human data, (2) compare the performance of different marker-based kinship matrices, (3) understand the effect of each component of these mixed-model approaches in genetic association mapping. To achieve these goals, we generate simulated datasets based on HapMap data under various scenarios. Preliminary results indicate that the approaches based on mixed-model perform well in controlling for population stratification/admixture compared with other methods. However, these methods do not necessarily eliminate the confounding effect of population stratification/admixture in genetic association studies.

## 1740/T/Poster Board #289

**Bayesian Testing of Genetic Association in Meta-analysis.** *X. Wen[1], M. Stephens[1,2].* 1) Dept Statistics, Univ Chicago, Chicago, IL; 2) Dept Human Genetics, Univ Chicago, Chicago, IL.

Testing genetic association is a challenging problem in meta-analysis settings, where different studies may have different sample sizes and/or different genotyping platforms. We propose a new Bayesian procedure to perform single marker association test in case/control studies. Our formulation explicitly considers the retrospective nature of study design, and our hierachical modeling enables variation of effect sizes across studies. Furthermore, for untyped SNPs, our method applies an efficient imputation approach and take into account of the uncertainty of imputed allele frequencies.

## 1741/T/Poster Board #290

**Model Selection Strategies in Genome-Wide Association Studies.** *S.L. Keildson[1,2], A.P. Morris[2], M. Farrall[1,2].* 1) Cardiovascular Medicine, University of Oxford, UK; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, UK.

Unravelling the complex genetic architecture of many common diseases is a continual challenge in the field of human genetics. Complex disease phenotypes are often controlled by the action of multiple genes, the effects of which may be confounded by environmental factors and epistasis, and therefore traditional attempts to map the loci that play a role in complex disease susceptibility have been largely unsuccessful. While genome-wide association studies have proven to be successful in identifying many new disease-susceptibility loci, the extension of these studies beyond the scope of single SNP analyses have been limited. The incorporation of multi-locus methods of analysis may, however, have the potential to increase the power of genome-wide association studies to detect genes of smaller effect size as well as the genes that interact with each other and the environment. We have carried out large scale simulations of four multi-locus model selection techniques in order to compare the type I error rate and power of each method. We simulated quantitative phenotype data for a range of genetic models and generated genotype data for 10 SNPs from HapMap ENCODE regions using HAPGEN. To this data, we applied four regression techniques encompassing additive main effects, implemented using R software packages: forward selection, backward selection, Lasso and Bayesian model averaging. Adjustments were made to each method in order to reduce the initial type I error rate to approximately 5% and then the power of each method to detect associations in the simulated data at these adjusted type I error rates, was calculated over 1000 simulated populations. At a type I error rate of 0.053, the Lasso method generally showed the highest power over a wide range of genetic models, followed by forward and backward selection, both of which had estimated type I error rates of 0.058. While Bayesian model averaging proved to be the least powerful model selection technique, it also had the lowest frequency of false positive results (0.038). Forward selection and a combined method of lasso and bayesian model averaging was then applied to real lipoprotein(a) data and yielded results consistent with those published in the literature.

## 1742/T/Poster Board #291

**A non-parametric method for global test of associations for a large number of SNPs in case-control association studies.** *Y. Li, J.J. Liu.* Human Genetics , Genome Inst Singapore, 60 Biopolis street, #02-01, S138672, Republic of Singapore.

Gene-gene interactions have long been recognized to be indispensable in deciphering the genetic etiology of common complex human diseases. When single nucleotide polymorphisms (SNPs) are used as genetic variants in case-control association studies, the routine method for gene-gene interaction detection is to test pair-wise SNP interaction among all SNPs or among all SNPs in different genes. Due to the large number of interaction tests involved when a medium number of SNPs are considered, we are always encountered with the issue of low power incurred by the multiple testing. On the other hand, SNPs in different genes in a biological pathway may individually have small effects, or individual SNP pairs have small interaction magnitude, but they show detectable joint effects or joint interactions as they play roles in a concerted way. AML, Admixture Maximum Likelihood, is an efficient method to test joint effects, which assumes that all the SNPs convey the same effect and have the same probability to be associated. However, AML will fail to detect joint associations when all the main effects are weak. We here propose a non-parametric global test method for a large number of SNPs in case/control association studies which considers all the orders of interactions (including main effects) implicitly in one test. Specifically, we map the genotypes at the given SNPs to an infinite dimensional space, which contains all the orders of interactions among any of the SNPs, then compare the distribution of this mapped space between the control subjects and the case subjects by making use of the "kernel" trick, avoiding explicit computation in the mapped infinite dimensional space. The advantage of our method is that we do not need to specify a particular form of interaction (e.g., second order or third order), neither need to specify among which SNPs there exist interactions. Simulations showed that our method had higher power of association detection than existing methods when the joint interactions are detectable while all the main effects are weak.

## 1743/T/Poster Board #292

**Multi-locus analysis of genome-wide association data via novel application of multivariate statistical and model selection techniques.** *A. Morris.* Wellcome Trust Centre for Human Genetics, Univ Oxford, UK.

Genome-wide association (GWA) studies have been successful in identifying novel loci contributing to complex human traits. However, despite these successes, much of the genetic contribution to the trait variance remains unexplained. Typically, GWA studies are analysed using single SNP tests. However, a gain power might be expected by modelling the effects of multiple SNPs, simultaneously, within a gene or small genomic region. In such regions, "local axes" of genetic variation can be defined by applying multivariate statistical techniques to SNP genotype data. The leading axes encompass most of the genetic variation in the region, and take account of linkage disequilibrium between SNPs. Testing for association with these axes reduces the overall burden of multiple testing. Furthermore, by considering these axes, jointly, in a generalised linear regression framework, it is possible to model the effects of multiple causal variants, simultaneously.

A simulation study was undertaken to evaluate the power of multivariate-based analyses of SNPs in a 50 kb region in a study of 1000 cases and controls. Three approaches to the selection of axes of genetic variation for inclusion in the regression model were considered: (i) the leading axes explaining 80% of genetic variation in the region; (ii) the leading axes explaining 90% of variation; and (iii) the leading axes with minimum Bayesian information content (BIC) among those explaining 90% of variation. Over all simulations, selecting axes by means of the BIC is the most powerful multivariate-based analysis approach. When disease risk is determined by a single causal variant, there is little difference in power between single SNP and multivariate analyses. However, in the presence of two independent causal variants, there are noticeable gains in power for the multivariate analyses.

Multivariate-based analysis of a 50kb sliding window across a GWA study of type 2 diabetes from the Wellcome Trust Case Control Consortium (2000 cases and 3000 controls) revealed: (i) stronger signals of association than single SNP tests for many of the now established loci; and (ii) multi-locus signals of association, not identified through single SNP tests, that warrant follow-up.

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## 1744/T/Poster Board #293

**A Bayesian approach to genetic association studies with family-based designs.** *M. Naylor[1], S.T. Weiss[2], C. Lange[1].* 1) Dept Biostatistics, Harvard School of Public Health, Boston, MA; 2) Channing Lab, Brigham and Women's Hospital, Boston, MA.

For genomewide association studies with family-based designs, we propose a Bayesian approach. We show that standard TDT/FBAT statistics can naturally be implemented in a Bayesian framework. We construct a Bayes factor conditional on the offspring phenotype and parental genotype data and then use the data we conditioned on to inform the prior odds for each marker. For the construction of the prior odds, the evidence for association for each single marker is obtained at the population-level by estimating the genetic effect size in the conditional mean model. Since such genetic effect size estimates are statistically independent of the effect size estimation within the families, the actual data set can inform the construction of the prior odds without any statistical penalty. In contrast to Bayesian approaches that have recently been proposed for genomewide association studies, our approach does not require assumptions about the genetic effect size; this makes the proposed method entirely data-driven. The power of the approach was assessed through simulation. We then applied the approach to a genomewide association scan to assess association between single nucleotide polymorphisms and body mass index in the Childhood Asthma Management dataset.

## 1745/T/Poster Board #294

**Gene set analysis in genome-wide association studies.** *N. Tintle.* Mathematics, Hope College, Holland, MI.

Gene set analysis is now a standard method for analyzing gene expression data. In contrast to traditional gene expression data analysis which examines significance gene-by-gene, gene set analysis is used to establish the significance of biologically relevant sets of genes. Recently, gene set analysis has been proposed for the analysis of single nucleotide polymorphism data in genome-wide association studies (GWAS). First attempts to use gene set analysis in GWAS have proposed the use of Gene set Enrichment Analysis (GSEA) and methods based on Fisher's exact test (FET), arguably the two most popular gene set analysis methods for gene expression data. However, recent advances in gene set analysis for gene expression data have proven to be more powerful and robust than GSEA and FET. I will present results showing that these newer methods can be used for gene set analysis of GWAS to increase power compared to GSEA and FET. Results are based on analysis of real and simulated GWAS data. I will also present a number of open, practical questions about best practices for the use of gene set analysis in GWAS.

## 1746/T/Poster Board #295

**Phenotype Harmonization in a Multi-Site Genome-Wide Association Study: GENEVA.** *S.N. Bennett[1], A.L. Fitzpatrick[1], N. Caporaso[2], K. Williams[1], J. Udren[1], B. Weir[1], GENEVA.* 1) Biostatistics, Univ Washington, Seattle, WA; 2) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institute of Health, Bethesda, MD.

Purpose: GENEVA (Gene Environment Association Studies) is a multi-site genome-wide association study developed as part of the NIH-wide Genes, Environment and Health Initiative (GEI) aimed at accelerating understanding of genetic and environmental contributions to health and disease. Its aims are to identify genetic variants related to common complex diseases, identify variations in gene-trait associations related to environmental exposures, and address potential pathways to outcomes in various populations. The 8 Phase I and 6 Phase II studies in this consortium have disparate interests including oral clefts, addiction, cardiovascular disease, lung cancer, type 2 diabetes, maternal metabolism and birth weight interactions, dental caries, prematurity, lung health, blood pressure, prostate cancer, venous thrombosis, stroke and glaucoma. One of the major analytic challenges is the wide range of phenotypes. The GENEVA Coordinating Center (CC) is charged with coordinating activities related to data collection, harmonization and management, and facilitating cross-study analyses. This paper describes the processes undertaken by the CC and issues that have arisen. Methods: The CC created a web-based survey to identify phenotypic data common across studies. For identified areas of interest, the CC and Phenotype Harmonization Subcommittee (PHS) convened a series of investigator-led Working Groups (WGs) to evaluate feasibility and logistics for cross-study analyses. WGs identify and define variables to be shared, identify covariates, recommend the most appropriate analytic method and draft an analysis plan using a template developed by the CC and PHS chair. The CC assigns a unique GENEVA ID to each individual. The CC provides WGs with statistical summaries of phenotypic data and harmonizes the covariates. The CC has established a centralized relational database containing each study's phenotypic data, data dictionary, individual-level consent, public use or GENEVA-only use status, and harmonized data. Results: The PHS identified 8 areas of common interest: alcohol use, anthropometrics, caffeine use, type 2 diabetes, oral health, psychiatric history, reproductive history and tobacco use. With the added Phase II studies, new WGs are being established. The CC is harmonizing 9 covariates, has assigned 62,935 GENEVA IDs and incorporated 4 studies' data into a centralized database. Four WGs have completed analysis plans and are starting shared data analyses.

## 1747/T/Poster Board #296

**Accounting for sample structure in large scale genome-wide association studies using a variance component model.** *H. Kang[1,2], J. Sul[3], S. Service[4], N. Zaitlen[5], S. Kong[6], N. Freimer[6,7,8], C. Sabatti[6,9], E. Eskin[3,6].* 1) Computer Science and Engineering, University of California, San Diego, La Jolla, CA; 2) Department of Biostatistics, University of Michigan, Ann Arbor, Ann Arbor, MI; 3) Computer Science Department, University of California, Los Angeles, Los Angeles, CA; 4) Center for Neurobehavioral Genetics, University of California, Los Angeles, Los Angeles, CA; 5) Bioinformatics Program, University of California, San Diego, La Jolla, CA; 6) Department of Human Genetics, University of California, Los Angeles, Los Angeles, CA; 7) The Jane and Terry Semel Institute for Neuroscience and Human Behavior and Los Angeles, Los Angeles, CA; 8) Department of Psychiatry, University of California, Los Angeles, Los Angeles, CA; 9) Department of Statistics, University of California, Los Angeles, Los Angeles, CA.

In the last couple of years, genome wide association studies have opened up a series of intriguing research directions, identifying a number of loci possibly implicated in a variety of complex traits. A serious concern in the interpretation of these results is the possibility of confounding effects due to sample structure. Typically, this is modeled as either of two extreme manifestations: population stratification or hidden relatedness. We here propose an efficient variance component approach that allows to deal with general types of sample structures. Unlike approaches based on principal components, which rely on the major axes of the pairwise genetic relatedness matrix, our method uses the entire matrix in modeling correlation between phenotypes of sample subjects. A similar mixed effect strategy has proven successful in model organism association mapping. Here we take advantage of the fact that each locus involved in human polygenic traits has a relatively small effect and we estimate the variance components only once in the genome scan. This makes it computationally feasible to process large sample sizes. We analyze data on 10 quantitative traits in the Northern Finland Birth Cohort 1966 (NFBC66), and 7 disease phenotypes in the Wellcome Trust Case Control Consortium (WTCCC). The proposed methodology consistently eliminates the over dispersion of the test statistics that cannot be fully resolved by including principal components in the regression tests. Moreover, simulation studies suggest that the proposed approach results in values of the test statistics at the tails of the distribution that might be more accurate than what is achievable with genomic control correction. The software to carry out described analysis is available at http://genetics.cs.ucla.edu/emmax.

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## 1748/T/Poster Board #297

**Principal Component Score Prediction for Related and Unrelated GWA Samples.** *S. Lee[1], F.A. Wright[1,2], F. Zou[1,2].* 1) Biostatistics, University of North Carolina, Chapel Hill, NC; 2) Center for Environmental Bioinformatics,University of North Carolina, Chapel Hill, NC.

Principal component (PC) analysis is used as one of the leading population stratification control approaches for genome-wide association studies (GWAS). In PC analysis, PC scores are used to estimate ancestry structure of study subjects. It is well known that PC analysis is sensitive to the presence of related samples, resulting in a distorted view of population structure. To avoid this problem, a subset of independent samples can be used for PC analysis, from which PC scores of excluded samples are subsequently estimated. However, this common PC score prediction method is substantially biased, in a sense that the predicted PC scores tend to be shrunken towards 0 compared to the scores computed using all samples. This bias is particularly large for studies with moderate population substructure, or unequal representation of samples from different subpopulations. In this talk, we will describe a novel method using recent results on random matrix theory to adjust for prediction bias, and is applicable to both sets of related and unrelated samples. We will demonstrate this bias correction method using both simulated and real GWAS data.

## 1749/T/Poster Board #298

**Cystic Fibrosis: An 'Inclusive' Disease - Demonstrated by Analysis of Population Stratification.** *W. Li[1], L. Sun[4,5], C. Taylor[1,4], R. Dorfman[2], A. Cojocaru[1], J. Zielenski[2], P. Durie[3], M. Corey[1,4], LJ. Strug[1,4].* 1) Child Health Evaluative Sciences, The Hospital for Sick Children, Toronto, ON, Canada; 2) Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, ON, Canada; 3) Physiology and Experimental Medicine, The Hospital for Sick Children, Toronto, ON, Canada; 4) Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada; 5) Department of Statistics, University of Toronto, Toronto, ON, Canada.

**Introduction** A common misconception, shared by researchers and the general population alike, is that Cystic Fibrosis (CF) only affects people of European descent. This can be seen in the lack of detailed ethnicity information collected in CF research studies. The Carleton University Students' Association went as far as canceling CF fundraisers, claiming CF is not an "inclusive" enough disease for massive fundraising efforts. Although historically this may have been the case, the diverse ethnic make-up of Canada suggests that the face of CF might also be changing. Armed with genome-wide SNP data on 1437 unrelated Canadian CF patients with pancreatic insufficiency (CFPI) from the Canadian Genetic Modifier Study (GC2), shown to be representative of the Canadian CF population, we set out to better understand the ethnic make-up of the Canadian CF population using Principal Component Analysis (PCA). **Methods** EIGENSTRAT (Price et al. 2006) was used to perform PCA, and the top 3 significant PCs were examined in 3D plots. We also seeded the CFPI data with SNPs from 6 HapMap3 (http://www.hapmap.org) populations including the Europeans (CEU), East Asians (CHB+JPT), Yoruba (YRI), African Americans (ASW), Indians (GIH) and Mexicans (MEX). **Results** No clear structure was observed in the top 3 PCs extracted from the CFPI samples alone. Seeding with the HapMap3 data, CEU, YRI and CHB+JPT formed three separate tight clusters, while GIH, MEX and ASW formed three short continuous arms pointing to the CEU cluster. CFPI exhibited a tripod structure with most of the subjects forming a cluster at the apex of the tripod, on top of the HapMap3 CEU cluster. Indeed, this CFPI cluster consisted of mostly self-reported 'Caucasian' subjects. However, the rest of the subjects scattered along the CEU-GIH, CEU-MEX and CEU-ASW arms, suggesting population admixture. Furthermore, neither CHB+JPT nor YRI encompassed any of the variation. **Discussion** CFPI samples that distributed along the CEU-ASW arm were either self-reported 'African' or 'mixed'; and those distributed along the CEU-MEX and CEU-GHI arms were either 'Caucasian' or of 'Other' ethnicity. **Conclusion** Our PCA suggests that the Canadian CF population contains many individuals with mixed ancestries. Given the ethnic complexity of this population, it is crucial to guard against potential spurious association in CF studies by incorporating either detailed ethnicity information or PCs.

## 1750/T/Poster Board #299

**Clustering based on Genetic Ancestry.** *N. Timofeev[1], S.H. Hartley[1], C.T. Baldwin[2], D.A. Dworkis[3], L.F. Farrer[1,3], M. Gladwin[4], E.S. Klings[3], J.N. Milton[1], T.T. Perls[3], M.H. Steinberg[3], P. Sebastiani[1].* 1) Department of Biostatistics, Boston University, boston, MA; 2) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 3) Department of Medicine, Boston University School of Medicine, Boston, MA; 4) Vascular Medicine Branch, National Heart, Lung and Blood Institute and Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, MD.

Genome wide association studies (GWAS) have become increasingly popular in the genetic community to identify disease associated genes. Population stratification is a major confounder causing spurious associations in GWAS and occurs when differences in allele frequencies of single nucleotide polymorphisms (SNPs) are due to the ancestral differences between cases and controls rather than the disease. Principal components analysis (PCA) is a widely used approach to detect population substructure using genome wide data and to adjust genetic association for stratification by including the top principal components in the analysis. An alternative solution to the population stratification problem in association studies is genetic matching of cases and controls that requires, however, well defined population strata for appropriate selection of cases and controls. With this objective in mind, we developed a novel algorithm to cluster individuals into groups based upon similar ancestral backgrounds using the principal components computed by PCA. Our algorithm utilizes k-means clustering of the most informative principal components to iteratively group individuals into clusters and a novel index that includes accuracy, stability and between-cluster distance to choose the appropriate number of clusters. We tested the algorithm on 151 subjects from 7 African populations in the Human Genome Diversity Project to demonstrate that the algorithm accurately clusters individuals. Our algorithm also discriminated fine groupings of Caucasians of European descent in a large cohort of Caucasian centenarians and their controls. In addition, examination of the ancestral substructure and the amount of Caucasian admixture in a large cohort of African American sickle cell disease patients using this algorithm revealed that African Americans with sickle cell disease are significantly less admixed than African Americans without sickle cell disease and have ancestries similar to either the Yorubans and Mandenkas or the Bantu population, ethnic groups located on the western coast of Africa.

## 1751/T/Poster Board #300

**Local Ancestral Inference in Admixed Populations.** *c. Zhu[1], S. Han[2], X. Liu[3].* 1) Department of Agronomy, Kansas State University, Manhattan, Kansas 66506,USA; 2) Division of Human Genetics, Department of Psychiatry, School of Medicine, Yale University, New Haven, Connecticut 06511, USA; 3) Human Genetics Center, School of Public Health, University of Texas at Houston, Houston, Texas 77030, USA.

The genome of an admixed individual represents a mixture of alleles inherited from multiple ancestral populations. Inferring ancestral information along the chromosomes is particularly useful in admixture mapping or correcting population stratification for genomewide association study in recently admixed populations. The most popular Hidden Markov chain model based methods for inferring local ancestry require that each pair of ancestry informative markers (AIMs) should be independent of each other in the ancestry populations. In the current study, we introduced a new method for inferring locus ancestry state based on copying process model which can handle highly correlated marker sets. Copying process model assumes that a new haplotype is produced by ancestral haplotype through copying, recombination and mutation. When combined with penalty score model and dynamic programming approach, the method could infer a single most likely combination of ancestral states (i.e., the pathway of copying process) of all polymorphic sites of the derived haplotype. Using a simple hybrid-isolation admixture model, we evaluated the existing methods for inference of population structure under dense SNPs marker sets, especially in the scenario of highly correlated SNPs. We investigated the performance of our proposed method using a series of simulation experiments and empirical datasets and found that the new method is accurate and robust when highly correlated dense marker set was used. A through comparison of the new method with the existing commonly used methods such as STRUCTURE or ANCESTRYMAP is still undergoing.

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## 1752/T/Poster Board #301

**Risk Prediction Model Establishment for Complex Genetic Diseases.**
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Background: Recent advances in high-density genotyping technology have made it possible to conduct genome-wide association studies (GWAS) to detect genetic variants that are associated with risk for complex diseases. However, the prediction of genetic risk of a complex disease using GWAS data remains a challenging problem because complex traits are likely affected by many genes, environmental risk factors, and interactions among them where most of which only confer a very small effect on disease risk. In addition, small sample size relative to the number of markers available is another issue in building meaningful risk models, where the number of features far exceeds the number of samples. When sample size and effect size are both small, the effect size for a given variant is often estimated with considerable uncertainty, which leads to subpar prediction performance because it is critical to identify genetic variants truly associated with disease and also appropriately quantify their effects. Method: We have conducted a systematic study to evaluate the effects of sample size, effect size, the number of selected features, and the specific prediction methods on prediction accuracy. Based on our observations, we have proposed a novel statistical method for disease risk prediction based on GWAS data. To assess of the utility of our methods in real data, we constructed a risk prediction model based on GWAS data collected for Crohn's Disease, a subtype of inflammatory bowel disease that is believed to have a complex etiology. We evaluated the performance of the prediction model by examining its area under ROC (Receiver Operating Characteristic) curve. Results: Our approach has been found to work well under a number of simulation scenarios. For Crohn's disease data, using a cross validation scheme, the median AUC (area under curve) among the twenty runs was $0.766 \pm 0.018$. Comparisons between our proposed method and other methods demonstrated the improved performance of our method.

## 1753/T/Poster Board #302

**Pathway-Based Analysis of Genome-Wide Association Studies using Random Forests.** *Y. Meng.* Metabolic Disease Initiative, Broad institute of Harvard and MIT, Cambridge, MA.

Most current approaches to analyzing genome-wide association studies (GWAS) test each variant individually and without regard to their genetic context. Although this comprehensive and unbiased approach has advantages and has proved to be successful in identification of candidate genes in many complex diseases, it is widely accepted that not all variants have been identified. Recently, more genes are being identified by testing copy number variants (CNVs) and copy number polymorphisms (CNPs), and by increasing samples sizes through joint analysis of multiple GWAS. While such approaches are yielding new associations, power for identifying common variants of weak association is still limited. An alternative and complementary approach is to use prior biological information to analyze GWAS data at the level of genes, sets of genes, and biological pathways. This may contribute to the statistical and explanatory power of discovering new genes and biological processes that influence complex human diseases and traits, especially when high degrees of allelic and locus heterogeneity exist as is the case for many complex human diseases.

Pathway-based analyses have generated some success in gene expression studies, and have been recently applied to GWAS data using similar approaches - GSEA (Gene Set Enrichment Analysis), and hypergeometric distribution. Many machine learning methods, including Random forests (RF) can take into account SNP interaction effects as well as main effects without requiring model specification, therefore have the advantage of capturing the interaction effect over the other approaches. The machine learning methods have been applied often in expression studies, but have just started to gain popularity in genetic analysis, but still not on the genome-wide scale due to large volume of the data (1 million SNPs). However, if we test SNPs on the pathway level, the computation burden is much smaller, therefore practically feasible. In this paper, we apply the pathway-based analysis using Random Forests, which was first developed for gene expression data analysis, to GWAS data analysis. We also compared Random Forests with GSEA and hypergeometric method. Here, we discuss the methodological factors of pathway-based analysis using Random Forest by applying these approaches to TGEN GWAS data of Alzheimer's Disease.

## 1754/T/Poster Board #303

**Assessment of Associations in Neuroimaging GWA Studies with Multi-dimensional Phenotypes.** *J.C. Roddey[1], O.A. Andreassen[2,3], A.M. Dale[1,4], Alzheimer's Disease Neuroimaging Initiative.* 1) Dept. of Neurosciences, University of California, San Diego, La Jolla, CA; 2) Division of Psychiatry, Ulleval University Hospital, Oslo, Norway; 3) Institute of Psychiatry, University of Oslo, Oslo, Norway; 4) Dept. of Radiology, University of California, San Diego, La Jolla, CA.

A neuroimaging genome-wide association study (GWAS) can involve high dimensional genetic data (e.g. 1,000,000+ SNPs) and potentially even higher dimensional imaging phenotype data. The number of subjects in a neuroimaging GWAS is typically less than 1000, rarely more than several thousand, and the reported effect sizes in prior studies have been relatively small. In light of these factors, an exhaustive search for highly significant associations between genetic variants and univariate imaging phenotypes probably will suffer from low statistical power after correction for multiple comparisons. This problem can be ameliorated via a priori selection and analysis of weighted combinations, or projections, of the multidimensional imaging data (e.g. choosing a single imaging feature, or the mean of all features). However, this approach will be insensitive to real associations involving unselected phenotypic combinations. Therefore, methods that minimize prior assumptions about optimal projection directions while maximizing the sensitivity of association tests are required. We have developed a set of algorithms similar to ridge regression and regularized canonical components analysis (CCA) for identifying significant associations of genetic variants with projections of multidimensional phenotypes. We applied the algorithms to data from two independent studies: Thematically Organized Psychosis research (TOP), and the Alzheimer's Disease Neuroimaging Initiative (ADNI). The genetic variants were SNPs (500,000+ SNPs in each study), and the phenotypes were brain structure measures derived from 3D MRI data. For each SNP, an optimal projection direction in multidimensional phenotype space was learned from training data, and it was used to obtain a derived univariate phenotype for subjects in the test set. Assaying the associations in test sets minimized the risk of false positives. We identified SNPs located within and near genes involved in brain development, including several implicated in microcephaly (MCPH), that were significantly associated with specific patterns of cortical areal expansion in the discovery sample (TOP). A subset of the findings replicated in the second sample (ADNI). Since the genotyping platform was not the same in the two studies, we used MACH to impute genotypes at unassayed SNPs in the HapMap CEU reference sample. We used EIGENSTRAT to control for ancestral differences among subjects.

## 1755/T/Poster Board #304

**Improved Ranking and Selection of Single Nucleotide Polymorphisms in Association Studies.** *H. Schwender[1], K. Ickstadt[2], I. Ruczinski[1].* 1) Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 2) Department of Statistics, TU Dortmund University, Dortmund, Germany.

A major goal of the analysis of data from association studies is the detection of SNPs exhibiting an impact on the risk of developing a disease. The typical strategy for identifying such SNPs is to carry out SNP-specific (marginal) tests such as the Cochran-Armitage trend test, rank the SNPs by their p-values, and declare SNPs with a Bonferroni corrected p-value less than a prespecified level (typically, 5%) to be significant. Although this certainly is an acceptable strategy for a first pass through the data, it has several drawbacks. On the one hand, employing a Bonferroni correction is much too conservative when simultaneously testing hundreds or thousands of SNPs that are partly highly correlated due to linkage disequilibrium. On the other hand, this strategy does not take the multivariate data structure of the SNPs into account, and therefore does not allow to identify SNPs that only show an effect on the disease risk when interacting with other SNPs. A solution to these problems is to employ regression or discrimination methods such as logic regression that explicitly search for interactions of variables, and thus enable the detection of disease-associated SNPs without a marginal effect. Based on the output of such procedures the importance of each SNP for a good prediction of the response of a study can be quantified, which in turn can be used to test the SNPs. In this presentation, we will propose a testing method that makes essential use of this idea to enable a more appropriate ranking of SNPs than univariate testing, and thus to improve the detection of disease-associated SNPs. We will furthermore show how an empirical Bayes approach can be applied to the resulting test statistics to control the false discovery rate at a certain level leading to a less conservative approach than the Bonferroni correction. Finally, it will be described how the proposed method can be extended to test biological sets of SNPs (e.g., SNPs belonging to the same LD-block or gene) and other types of responses (e.g., quantitative and multi-categorical responses).

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## 1756/T/Poster Board #305

**Tiled Logistic Regression for Response to Antidepressant Treatment.**
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A new method called "Tiled Regression" has been developed and is described elsewhere for quantitative traits [Wilson et al., pers. comm.]. In this method, the genome is divided into independent regions, or tiles, and the SNPs or sequence variants within each tile are analyzed with multiple and stepwise regression to identify only those markers within the tile that have independent and significant additive effects on the phenotype. Markers identified in independent tiles are then reanalyzed, again with stepwise regression, in an attempt to identify all independent markers that have a significant effect on the phenotype. In this study, tiled regression is extended to include logistic regression for qualitative or dichotomous traits. Tiled logistic regression was applied to a binary trait "responder status" in data from the STAR*D (Sequenced Treatment Alternatives to Relieve Depression) study [Rush et al., 2004]. This trait was derived from the relative improvement in QIDS-C16 (a standard depression score) over a period of antidepressant treatment. 1096 white subjects were categorized according to high or low levels of improvement and were analyzed with 705 SNP markers in candidate genes. The analyses were done with tiles defined in two different ways: by hotspot regions (within and between high-recombination "hotspots") and by gene (within and between known genes). In most cases, no more than one marker was chosen from any tile. At a significance level of 0.05, seven SNPs were selected for the final model using hotspot-based tiles and eight using gene-based tiles, with five SNPs appearing in both models. In both models, the SNP rs7997012 on chromosome 13, part of the HTR2A gene which encodes the serotonin 2A receptor, had the most significant p-values (0.00116 and 0.00163 using hotspot- and gene-based tiles, respectively). This is the same SNP previously noted by McMahon et al. [2006].

## 1757/T/Poster Board #306

**A case study of a statistical tool for fine-scale mapping.**
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Various methods have been proposed to identify the genetic basis of diseases that are caused by both genetic and environmental conditions, with the treatment of multiple testing often in the foreground. The effectiveness of four approaches is here assessed, in particular (1) 'Treescan' proposed by Templeton et al.(*Genetics 169:441-453*), (2) a single site analysis based on Treescan (3) analysis adjusted by Bonferroni correction and (4) a novel method that uses Bayes Factors in place of the F statistics used in the Treescan and single SNP methods. Data sets have been simulated under a wide variety of conditions and the simulator and analyses have been implemented in R, with a graphical user interface provided by 'rpanel'. These data sets were used to test the effectiveness of each method in finding a simulated known causal variant that increases a continuous phenotypic score, thus avoiding categorizing individuals as either case or control. Initial results suggest that the most suitable method depends strongly upon the conditions imposed on the simulated data, which are chosen according to numeric values that are consistent with real data. There is however an indication that the use of Bayes Factors in this context will provide some advantages over the frequentist methods, depending on hyperparameter choices. Each method is also applied to a real data set involving heart rate and blood pressure measurements, and the use of the Bayes Factors methods detects a possible causative SNP that the other methods have insufficient power to detect.

## 1758/T/Poster Board #307

**Using a local extension of HapMap 3 to raise SNP imputation quality and to identify CNV tag-SNPs in population isolates.**
*S. Ripatti[1,2], I. Surakka[1,2], K. Kristiansson[3], V. Anttila[3], M. Innouye[3], M. Daly[4], L. Moutsianas[5], M. Hurles[3], A. Palotie[1,3,4], L. Peltonen[1,2,3,4].* 1) Institute for Molecular Medicine Finland FIMM, Helsinki, Finland; 2) Public Health Genomics Unit, National Institute for Health and Welfare, Helsinki, Finland; 3) Wellcome Trust Sanger Institute, Hinxton, UK; 4) The Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA; 5) University of Oxford, Oxford, UK.

The introduction of HapMap 3 reference data set combining data from two commercially available dense marker chips has made it possible to create similar local reference samples with relatively low cost. This is particularly useful for populations not included in the HapMap 3 and for genetically homogeneous population isolates. In this work we illustrate with Finnish data how a local reference of 81 Finns (40 individuals from general population and 41 individuals from late settlement Kuusamo region) with 1.6 million SNP markers and 2.8 million probes for CNV detection can efficiently be used for two aims: 1) to gain precision in imputation quality of population samples with lower density markers, and 2) to identify SNPs tagging CNV regions in local populations. With a Finnish population-based sample of 2138 individuals, we show that the average $R^2$ between imputed and directly genotyped markers raises from 0.85 to 0.91 with Hapmap3 CEU +TSI reference and joint Hapmap3 + Finnish reference, respectively, when imputing SNPs in Illumina 370K resolution to Illumina 610K resolution. In a subset of 429 individuals with both parents being born in a regional bottleneck population originating from some 400 chromosomes 400 years ago, the $R^2$ raised from 0.85 to 0.94. The imputation quality gain was largest in the lower frequency SNPs with 1% < MAF < 5%. The strong LD structure and dense probe set also allowed us to identify SNPs tagging CNV regions. We investigated 13500 putative CNV loci and initially identified 4980 regions in our Finnish reference data. For these 4980 loci, we tested for an association between summarized CNV probe signal intensities and SNP genotypes. We found 908 distinct CNV loci which were tagged by one or more SNPs with $R^2 > 0.50$ and 475 CNV loci tagged with $R^2 > 0.80$. To evaluate the gain in using population specific data in CNV tagging, we compare the tag-SNPs identified by Hapmap3 CEU and TSI CNV genotyping, and tag-SNPs discovered in analysis of our Finnish reference data. Finally, we illustrate how the better imputation quality and tag-SNP identification provide statistical power gain for detecting associations with metabolic traits in our Finnish population-based cohorts with special emphasis on lower frequency SNPs and CNVs. The population specific HapMap 3 datasets are thus beneficial for providing improved resolution for imputing and increase in statistical power for detecting association.

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## 1759/T/Poster Board #308

**MSGene - A novel and publicly available database of genetic association studies in multiple sclerosis.** *C.M. Lill[1], M.B. McQueen[2], B.-M.M. Schjeide[4], U. Zauft[4], S. Bagade[3], F. Zipp[1], L. Bertram[3,4].* 1) Cecilie Vogt Clinic for Neurology, Charité - University Hospital, Berlin, Germany; 2) University of Colorado, Boulder, CO, USA; 3) Genetics and Aging Research Unit, MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Charlestown, MA, USA; 4) Neuropsychiatric Genetics Group, Max Planck Institute for Molecular Genetics, Berlin, Germany.

Multiple sclerosis (MS) is a genetically complex disorder that shows familial aggregation without following Mendelian inheritance. Rather, it is governed by a variety of genetic and non-genetic factors, which define an individual's risk to develop the disorder. In the past two decades, hundreds of reports have been published claiming or refuting genetic association between putative MS genes and disease risk. In addition, several genome-wide association studies (GWAS) have been reported, highlighting putative novel MS loci that need further validation. Similar to the situation in other genetically complex diseases, this wealth of information is becoming increasingly difficult to follow, evaluate, and most importantly to interpret. To this end, based on methodology previously developed by our group (Bertram [2007] Nat Genet 39(1):17-23), we are currently building a database ("MSGene"), which will serve as an exhaustive, unbiased, and regularly updated resource of all MS genetic association studies published in peer-reviewed journals. Data for MSGene will be gathered following systematic searches of the scientific literature, and will summarize essential characteristics of each study, including sample size and ethnicity of the investigated populations, as well as gene-specific results and genotyping details (e.g. genotype and allele frequencies). Following this strategy, we have now systematically collected and summarized over 800 MS genetic association papers reporting on over 2000 polymorphisms across 445 independent loci. In addition to these candidate gene-based studies, MSGene also provides detailed summaries of the five currently published GWAS. An integral part of MSGene is the systematic computation of random-effects meta-analyses for all polymorphisms with sufficient available genotype data. Of the 93 meta-analyses currently available in MSGene, 17 genetic variants in 14 loci show significant effects across all published studies (with average odds ratios of ~1.3, ranging from 1.1 to 1.6); nearly half of these were originally implicated by GWAS (e.g. *CLEC16A*, *IL7R*). All data collected as part of this project - including the results of the up-to-date meta-analyses - will be made publicly available at http://www.msgene.org, scheduled to go online in September 2009. At the meeting, we will provide a synopsis of the current status of the MS genetics field, with a particular focus on those loci showing significant summary odds ratios.

## 1760/T/Poster Board #309

**C-Reactive Protein levels and body mass index: elucidating direction of causation through a reciprocal Mendelian Randomization design.** *N. Timpson[1], B. Nordestgaard[2], R. Harbord[3], J. Zacho[2], T. Frayling[4], A. Tybjaerg-Hansen[5], G. Davey Smith[1].* 1) MRC CAiTE Ctr/Social Med, Bristol Univ, Bristol, UK; 2) Department of Clinical Biochemistry, Herlev Hospital, Copenhagen, Denmark; 3) Department of Social Medicine, Bristol Univ, UK; 4) Institute of Biomedical and Clinical Science, Penninsular Medical School, Exeter Univ, Exeter, UK; 5) Department of Clinical Biochemistry, Rigshospitalet, Copenhagen Univ Hospital, Faculty of Health Sciences, Copenhagen, Denmark.

We used genetic data to help assign direction of causality within a network of observational associations; a procedure which is problematic outside of randomised experiments. Through the application of reciprocal Mendelian randomisation, we employ confirmed genetic associates of BMI (FTO, rs9939609) and CRP (CRP, rs3091244) to interrogate observational associations between C-reactive protein (CRP) and BMI (body mass index) in the Copenhagen General Population Study (n>37,000). In this population, the observational change in logCRP for a standard deviation increase in BMI can be summarised in a ratio of geometric means of 1.36(1.35, 1.37), which approximates to a 0.65 unit increase in circulating CRP (at the median and in sex-adjusted analyses). In efforts to avoid measured confounding in observational studies of BMI and CRP, analyses using CRP variation to re-estimate the causal effect of circulating CRP on BMI yielded null effects (change in BMI per unit change in logCRP -0.30(-0.78, 0.18), p=0.2). However, those using FTO variation to assess this association as driven by BMI, confirmed observational associations between BMI and CRP (ratio of geometric means of CRP per unit change BMI 1.06(1.02, 1.11), p=0.002). These data suggest that the observed association between circulating CRP and BMI is driven by BMI, with CRP being a simply a marker of elevated adiposity. Reciprocal Mendelian randomisation has applicability in determining direction of causality within inter-correlated biological networks. Methods such as this provide an approach for delivering immediate and clinically applicable information, which operates at a level above that of basic genetic associations.

## 1761/T/Poster Board #310

**A New Statistic in Evaluating Imputation Reliability.** *P. Lin[1], Z. Zhang[1], S. Hartz[1], S. Saccone[1], J. Wang[1], M. Schuckit[2], J. Tischfield[3], L. Bierut[1], J. Rice[1], COGA Collaborators, COGEND Collaborators, GENEVA.* 1) Washington Univ in St L, St. Louis, MO; 2) School of Medicine, University of California, San Diego, CA; 3) Dept of Genetics/Human Genetics Institute, Rutgers University, Piscataway, NJ.

Imputation is commonly used in genome wide association studies to boost power. Statistics for measuring the accuracy of imputation are frequently inadequate for evaluating the quality of imputation because concordance due to chance increases dramatically as alleles become more rare. Specifically, imputation accuracy, a measure of the concordance rate between the imputed and observed genotypes for each SNP, as well as average maximum posterior probability, dramatically over-estimates reliability when minor allele frequencies are low. Here we introduce a new statistic, the imputation quality score (IQS), as an improved measure to differentiate between well-imputed SNPs and poorly-imputed SNPs. IQS adjusts for chance agreement and addresses the inflation of false positive rate arising from imputation error. We used about 4000 individuals genotyped on the Illumina 1M array for SAGE, one study in the Gene Environment Association (GENEVA) project. We randomly divided the data into two subgroups labeled cases and controls. In cases, original genotypes were retained only for SNPs on the Illumina 550K array; these were then imputed to the full Illumina 1M array. In controls, original genotypes were retained for all SNPs on the Illumina 1M array. The initial Q-Q plots showed many false positives with values of -log(P) exceeding 100, reflecting imputation error. In order to improve the Q-Q plots, we compared the effect of filtering by traditional imputation quality measures to filtering by IQS. Traditional measures were unable to distinguish well-imputed SNPs from poorly-imputed SNPs. But, after using IQS to filter out poorly-imputed SNPs, we successfully adjusted the Q-Q plot back to an acceptable level. This demonstrates the IQS's potential usage in combining genotype data from different arrays.

## 1762/T/Poster Board #311

**Methylation and gene expression microarray data integration.** *S. Sun.* Math Biosci Inst, Ohio State Univ, Columbus, OH.

DNA methylation plays a very important role in the silencing of tumor suppressor genes in various tumor types. In order to gain a genome-wide understanding of how changes in methylation affect tumor growth, the differential methylation hybridization (DMH) protocol has been developed and large amount of DMH microarray data have been generated. However, it is still not clear how to integrate these high throughput methylation data with gene expression data to obtain a clear picture of the role of methylation in the whole genome. In this poster, we propose a novel three step procedure for data integration: (1) using a quantile regression method to identify genes that are commonly hyper-methylated, (2) identifying the co-expression network, and (3) using the idea of one-to-many mapping to find the methylated hub genes that are significantly associated with genes in each network. This procedure is illustrated in breast cancer cell line data.

## 1763/T/Poster Board #312

**How much variation is there among copy number variation algorithms? A case study using the Affymetrix SNP Array 6.0.** *E.J. Atkinson[1], M. de Andrade[1], J. Eckel-Passow[1], W. Bamlet[1], M. Matsumoto[1], S. Maharjan[1], S. Kardia[2].* 1) Div Biomedical Statistics, Mayo Clinic, Rochester, MN; 2) Epidemiology, University of Michigan, Ann Arbor, MI.

As the number of genome wide association studies (GWAs) increase, an additional benefit is the ability to investigate copy number variation (CNV). CNV data obtained from GWA SNP chips is a relatively new technology and there are an increasing number of software packages devoted to extracting CNV data. However, there has been no formal comparison exploring the impact that these different software packages have on the corresponding results. We extracted CNV data from the Affymetrix SNP Array 6.0 on 854 samples of hypertensive sibships from Rochester, MN, using PennCNV, Canary, and the R packages CRLMM and aroma.affymetrix. Variations among normalization, impact of "bad" samples, and choice of a reference sample were explored. A comparison of these four software packages will be discussed using the hypertensive data.

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## 1764/T/Poster Board #313

**Genome-Wide Association Studies (GWAS): Performance comparison between Illumina® 1M BeadChip and Affymetrix Genome-Wide Human SNP Array 6.0 (Affy 6.0) GWA arrays.** *K. Hetrick[1], B. Marosy[1], M. Zilka[1], C. Oncago[1], J. Romm[1], H. Ling[1], K. Barnes[2], E. Pugh[1], K. Doheny[1].* 1) Center for Inherited Disease Research (CIDR) and Genetic Resources Core Facility (GRCF) SNP Center, IGM, JHUSOM, Baltimore, MD; 2) Johns Hopkins Asthma and Allergy Center, Johns Hopkins University, 5501 Hopkins Bayview Circle, Baltimore, MD.

CIDR provides high quality genotyping services and statistical genetics consultation to investigators working to discover genes that contribute to common disease. Previously, we only offered GWA SNP genotyping services using Illumina products. As part of our process to evaluate offering an Affy 6.0 array service to our investigators, we performed a side by side experiment investigating performance between the two arrays. Sample and SNP statistics were analyzed on a sample set consisting of related and unrelated study and HapMap samples with 4 replicate pairs (136 total samples). Illumina data was analyzed with Illumina's BeadStudio and Affymetrix was analyzed with birdseed v2 in Affymetrix's Genotyping Console. In order to determine a call rate threshold for Affymetrix data, we looked at the discordance rate between both sets of data where the SNPs matched. As a sample's call rate fell to 98% on Affymetrix (matching Illumina call rate >99%), the discordance rate rose above 1%. Mean sample call rates were 99.0% (SD 0.6%) for Affymetrix and 99.5% (SD 0.2%) for Illumina. Error rates in replicates were 0.25% (Affymetrix) and 0.02% (Illumina). Mendelian error rates were 0.71% (Affymetrix) and 0.04% (Illumina). After applying a SNP technical filter (call rate <85%, >2 replicate errors), mean autosomal SNP call rates were 99.29% (SD 1.84%) for Affymetrix and 99.80% (SD 0.97%) for Illumina. Estimates of the proportion of unusable SNPs for an association study (call rate <90%, HWE <10-6 or MAF <1% in autosomal SNPs) were 12.4% (Affymetrix) and 10.4% (Illumina). Further experiments were done to evaluate the ability to detect mixed genomes as well as the effect of DNA concentration on both arrays. In related mixtures (50/50, 75/25, 90/10 and 95/5), >10% mixture failed call rate on Illumina with error rates <0.04% for passing mixtures; while >25% mixture failed call rate on Affymetrix with error rates <0.23% for passing mixtures. In unrelated mixtures (50/50, 66/33, 85/15, 90/10), all call rates were <98%. Nine identical HapMap aliquots were assayed using a DNA concentration gradient ranging from 10 ng/ul to 300 ng/ul. QC measures were stable across the range for Illumina while a decrease in data quality was demonstrated for Affymetrix below 50 ng/ul. These experimental results provided us the confidence to provide Affy 6.0 as a genotyping service. (Please see Romm, et al., Barnhart, et al., Ling et al. abstracts for related information).

## 1765/T/Poster Board #314

**Quality score recalibration of next-generation sequencing reads : application to the 1000 Genomes data.** *A. Menelaou, J. Marchini.* Department of Statistics, University of Oxford, Oxford, United Kingdom.

Next-generation sequencing technologies are making it possible to carryout whole-genome sequencing at the population scale and are likely to be widely used in all aspects of human genetics research. These technologies produce short sequencing reads together with Phred scores for the read bases. These Phred scores encapsulate the uncertainty of read bases and should ideally be propagated through to any subsequent analysis of the data, such as novel SNP detection. We have investigated whether improvements in the calls and their estimates of Phred scores can be improved through the use of contextual information about the read such as the flanking sequence content, flanking base Phred scores and the position in the read of the base. To do this we selected all the aligned reads from 10 2Mb regions on chromosome 20 in 16 CEU individuals from the April release low coverage pilot project of the 1000 Genomes data. We used the program samtools to retrieve the subset of reads overlapping SNPs present in both the HapMap2 and HapMap3 datasets. In each individual we then focussed exclusively on reads where the HapMap2 and HapMap3 genotypes both reported the same homozygote genotype and we took the genotyped allele to constitute the truth underlying that read. We excluded reads that showed evidence of indels and had low mapping quality (< 60). The raw error rate for this set of reads was 1.018%. Examination of the raw data indicated noticeable variation in error rate according to the context of the sequence read and the position of the base in the read. We fit several different neural network models to try to predict the true allele based on the available contextual information about each read. The best model we found consisted of 1 level of 4 hidden units and included the following predictors : the read base, the flanking read bases, the Phred score at the read base, the sum of the Phred scores at the flanking bases and the relative position of the read base in the read. This model gave a cross-validated error rate of 0.994%. All the reads that were corrected by this recalibration had Phred scores below 10. The re-calibrated Phred scores from our model are well calibrated at scores above 20 but show a slight underestimation of accuracy at lower scores. Our results illustrate that contextual information can be used to improve the overall all error rate of the reads and have the potential to save as many as $10^{10}$ reads in the 1000 Genomes project.

## 1766/T/Poster Board #315

**Analysis of homozygous runs of SNPs in the 11 HapMap 3 populations.** *F.J. Boehm, T.R. Bhangale, B.S. Weir, GENEVA.* Dept Biostatistics, Univ Washington, Seattle, WA.

Extended tracts of homozygous single nucleotide polymorphisms (SNPs) arise due to a multi-generational dynamic interplay among inbreeding, recombination, and other processes. Lencz et al. and others have developed methods for association mapping using runs of homozygosity. Characterization of homozygous runs in the eleven diverse HapMap3 populations represents a first step in the process of identifying potential disease-associated genomic regions in populations of differing continental ancestry. Additionally, our methods may aid in the detection of genotyping errors. We identified runs of homozygosity in the 11 HapMap3 populations. One novel aspect of our approach is that we implemented a hidden Markov model to identify sites of genotyping errors within homozygous runs, thus allowing more accurate determination of run lengths. In this sense, our methods may be applicable to data cleaning procedures in genome-wide association studies. After we identified the runs for each subject, we compared the number of runs and lengths of runs per subject by continental ancestry group. Furthermore, we investigated regions of shared homozygosity among subjects of a given continental ancestry. We further characterized the homozygous runs by examining the total probe intensities of those SNPs on the Affymetrix 6.0 chip. A region of decreased total probe intensity that coincides with a run of apparent homozygosity suggests that that particular region of homozygosity is explained by a hemizygous deletion that led to a monoallelic state, while no change in the total probe intensity over a run of homozygosity suggests that the region is one of biallelic homozygosity. Thus, our methods allow for detection of a second class of genotyping error. Given the current interest in genome-wide association studies (GWAS), we feel that our multifaceted characterization of runs of homozygosity in genome-wide SNP data is a valuable resource to those investigators who seek to identify disease-associated genomic regions and has potential value in GWAS data cleaning procedures.

## 1767/T/Poster Board #316

**Genotype Calling for Trisomic Samples.** *S. Cheong[1], E. Feingold[2].* 1) Dept Biostatistics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

Genotypes are "called" from raw intensity data using statistical clustering techniques, and software packages are available to do this for various genotyping technologies. For individuals with chromosomal abnormalities, however, standard genotype-calling software cannot be used. We describe several approaches to calling genotypes for a large sample of individuals with Down syndrome. We develop a method that first applies standard genotype-calling algorithms and then adjusts the calls to handle the trisomy, and compare it to a previously-published method that clusters the trisomic genotypes de novo. We illustrate and test both approaches with on several datasets.

## 1768/T/Poster Board #317

**Accurate IBD inference identifies cryptic relatedness in 9 HapMap populations.** *A. Dimitromanolakis[1], A.D. Paterson[2,1], L. Sun[1,3].* 1) Dalla Lana School of Public Health, University of Toronto; 2) Genetics and Genomic Biology, The Hospital for Sick Children, Toronto; 3) Department of Statistics, University of Toronto.

The HapMap project has been very successful in mapping human genetic variation. If numerous cryptic relationships exist among founder individuals in the dataset, the accuracy of allele frequency calculations, estimation of LD patterns, selection of tag SNPs, population stratification analysis and association studies could all be adversely affected. We extended our previous work (McPeek and Sun, 2000), developed for genome-wide linkage data, with PREST-plus, suitable for both population and family based genome-wide association studies, and we applied an accurate likelihood-based IBD inference via the EM algorithm to the most recent release (#27, phase 3) of HapMap data. Cryptic relatedness was detected in 195 of the 1002 founders (20%) . Among the 271 founder pairs that were shown to share more that 10% of their genome (p.IBD.0 < 0.9), we found strong evidence for first-degree relatives, including 21 parent-offspring and 27 full-sibling pairs. In particular, among the 4 phase II populations, we identified 4 related pairs in the CEPH founders and 3 related pairs in Yorubans in Ibadan. Among the 11 phase III populations, a very extended family structure was uncovered in Maasai, where 119 of the 180 founders were found to be related. Additional closely related pairs were present in 6 other populations. The results were validated using the method of moments IBD inference algorithm implemented in PLINK (Purcell et al., 2007). We would like to emphasize the importance of checking for cryptic relatedness in genetic studies and provide an outline of the necessary steps to achieve this, by the use of PREST-plus and companion post-processing R scripts assisting the interpretation of the results.

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## 1769/T/Poster Board #318

**Overcoming Data Quality and Copy Number Detection Issues in Genome-Wide CNV Association Studies.** *C. Lambert[1], G. Linse[1], J. Grover[1], D. Hawkins[2], J. Forsythe[1].* 1) Golden Helix, Inc, Bozeman, MT; 2) School of Statistics, University of Minnesota, Minneapolis, MN.

Incorporating copy number variations into genome-wide association studies promises to explain more of the heritability of common diseases than that accounted for by SNPs alone. This potential goldmine however, has been plagued by myriad of technical and experimental challenges. We examine the most persistent issues observed in over 20 CNV GWAS studies conducted by us and our collaborators. These include huge batch effects, genomic waves, mosaicism, T-cell artifacts and poor signal-to-noise ratios, all of which can lead to false positive and negative CNV detection and subsequent association findings. To address these issues we describe a novel principal component analysis approach that simultaneously corrects for batch and wave effects and population stratification, while significantly improving signal-to-noise ratios. We address the challenge of lingering batch effects in CNV regions. We then describe optimal segmentation methods which use dynamic programming to detect copy number segment boundaries on either a per-sample (univariate) or a multi-sample (multivariate) basis. Unlike Hidden Markov Model methods, which assume the means of different copy number states are consistent, optimal segmenting methods properly delineate segment boundaries in the presence of mosaicism, even at a single probe level, and with superior sensitivity and false discovery rates. We then outline several approaches to genome-wide scans for CNV association, demonstrating the utility of these methods on a series of large-scale GWAS.

## 1770/T/Poster Board #319

**Comparisons of calling strategies on Affymetrix® 6.0 arrays (Affy 6.0) using Birdseed v2.** *H. Ling, K. Hetrick, B. Marosy, S. Pottinger, J. Gearhart, C. Boehm, J. Romm, E. Pugh, K. Doheny.* CIDR, Johns Hopkins University, Baltimore, MD.

The Center for Inherited Disease Research (CIDR) provides high quality genotyping services and statistical genetics consultation to investigators working to discover genetic variants that contribute to common diseases. Recently, we have begun to offer an Affy 6.0 genotyping service and use Birdseed v2 to call genotypes. As Birdseed is a multiple-chip calling algorithm, genotype calls can be made either by using all samples (call-by-project) or a subset of samples that were processed in the lab together (call-by-batch) for each study. To find an appropriate calling method for our lab, we assessed the performance of the two calling methods by examining a set of SNP as well as sample level QC metrics. The analysis was performed on a case-control study of T2DM, our 1st Affy project, which includes 2,007 AA samples. After excluding samples that failed our daily QC filters, 2,143 samples including 46 blind duplicates and 90 HapMap controls were called by Birdseed v2 using the two SNP clustering approaches. Data were analyzed in PLINK and SAS. The mean sample call rate (CR) was 99.5% for call-by-project and 99.72% for call-by-batch with an average genotype concordance rate of 99.92% between the two methods. 21 samples showed a CR difference greater than 1%. Among the 70 related HapMap samples, the mean Mendel error rate was 0.10% for both methods. The error rates per blind duplicate pair were less than 1% in both methods. In total, 906,600 SNPs (868,157 autosomal) were called for each sample. Similar to sample level QC metrics, call-by-batch showed a higher autosomal SNP CR but lower concordance rate (both HapMap and blind duplicate) than call-by-project. Although call-by-project had a slightly higher number of Mendel errors than call-by-plate (27,641 vs. 27,004), after removing SNPs with CR below 95%, the number of Mendel errors drops 31% for call-by-project and 7.8% for call-by-batch. In addition, nearly 59% and 29% SNPs with CR below 95% have Mendel errors when called by project and by batch, respectively. By comparing these statistics between the two calling methods, we found 4.2% SNPs have CR difference greater than 1%, and 0.08% SNPs have a MAF difference greater than 10%. 10,224 and 8,696 SNPs had HWE P-value $< 10^{-5}$ for call-by-project and call-by-batch. Among 861,495 SNPs that were above this threshold for at least one method, 6,655 showed a difference in -logP greater than 5. Our current practice is to release genotypes called using both strategies.

## 1771/T/Poster Board #320

**Single-sample QC for the Affymetrix Next Generation Genotyping Platform.** *Y. Lu, E. Wang, M. Moorhead, T. Webster, S. Cawley.* Affymetrix, Inc. 3420 Central Expressway Santa Clara, CA 95051.

Sample QC is an important analysis step in any Genome Wide Association Study. It is well known that the quality of genotyping results is affected by the sample quality and the data quality of samples used in the genotype clustering. Therefore, it is particularly useful to be able to perform single-sample QC analysis to identify and exclude all low-quality samples before running genotype clustering.

We have developed a new set of single-sample QC metrics for the Affymetrix next generation genotyping platform, a two-color ligation based genotyping assay. The metrics assess the single sample quality at both the single-color level and the two-color level. Our results show that the two-color level QC metric is strongly correlated with the overall data quality for the sample and effectively identifies and removes low quality samples. These metrics are helpful both in the process of optimizing the system and in for use in future association studies.

## 1772/T/Poster Board #321

**Impact of sample pretesting in a high through-put genotyping facility.** *B. Marosy, C. Boehm, B. Craig, J. Romm, C. Oncago, M. Zilka, M. Adams-Carr, Y. Osimokun, K. Hetrick, H. Ling, E.W. Pugh, K.F. Doheny.* CIDR/GRCF-IGM, JHU-SOM, Baltimore, MD.

DNA samples received by CIDR undergo pre-testing (if funding supports this step) prior to production genotyping to confirm gender, detect Mendelian inconsistencies, assess sample quality and provide a genotype barcode to track samples through production lab processing and data release. Problems are reported allowing for samples to be replaced, dropped or information to be corrected. We present results for 12 GWA projects and 17 linkage and custom SNP projects that were genotyped using the Illumina® Infinium and GoldenGate platforms. Data was collected on sample problems identified at pre-testing and prior to data release. Investigator responses to problems reported at the pre-testing phase were categorized based on the outcome for each sample problem and whether replacing a sample or changing sample information fixed the problem.

| Total samples | 41748 (GWA) | Avg/ GWA proj | Range/ GWA proj | 22786 (non-GWA) | Avg/non-GWA proj | Range/non-GWA proj |
|---|---|---|---|---|---|---|
| Testpanel problem | 1676 | 4.0% | 0.3%-10.7% | 822 | 3.6% | 0%-8.6% |
| Problem fixed | 1552 | 3.7% | 1%-10.6% | 578 | 2.5% | 0%-6.1% |
| Problem not fixed | 124 | 0.3% | 0%-0.8% | 244 | 1.1% | 0%-3% |
| New at release | 359 | 0.9% | 0%-2% | 485 | 2.1% | 0%-9.1% |

Pre-testing has proven to be a helpful tool to aide in providing high quality genotyping data by validating sample information, evaluating sample quality and minimizing costs. For example, for an average GWA study of 3,000 samples, the pre-testing process saves $40,000. For the GWA project with the greatest number of identified problems, over $300,000 was saved.

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## 1773/T/Poster Board #322

**Evaluating the Effects of Batch Composition and Size on BRLMM Genotype Calls for the Affymetrix 500K Array.** *K. Miclaus, MAQC Genome-Wide Association Working Group.* Statistics, SAS Inst Inc, Cary, NC.

The Affymetrix GeneChip Human Mapping 500 K array is a popular choice for Genome-Wide Association Studies (GWAS). Recent findings have highlighted the importance of accurate genotype calling algorithms to reduce inflation in Type I and Type II error rates. Differential results due to genotype calling errors can introduce severe bias into case-control association study results. With data from the Wellcome Trust Case Control Consortium, 1991 individuals with coronary artery disease (CAD) and 1500 controls from the UK Blood Services (NBS) genotyped on the Affymetrix 500 K array, different batch sizes and compositions were used in the Bayesian Robust Linear Model with Mahalanobis distance classifier (BRLMM) genotype calling algorithm in order to assess the batch effect on downstream association analysis results. Results show that the composition (cases and controls genotyped simultaneously or separate) as well as size (number of individuals processed by BRLMM at a time) can create 2-3% discordance in the SNP results for quality control and statistical analysis and may contribute to the lack of reproducibility between GWA studies. Changes in batch size are largely responsible for differential SNP results, yet we find evidence of an interactive effect of batch size and composition that contributes to discordant results in the list of significantly association loci.

## 1774/T/Poster Board #323

**Automated evaluation of signal intensities - cluster validity measures are great.** *A. Ziegler[1], A. Schillert[1], O.-J. Frahm[1], T. Zeller[2], D.F. Schwarz[1], S. Blankenberg[2].* 1) Inst Med Biometry & Statistics, Univ Luebeck, Luebeck, Germany; 2) Johannes Gutenberg University Mainz, Germany, Dept. Of Medicine II.

The visual inspection of signal intensity plots (cluster plots) by two experienced independent readers is standard in the quality control of genome-wide association studies (GWAs). This process is time-consuming and expensive, and therefore restricted to candidate single nucleotide polymorphisms (SNPs) in practice. Thus, the valid judgement of signal intensities for all SNPs from a GWA through an automated procedure would be helpful. Even more so, meta-analyses which require imputed genotypes and machine learning approaches like random forests would greatly profit from this. So far, only few approaches for automatically evaluating signal intensities have been proposed [Plagnol et al. 2007 PLoS Genet, 3:e74, Schillert et al. 2009 BMC Proc, in press; Teo et al. 2008 Ann Hum Genet, 72:368-74]. We embed the problem of signal intensity plot inspection in the well-developed theory of measuring cluster validity in cluster analyses. We propose various measures for judging cluster compactness, cluster homogeneity, cluster connectedness, cluster separability and combinations of these criteria. We jointly evaluate these criteria through a random forest approach and propose a simple combination of the aforementioned criteria. The criteria are evaluated using 3300 samples from the Gutenberg Heart Study which has been genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0 in conjunction with Birdseed 2. The gold standard for signal intensity plots is provided through ratings from two independent readings of experienced readers for 5000 SNPs which successfully passed the standard quality criteria (call rate, minor allele frequency and deviation from Hardy-Weinberg equilibrium). We show that a simple combination of cluster validity measures show satisfactory agreement between visual and automated inspection of signal intensity plots. The proposed approach is extremely helpful for reducing the laboratory workload.

## 1775/T/Poster Board #324

**Does it matter which genotype calling algorithm is used for the Affy SNP 6.0?** *M. de Andrade[1], E. Atkinson[1], W. Bamlet[1], M. Matsumoto[1], S. Maharjan[1], S. Kardia[2].* 1) Div Biomed Statistics, Mayo Clinic, Rochester, MN; 2) Epidemiology, University of Michigan, Ann Arbor, MI.

As the number of genome wide association studies (GWAs) increases, SNP results are being obtained using different platforms and different calling algorithms. However, there has been no investigation focusing on the impact these algorithms might have on the results of a genome-wide association study. We will focus our work on the Affymetrix 6.0 platform where there are two genotype calling algorithms (GCA) available: Birdseed and CRLMM. These two algorithms have different recommended workflows including SNP and sample quality control selection criteria. We have evaluated these two GCAs and have come up with some best practice guidelines. For instance, Affymetrix suggests filtering samples based on contrast quality control (CQC) values prior to running Birdseed, using only samples with CQC > 0.4. On the other hand, CRLMM uses the sample quality score (SNR) and recommends that samples should be flagged with SNR values < 5. Based on our own experience we conclude that it is important to use these screening algorithms and that there is a strong correlation between these two algorithms. We used results from both GCA in our GWA and found that the study results were similar and that there appeared to be no systematic difference. We will present our results using 854 samples of hypertensive sibships from Rochester, MN.

## 1776/T/Poster Board #325

**A simple test for detecting genotyping errors using linkage disequilibrium information.** *B. Han[1], B. Hackel[2], E. Eskin[2,3].* 1) Dept Computer Sci, Univ California, San Diego, La Jolla, CA; 2) Dept Computer Sci, Univ California, Los Angeles, Los Angeles, CA; 3) Dept Human Genet, Univ California, Los Angeles, Los Angeles, CA.

In genetic association studies using high-throughput genotyping technologies, genotyping errors can occur at certain positions of single nucleotide polymorphisms (SNP) resulting in spurious associations. Tests based on Hardy-Weinberg equilibrium (HWE) may fail to detect these errors if the error SNPs do not deviate from HWE. Recent availability of reference datasets allows us to estimate the linkage disequilibrium (LD) between SNPs. The LD information can be used to detect genotyping errors under the following reasoning. If a significant peak of p-values is due to a true association, nearby correlated SNPs will show a mountain-looking shape of significance. If a significant peak is due to genotyping error, nearby correlated SNPs will show a flat non-significant p-values. However, there is no known method that incorporates this information, which often leads to a non-systematic manual examination of errors. We propose a simple statistical testing called TED (genoTyping Error Detection) that systematically detects genotyping errors using the LD information. Given a significant SNP, commonly used statistics at nearby SNPs asymptotically follow a multivariate normal distribution (MVN). The null and alternative MVN densities can be approximated using the LD information and the effect size estimated at the significant SNP. Then, the likelihood ratio of two densities conforms a TED statistic. Assuming reference data is correct, TED is optimal if the effect size is correctly estimated. Even if the effect size is incorrectly estimated, TED still controls type I error rate. The performance of TED is stable and dependent little on the choice of nearby SNPs. TED is applied to the WTCCC data and systematically removes SNPs that are previously removed by a manual examination.

## 1777/T/Poster Board #326

**A Method to Impute Genotypes at Untyped SNPs.** *Z. Zhang[1,2], H. Qin[1], S. Zhang[1,3], Q. Sha[1].* 1) Mathematical Sciences, Michigan Technological Univers, Houghton, MI; 2) School of Computer Science and Technology, Heilongjiang University, Harbin,150080, China; 3) Department of Mathematics, Heilongjiang University, Harbin 150080, China.

In genome-wide association studies, only the genotypes of tagging single nucleotide polymorphisms (SNPs) are typed only. If we can fill in the gap of genotypes of causal untyped SNPs, the genome-wide association study can be more powerful. In this article, we propose an efficient ImPutation Expectation-Maximization (IPEM) algorithm to infer the genotypes at untyped marker loci. We utilize the linkage disequilibrium (LD) structure of an available reference dataset to infer genotypes at untyped SNPs in the dataset of a new study. The proposed approach is independent of statistical models and significantly outperforms the popular hidden Markov model (HMM) approach (Marchini et al., 2007) in terms of inferring accuracy and computational efficiency. We illustrated the practical advantages of our algorithm by applying it to 22 chromosomes on the amyotrophic lateral sclerosis (ALS) dataset and using the HapMap dataset as the reference dataset. Compared with the popular HMM method, the proposed algorithm improved inferring accuracy by 2-7% and doubled the computational efficiency.

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## 1778/T/Poster Board #327

**Parallel genotype calling using neighboring loci for Affymetrix SNP 6.0 platform.** *Y.T. Chen[1], S.N. Hsu[2], C.Y. Tang[1], W.P. Hsieh[2].* 1) Department of Computer Science, NTHU, Hsinchu, Taiwan; 2) Institute of Statistics, NTHU, Hsinchu, Taiwan.

Current high throughput technologies allow researchers to genotype a huge number of Single Nucleotide Polymorphisms (SNPs) for several thousands of samples with arrays. The array-based data needs a genotype calling step before the data can be used in the genome-wide association study. The transformation from continuous intensity to discrete genotypes usually requires classification techniques on the features combined from several probes in the Affymetrix platforms. Most of the algorithms work on a SNP-by-SNP base and process multiple arrays at the same time. The most popular approaches include BRLMM, CRLMM and Birdseed. The call rates and concordance rates are generally very high for most experiments with SNP arrays using any one of the methods, but none of these methods give a satisfying solution to SNPs of low quality. In addition, only Birdseed has detailed performance reported on Human SNP 6.0 arrays. Hence, we try to include extra information and propose two improvements in this study. The first one is to include the features from the nearby SNPs. It implicitly integrates the strength of linkage disequilibrium as the features are transmitted together and have linkage structure in it. The idea has been widely used in the imputation techniques, which is based on the genotype data. We work on the intensity data directly instead of working on the genotype data as a second step to correct the errors in the calling step. The second suggestion is to include arrays of high quality from other experiments. Based on our empirical study, the call rates can be enhanced by increasing the sample size in the batch when adopting multi-array genotype calling methods. There is no need to match the sample sources as long as the extra arrays are of good quality. We will report the overall performance with samples from the HapMap project and genotyped with SNP 6.0. There are two experiments conducted by both the Affymetrix and Broad Institute and 906,600 SNP markers are assessed. The comparison is made for our proposed method as well as CRLMM, BRLMM and Birdseed. The result shows that the inclusion of neighboring SNPs has an edge on tightly linked regions where $r^2 > 0.8$.

## 1779/T/Poster Board #328

**BRLMM-P: an adaptable SNP genotyping algorithm for a variety of Affymetrix arrays.** *S. Cawley, P. Dickinson, R. Lu, A. Pirani, T. Webster, E. Hubbell.* Affymetrix, 3420 Central Expressway, Santa Clara, CA 95051, USA.

"BRLMM-P" is a genotyping method that takes normalized data from two alleles across multiple arrays and generates genotypes and confidences for each experiment and SNP. In the context of its development, much consideration has been given to extension of the framework to new situations, allowing for relatively straightforward adaptation to a variety of different Affymetrix array applications including the SNP5.0, SNP6.0 and DMET Plus arrays, in addition to some model organism arrays and the next-generation ligation-based genotyping assay. BRLMM-P performs genotyping using three subcomponents derived from the "BRLMM" method: generation of tentative genotypes from either reference data or the DM methodology, creation of cluster models from the tentatively labeled data points and prior information, and the application of the cluster models to yield genotypes. BRLMM-P uses the same underlying structure, but generates the tentative genotypes directly from the data. The first subcomponent generates tentative genotypes for the data points based on a 1-dimensional Gaussian mixture model. The likelihood model includes several special features: a Bayesian prior, which allows incorporation of previous information, a penalty for disagreeing with reference data, which allows for errors in training data, and isotonic regression, which prevents clusters from occurring in incorrect orders. These exposed features of the likelihood are "affordances" which simplify training. The second sub-component takes tentative genotypes and updates a Bayesian prior normal-inverse-Wishart mixture model resulting in a posterior mixture model of the same distributional form. Because the prior and posterior have the same form, an output posterior from one run of the code can serve as a prior in another run. The third sub-component utilizes the mixture model to make genotype calls based on the likelihood that a data point belongs to a specific cluster. This component can be run in "single-sample" mode, in which there has been no update of the mixture model, and we are only interested in generating genotype calls. BRLMM-P has been successfully used over multiple generations of products and completely different assays, not because it has the optimal clustering designed for any particular application, but because it has affordances allowing the full cycle of training to take place quickly and accurately. This makes it a useful research tool as well as a useful genotyping method.

## 1780/T/Poster Board #329

**The PhenX Toolkit - get the most from your measures.** *C. Hamilton[1], I. Strader[1], J. Pratt[1], J. Hammond[1], T. Hendershot[1], W. Huggins[1], D. Jackman[1], R. Kwok[1], D. Maiese[1], D. Nettles[1], H. Pan[1], D. Wagener[1], M. Zmuda[1], H. Junkins[2], R. Li[2], E. Ramos[2], W. Harlan[3], J. Haines[4].* 1) RTI International, Res Triangle Park, NC; 2) NHGRI, Bethesda, MD; 3) Retired, NIH; 4) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Despite the vast potential for cross-study comparisons, the lack of standardized or comparable phenotypic and environmental measurements has limited the ability to combine data from GWAS and other genomic studies. To enhance cross-study analyses, RTI International and the National Human Genome Research Institute (NHGRI) are collaborating on a three- year project called PhenX (consensus measures for Phenotypes and eXposures). The goal of PhenX is to identify 15 high-priority, well-established, measures in each of 20 research domains. Working Groups (WGs) of domain experts select these measures via a consensus-building process that also considers input from the broader scientific community. The measures selected by the WGs are made publicly available via the PhenX Toolkit. Researchers will want to visit the PhenX Toolkit to select measures as they plan a new study or to add measures to an existing study. Thus, the Toolkit provides a common currency for investigators who want to be able to effectively combine and/or compare their data with data from other studies. The Toolkit makes it easy for investigators to broaden their study by adding measures that are outside of their primary research focus. Visitors can search or browse the Toolkit to view PhenX measures, and then add measures of interest to a Cart. As measures are chosen, the Toolkit prompts the User to review other measures that are essential or related. Registered Users may name and save their Cart(s), and then come back later to review and/or revise. Registered Users will also have the option of sharing their Cart(s) with other Registered Users. For each PhenX measure the Toolkit provides a brief description of the measure, the rationale for including the measure in the Toolkit, protocol(s) for collecting the measure, supporting information and references. The User can generate a report that incorporates all of this information. By the end of 2009, the PhenX Toolkit is expected to include measures that were selected by 11 WGs, including Demographics, Anthropometrics, Alcohol, Tobacco and Other Substances (ATOS), Cardiovascular, Nutrition and Dietary Supplements, Environmental Exposures, Oral Health and Cancer. Broad acceptance and use of PhenX measures should greatly facilitate cross-study analysis. You can visit the PhenX Toolkit at https://www.phenxtoolkit.org/. Supported by: NHGRI, Award No. 1U01 HG004597-01.

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## 1781/T/Poster Board #330

**A new PAGE in understanding complex traits: study design for analysis of Population Architecture using Genomics and Epidemiology.** *T.C. Matise[1], J.L. Ambite[2], S.A. Cole[3], D.C. Crawford[4], C.A. Haiman[5], C. Kooperberg[6], L. Le Marchand[7], T.A. Manolio[8], K.E. North[9], U. Peters[6], M.D. Ritchie[4], L.A. Hindorff[8], J.L. Haines[4] for PAGE.* 1) Dept Genetics, Rutgers Univ, Piscataway, NJ; 2) Information Sciences Institute, Univ of Southern California, Los Angeles, CA; 3) Dept Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 4) Center for Human Genetics Research, Vanderbilt Univ, Nashville, TN; 5) Keck School of Medicine, Univ of Southern California, Los Angeles, CA; 6) Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 7) Cancer Research Center, Univ of Hawaii, Honolulu, HI; 8) Office of Population Genomics, NHGRI, NIH, Bethesda, MD; 9) Dept Epidemiology, Univ of North Carolina, Chapel Hill, NC.

By relating hundreds of thousands of genotypes to only a few phenotypes, mostly in individuals of one ancestry, genome-wide association (GWA) studies are identifying a rapidly growing number of associations. The Population Architecture using Genomics and Epidemiology (PAGE) Study is designed to further characterize the most promising variants along an epidemiological dimension that is substantial in its sample size, ethnic diversity, breadth of phenotypes, and exposures. PAGE includes scientists and population samples from large ongoing cohort studies: CALiCo (Causal Variants Across the Life Course, a consortium of ARIC, CARDIA, CHS, HCHS/SOL, Strong Heart Cohort Study, Strong Heart Family Study), EAGLE (Epidemiologic Architecture for Genes Linked to Environment, based on 3 National Health and Nutrition Examination Surveys (NHANES)), MEC (Multiethnic Cohort) and WHI (Women's Health Initiative), with logistical and scientific support contributed by a Coordinating Center and the NHGRI Office of Population Genomics. These studies combined include over 290,000 participants, and populations represented include Asian Americans, African Americans, European Americans, Hispanic Americans, Native Hawaiians and American Indians. Health outcomes and traits of interest are prioritized, followed by replication of trait-genotype associations and generalization of their effects across population groups and environmental contexts. The first round of genotyping focused on 256 high-profile SNPs having replicated associations with phenotypes related to diabetes, obesity, cardiovascular disease, lipids, inflammation and autoimmunity that are being genotyped in over 90,000 participants, as available for trait-specific analyses. Subsequent genotyping will examine new variants in these and other traits such as cancers. SNP genotyping, quality control and population-specific association analyses are performed within each cohort, followed by meta-analyses using harmonized phenotypes and standardized analytic methods. Novel display tools are being developed to facilitate exploration and dissemination of the large number of association tests being conducted. Individual-level or summary data will be deposited to dbGaP. PAGE will contribute context-specific population level annotation for promising variants from GWAS, addressing gene-gene and gene-environment interactions and intermediate outcomes. More information at http://www.pagestudy.org.

## 1782/T/Poster Board #331

**Assessing the accuracy of ancestry reported in a biorepository linked to electronic medical records for genetic association studies.** *M.D. Ritchie[1,2], L. Dumitrescu[1], K. Brown-Gentry[1], J. Pulley[3], M. Basford[3], J. Denny[4,5], J.R. Oksenberg[6], D.M. Roden[3,5,7], J.L. Haines[1,2], D.C. Crawford[1,2].* 1) Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 3) Office of Personalized Medicine, Vanderbilt University, Nashville, TN; 4) Department of Biomedical Informatics, Vanderbilt University, Nashville, TN; 5) Department of Medicine, Vanderbilt University, Nashville, TN; 6) Department of Neruology, University of California at San Francisco, San Francisco, CA; 7) Department of Pharmacology, Vanderbilt University, Nashville, TN.

The Vanderbilt DNA Databank (BioVU) is a biorepository of >57,000 DNA samples linked to electronic medical records in Nashville, TN. While BioVU is a valuable source of samples and phenotypes for genetic association studies, it is unclear whether administratively assigned race/ethnicity in BioVU is accurate in describing genetic ancestry for association studies. Previous studies suggest that self-reported ancestry is highly concordant with genetic ancestry. In contrast, there is much variation in the accuracy of observer-reported ancestry, and this variability can be highly dependent on the study, site, and method of interview. To characterize the accuracy of observer-reported ancestry compared with self-reported ancestry, we genotyped 360 SNPs on the Illumina DNA Test Panel containing ancestry informative markers (AIMs) in 1910 BioVU samples with observer-reported ancestry [1503 European-American (EA), 201 African-American (AA), 62 other, and 144 missing data] and 384 samples from the Multiple Sclerosis Genetics Group with self-reported ancestry (192 EA and 192 AA). After filtering for minor allele frequency (MAF<1%), deviations from Hardy-Weinberg expectations (p<10-4), and genotyping efficiency (<95%), 294 SNPs in the samples with self-reported ancestry were clustered by ancestry using STRUCTURE 2.2. After assuming K=3 and allowing 50,000 iterations and 10,000 burn-in cycles, 95.7% of the self-reported EA DNA samples were assigned to one cluster. After implementing similar quality control measures and STRUCTURE settings, 341 SNPs were used to cluster the observer-reported DNA samples. Similar to the self-reported clustering results, 96.9% of the EA observer-reported samples clustered together. For AA, 89.2% and 89.7% of samples with self-reported and observer-reported ancestry, respectively, clustered together. Among the BioVU samples with missing data, 90.1% clustered with EA samples and 9.30% clustered with AA samples. Using a two-sample test of proportions, we determined that the concordance rate of observer-reported ancestry was not significantly different from the concordance rate of self-reported ancestry in either population (z=-0.885, p=0.376 in EA and z=-0.161, p=0.872 in AA). This finding suggests that for EA and AA populations in BioVU, observer-reported ancestry is highly correlated with genetic ancestry determined by AIMs and thus probably sufficient for use in genetic association studies.

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### 1783/T/Poster Board #332

**A multi-stage genome-wide association study detects a novel risk locus near *IRS1* for type 2 diabetes, insulin resistance, and hyperinsulinemia.** *S. Cauchi[1], J. Rung[2,3], A. Albrechtsen[4], G. Rocheleau[2,5,6], C. Cavalcanti-Proença[1], A. Vaag[7,8], O. Pedersen[9,10], P. Froguel[1,11], R. Sladek[2,5,12], The IRS1 consortium.* 1) CNRS-UMR-8090, Institute of Biology and Lille 2 University, Pasteur Institute, F-59019 Lille, France; 2) McGill University and Génome Québec Innovation Centre, Montréal, Canada H3A 1A4; 3) European Bioinformatics Institute, Hinxton, Cambridge, CB10 1SD United Kingdom; 4) Department of Biostatistics, University of Copenhagen, DK-1014 Copenhagen K, Denmark; 5) Department of Human Genetics, Faculty of Medicine, McGill University Montreal, Canada H3A 1B1; 6) Prognomix Inc., Montreal, Canada H1Y 3L1; 7) Steno Diabetes Center, DK-2820 Gentofte, Denmark; 8) Unit of Diabetes and Endocrinology, Department of Clinical Sciences, Malmö University Hospital, Lund University, Malmö, Sweden; 9) Hagedorn Research Institute and Steno Diabetes Center, DK-2820 Gentofte, Denmark; 10) Department of Biomedical Sciences, University of Copenhagen, DK-2200 Copenhagen N, Denmark; and Faculty of Health Sciences, University of Aarhus, DK- 8000 Aarhus C, Denmark; 11) Centre and Department of Genomic Medicine, Hammersmith Hospital, Imperial College London, W12 0NN, London, UK; 12) Department of Medicine, Faculty of Medicine, McGill University, Montreal, Canada H3A 1A4.

Genome-wide association studies (GWAS) have identified a number of robust associations between specific chromosomal loci and type 2 diabetes (T2D). To fully utilize the GWA data that we had obtained in 1,376 French individuals (Sladek and colleagues, Nature, 2007), we designed a large stage 2 study to capture other possible association signals. From our existing datasets, we selected 16,360 SNPs nominally associated with T2D and genotyped these in an independent set of 4,977 French normoglycemic and T2D subjects. Based on their association strength, we selected 28 SNPs for replication in 7,698 Danish individuals and identified four SNPs showing strong association with T2D. Three of these represent signals in known loci and one is in a novel risk locus adjacent to the insulin receptor substrate 1 gene (*IRS1*: rs2943641, $p = 9.3 \times 10^{-12}$, OR = 1.19). IRS1 plays a key role in transmitting signals from the insulin and insulin-like growth factor-1 receptors to intracellular PI3K/Akt and Erk MAP kinase pathways. Unlike previously reported T2D risk loci, which predominantly associate with impaired beta-cell function, the C-allele of rs2943641 is associated with insulin resistance and hyperinsulinemia in 14,358 French, Danish and Finnish non-diabetic participants from population-based cohorts. Importantly, this risk allele is also associated with reduced basal levels of IRS1 protein and decreased insulin-induction of IRS1-associated PI3-kinase activity in human skeletal muscle biopsies. In conclusion, our stage 2 design and analysis approach allowed us to identify, for the first time, a novel T2D risk locus associated with whole body insulin resistance, hyperinsulinemia and impaired insulin signalling. Our study emphasizes the importance of using different strategies for genome-wide discovery of genetic loci related to T2D.

### 1784/T/Poster Board #333

**Evaluation of Factors Associated with Occurrence of Congenital Malformations in an Area Close to a Large Municipal Dump Site in Cali, Colombia.** *C. Isaza[1], F. Mendez[2], Y. Ariza[2], N. Benitez[2], Y. Orejuela[2], H. Pachajoa[1].* 1) Dept Morphology, Univ del Valle, Cali Valle, Colombia; 2) Dept Epidemiology, Universidad del Valle, Cali, Valle, Colombia.

Objective: A hospital based surveillance system was established since 2004 in Cali, Colombia, and geographical analysis of cases distribution showed malformations are clustered in poorer areas which are close to the main river of the city. This river is polluted by contaminants from the municipal waste disposal site and agricultural pesticides. A study is developed to evaluate environmental factors potentially associated to occurrence of clusters of congenital malformations in this area. Materials and Methods: We have collected information to describe routes and pathways of exposure to contaminants. Specifically, water, soil and air samples were analyzed. In addition, to understand population believes and practices which may facilitate intake of pollutants, we developed a survey among 380 women. Results: Neural tube defects and vascular disruption defects are among the most frequent malformations in the area (5 to 10 times higher occurrence as compared with similar surveillance systems in other cities of the world). Ground water and soil samples demonstrated presence of metals, particularly cadmium, lead and mercury. These contaminants and other pollutants, including pesticides, can reach pregnant women by several routes and pathways, including fish and crops in diet (oral) and also indoor exposure to batteries of vehicles and other chemical products (inhalation and dermal contact). Conclusions: Multiple routes and pathways allow exposure of women to pollutants and explain clustering of congenital malformations in this area. Indoor exposures and behavioral factors, as fish consumption during pregnancy, need to be considered to understand causes of congenital malformations.

### 1785/T/Poster Board #334

**Genome-wide studies of complementary designs identify coagulation and fibrinolytic loci and variants potentially implicated in venous thromboembolism.** *F. Gagnon[1], G. Antoni[1,2,3], N. Saut[4], Y. Luo[1], A. Tuite[1], P.S. Wells[5], J. Emmerich[6], P.E. Morange[4,7], D.A. Tregouet[2,3].* 1) School of Public Health, Univ of Toronto, Toronto, ON, Canada; 2) INSERM, UMR_S 937, F-75013, Paris, France; 3) UPMC Univ Paris 06, UMR_S 937, F-75013, Paris, France; 4) INSERM, URM_S 626, F-13385, Marseille, France; 5) Ottawa Health Research Institute, Ottawa, ON, Canada; 6) INSERM U765, Universite Paris-Descartes, France; 7) Universite de la Mediterranee, F-13385, Marseille, France.

A multi-stage/multi-design strategy is being used to identify new loci and variants that would contribute to venous thromboembolism (VTE) susceptibility by modulating coagulation and fibrinolytic factors known to be implicated in thrombosis and hemostasis. Linkage analyses of 26 quantitative traits were first performed on 5 extended French-Canadian families ascertained on a single proband with both VTE and the F5L variant. These analyses included 261 individuals genotyped for 1079 microsatellites. Using Bayesian MCMC-based oligogenic methods, we identified a total of 25 loci for 14 quantitative traits with Bayes Factors (logBF) >1.5 (strong evidence for linkage). Anti-coagulant and other treatment effects were taken into account in follow-up analyses. These regions were then validated by *in silico* association analysis of published GWAS data on VTE with the aim of narrowing the linkage signals by focusing on candidate regions for VTE. Among the results, the replication of the FXII chromosome (chr) 5 locus previously identified in the GAIT (Genetic Analysis of Idiopathic Thrombosis) study, and a novel locus on chr 8, with logBF of 2.9 and 1.9, respectively. One and two SNPs for chr 5 and 8, respectively, were associated with VTE at $p<10^{-4}$ in the GWAS. Prioritized analyses investigating their association with FXII in the Stanislas cohort, a sample of 123 French nuclear families, and with VTE in two independent French case-control studies, are underway. In conclusion, using a multi-stage approach based on pedigree linkage analysis of quantitative traits, followed by a GWAS of VTE in an independent sample, was critical in identifying putative variants involved in the susceptibility to VTE, as well as providing clues into the underlying mechanisms.

### 1786/T/Poster Board #335

**Effect of Genetic Architecture on Prediction of Complex Diseases.** *D. Hu[1, 2, 3], E. Ziv[1, 2, 3].* 1) Dept Medicine, Univ California, San Francisco, CA; 2) Institute for Human Genetics, Univ California, San Francisco, CA; 3) Hellen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA.

A common model of complex diseases, the “common disease common variant” model, suggests that by identifying common variants that have modest effects on disease, geneticists will be able to combine these to introduce accurate disease prediction tools. In contrast, rare variants that substantially increase risk have traditionally been considered minimally useful from a public health perspective. We relate the allele frequency, the number of loci, the population attributable risk and area under the curve of the ROC curve. Using these relationships we demonstrate that rare variants with a strong effect on disease are actually more useful in a public health setting than common variants with modest effects. Furthermore, we demonstrate that under many plausible circumstances, most of the disease prediction potential in the genome is actually in the rare allele frequency. Finally, we consider the practical sample size limitations on discovery for both rare and common variants related to disease and, at what point, disease prediction potential in the genome has been optimally reached. Our results have several important consequences for study design and for clinical practice: (1) Study designs may have currently reached or are close to reaching the limit of common variants useful for disease prediction. In contrast, the majority rare variants with disease prediction potential remain undiscovered. (2) Designing clinical prediction tools to optimally take advantage of the disease prediction potential of the genome will require sequencing individual patients at some genetic loci.

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## 1787/T/Poster Board #336

**Families versus unrelateds: An empirical comparison of gene mapping power based on genetic analysis of expression profile data.** *H.H.H. Göring, E.I. Drigalenko, M.A. Carless, J.E. Curran, M.P. Johnson, S.A. Cole, T.D. Dyer, L. Almasy, M.C. Mahaney, E.K. Moses, J. Blangero.* Dept. of Genetics, Southwest Foundation for Biomedical Resesarch, San Antonio, TX.

More than a decade ago, Risch and Merikangas suggested that association analysis is more powerful for identification of complex trait genes than linkage analysis. However, linkage versus association is a false dichotomy, because families permit joint usage of both strategies. A more fundamental question is the performance of study designs based on families versus unrelated individuals. It is now apparent that the genetic variants discovered in singleton-based GWAS explain only a small proportion of the estimated heritability. A possible explanation is that rarer variants are cumulatively responsible for a substantial portion of the genetic risk. Family-based studies are expected to be more powerful to detect such variants since rare variants, if present in a sample, are more likely to be found in multiple copies amongst related individuals. Additionally, linkage power is a function of the cumulative effects of functional variants across a larger genomic interval. To address these questions, we have performed an empirical power comparison between singleton- and family-based study designs, focusing on gene expression phenotypes. Our study has two key strengths: 1) it is not based on simulation and its dependence upon unknown parameters, and 2) the true state of nature is known, namely that proximal (*cis*) genetic regulation is common. Focusing on autosomal RefSeq transcripts, we have performed proximal linkage and joint linkage/association analyses in families, and association analysis on a subset of unrelated individuals. Our sample is the San Antonio Family Heart Study, where lymphocyte-derived expression profiles (Illumina WG-6 Series I BeadChip) and SNP genotypes (Illumina HumanHap 550 BeadChip) are available for 858 individuals. At a 5% FDR, we identified >1,000 proximally regulated transcripts using linkage analysis alone, and >5,000 such transcripts in joint linkage/association analysis. A comparison between association analysis on unrelated individuals and joint linkage/association on the same number of individuals in large families shows that in the presence of linkage evidence, joint tests can exhibit superior power to localize causal genes. In the absence of linkage evidence, little power tends to be lost relative to association in unrelateds. Our results suggest that joint linkage/association analysis in families represents a rational strategy that is not dependent upon prior assumptions regarding the frequency spectrum of functional variants.

## 1788/T/Poster Board #337

**A design of multi-source samples as a shared control for association studies in genetically stratified populations.** *Y. He[1], S. Xu[1], C. Jia[2], J. Li[1,2].* 1) Computational Genomics Department, CAS-MPG Partner Institute for Computational Biology, SIBS, CAS, Shanghai 200031, China; 2) MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences, Fudan University, Shanghai 200433, China.

Statistical power is one of the key issues in study design of GWAS. Having a larger sample size of normal controls and cases is usually considered in achieving higher statistical power in association studies. Shared control for different studies, or common control, is a possible cost effective solution to achieve a larger sample size. However, this design may be impeded by the presence of population stratification in the samples. Since most of the patient samples, i.e., cases, were pre-collected from large hospitals in cosmopolitan areas, it is sensible to assume that they often come from mixed sources or subpopulations. Population stratification is known to exist in Chinese populations across geographic area. Principles in a better, if not optimal, design for shared controls in Chinese could benefit association studies in all countries and areas. In this study, performance of different designs for shared controls in Chinese was evaluated with genetic parameters from real data. A better design is expected to yield higher power for a given sample size, which was calculated using a simulation algorithm throughout this study, given a predefined type I error (0.05) and other population parameters including sample size. The simulation algorithm incorporated a hierarchical model to address genetic divergence in multi-source scenarios. For risk allele frequencies at 0.1 and 0.3, we showed that power increases with increasing sources of controls, and the single-source control yields the lowest power. Another question of great importance is whether the shared control could benefit future association studies in which the diversity of the sources of samples is expected to increase. Our results showed that the multi-source designs gain more power than the single-source designs when cases increase. We also observed that the power continually improves with increasing sources of control subjects for both types of designs. But, the power increase was nearly stopped for the single-source control design when number of studies is more than 3, while the multi-source control design continues to gain more statistical power. To summarize, this study showed that a good design of shared control for populations with genetic heterogeneity should include samples from multiple sources. A good multi-source design wills not only benefit GWAS study using the existing cases but also the future studies when more patient samples are added.

## 1789/T/Poster Board #338

**Rapid inexpensive genome-wide association using pooled whole blood.** *S. Macgregor[1], A. Hewitt[2,3], A. McMellon[2], A. Henders[1], L. Ma[2], L. Wallace[1], S. Sharma[2], K. Burdon[2], P. Visscher[1], G. Montgomery[1], J. Craig[2].* 1) Genetics and Population Health, Queensland Inst Med Res, Brisbane, Australia; 2) Department of Ophthalmology, Flinders University, Adelaide, SA, 5046, AUS; 3) Centre for Eye Research Australia, Melbourne University, Melbourne, VIC, 3002, AUS.

Genome-wide association studies (GWAS) have now successfully identified important genetic variants associated with many human traits and diseases. The high cost of genotyping arrays in large datasets remains the major barrier to wider utilization of GWAS. Previously we have shown it is possible to reduce the genotyping cost by pooling DNA samples for genotyping. Here we have developed a novel method in which whole blood from cases and controls respectively is pooled prior to DNA extraction for genotyping. We demonstrate proof of principle by clearly identifying using blood pooling based GWAS the associated variants for eye color, age-related macular degeneration and pseudoexfoliation syndrome in cohorts not previously studied. Blood pooling has the potential to reduce GWAS cost by several orders of magnitude and dramatically shorten gene discovery time. This method has profound implications for translation of modern genetic approaches to a multitude of diseases and traits yet to be analysed by GWAS, and will enable developing nations to participate in GWAS.

## 1790/T/Poster Board #339

**Design of Association Studies with Pooled Next-Generation Sequencing Data.** *S. Kim.* Integrative Biology, University of California, Berkeley, CA.

Most of the common hereditary diseases in humans are complex and multifactorial. Large scale genome-wide association studies based on SNP genotyping, have only identified a small fraction of the heritable variation of these diseases. One explanation may be that many rare variants (a minor allele frequency, MAF > 5%), which are not included in the common genotyping platforms, may contribute a substantial portion of the genetic variation of these diseases. Next-generation sequencing, which would allow the analysis of rare variants, is now becoming so cheap that it provides a viable alternative to SNP genotyping. In this paper, we present cost-effective protocols for using next-generation sequencing in association mapping studies based on pooled and un-pooled samples, and identify optimal designs with respect to total number of individuals, number of individuals per pool, and the sequencing coverage. We perform a small empirical study to evaluate the pooling variance in a realistic setting where pooling is combined with exon capturing. To test for associations, we have developed a likelihood ratio statistic that accounts for the high error rate of next-generation sequencing data. We also perform extensive simulations to determine the power and accuracy of this method. Overall, our findings suggest that with a fixed cost, sequencing many individuals at a more shallow depth with larger pool size achieves higher power than sequencing a small number of individuals in higher depth with smaller pool size, even in the presence of high error rates. Our results provide guidelines for researchers who are developing association mapping studies based on next-generation sequencing.

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## 1791/T/Poster Board #340

**Genomic and genealogic investigation of the French Canadian founder population structure.** *M.-H. Roy-Gagnon[1,2], C. Moreau[1], C. Bherer[1], P. St-Onge[1], D. Sinnett[1,3], C. Laprise[4], H. Vézina[5], D. Labuda[1,3].* 1) Sainte-Jusine Hospital Research Center, Montreal, QC, Canada; 2) Dept. of Social and Preventive Medicine, University of Montreal, QC, Canada; 3) Dept. of Paediatrics, University of Montreal, QC, Canada; 4) Dept. of Fundamental Sciences, Université du Québec à Chicoutimi, QC, Canada; 5) GRIG, Université du Québec à Chicoutimi, QC, Canada.

It is thought that genetic epidemiological studies in founder populations may have greater power to identify genes for complex diseases due in part to greater genetic homogeneity. A relatively large number (~3,300) of original founders contributed to the majority of the contemporary French Canadian (FC) gene pool and subsequent regional founder effects also occurred. Hence, the extent of genetic homogeneity in the FC population is unclear. In this study, we investigated genome-wide variation and structure in 143 individuals from 7 FC sub-populations characterized by different demographic histories. We also situated our FC samples among the HapMap samples and the French samples of the Human Genome Diversity Panel (HGDP-CEPH). An important advantage of the FC population is the availability of major population registers, such as the BALSAC register, allowing the reconstruction of ascending genealogies going back over four centuries. We used both genealogic data and genome-wide SNP data (Illumina 650K) to characterize genetic variation and structure in the FC population. Principal components analysis on a subset of genome-wide SNPs spaced at least 500kb apart yielded 4 FC sub-populations (Tracy-Widom p-value < 0.02), 3 of which are geographically close but culturally different. Genomic inflation factors between pairs of these 4 sub-populations (assuming study cases come from one and controls from another) ranged from 1.2 to 1.4, while they were greater than 1.09 for all sub-populations pairs. We observed higher numbers and longer extended runs of homozygosity when within-population relatedness was higher. We also observed higher median linkage disequilibrium (LD) levels and proportions of SNPs in high LD in the FC sub-populations compared to HapMap CEU and French HDP-CEPH. Information on relatedness and inbreeding calculated from genealogies (14 generations) supports and complements our genomic-based findings. Our results highlight the need to consider both geographical and cultural origin of FC participants in association studies. Our study also provides insights on approaches to exploit both genomic and genealogic data in the search for disease genes in the FC founder population. These approaches may be especially relevant for the identification of rare variants.

## 1792/T/Poster Board #341

**Analysis of Genetic Substructure of Han Chinese Using Genome-Wide SNP Arrays: Implication for Association Studies.** *S. Xu[1], S. Li[2], W. Jin[1], H. Lou[1], L. Yang[1], X. Gong[2], X. Zhang[3], X. Yin[3], Y. He[1], Y. Yang[2], Y. Wang[2], W. Fu[2], Y. An[2], J. Tan[2], J. Wang[2], J. Qian[2], X. Zhang[2], H. Wang[2], B. Wu[2,4], L. Jin[1,2,5].* 1) Chinese Academy of Sciences and Max Planck Society (CAS-MPG) Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China; 2) State Key Laboratory of Genetic Engineering and Ministry of Education (MOE) Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, China; 3) Institute of Dermatology and Department of Dermatology at No.1 Hospital, Anhui Medical University, Hefei, Anhui 230022, China; 4) Children's Hospital Boston, Harvard University, Boston, MA 02115, USA; 5) China Medical City (CMC) Institute of Health Sciences, Taizhou, Jiangsu 225300, China.

China will start this year a $30 million effort of genome-wide association studies (GWAS) of common diseases in Chinese populations which have been largely underrepresented in the similar effort worldwide. A general concern is population stratification (ancestry differences) among subpopulations which can cause false positive associations. Han Chinese is the largest ethnic group in the world, however, its population substructures are often expected and yet well characterized. In this study, we examined population substructures in a diverse set of >1,700 Han Chinese samples collected from 26 regions, each genotyped with at least 160K single nucleotide polymorphisms (SNPs). Our results showed that: (a) Han Chinese population is complicatedly substructured, with the main observed clusters roughly corresponding to northern Han, central Han and southern Han; (b) Han Chinese samples collected from large cities, such as Shanghai, Beijing and Guangzhou, show diverse source of ancestries including three aforementioned clusters; (c) HapMap samples (CHB & CHD) and HGDP samples (Han & Han-NChina) deliver a limited representation of Han Chinese people. Building on the above insights, we investigated false positive rates and statistical power in various study designs using both empirical and simulated data. We further explored sample collection strategies and public data usage for future association studies.

## 1793/T/Poster Board #342

**A common variant of tumor necrosis factor-alpha gene and pregnancy-induced hypertention in Japan.** *G. Kobashi[1,4], K. Ohta[1,2,3], H. Yamada[2], A. Hata[3], H. Minakami[2], N. Sakuragi[2], H. Tamashiro[4], S. Fujimoto[5], Hokkaido PIH Epidemiological Study Group.* 1) Research Center for Charged Pa, National Institute of Radiological Sciences, Chiba, Japan; 2) Dept. of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine; 3) Dept. of Public Health, Chiba University Graduate School of Medicine; 4) Dept. of Global Health and Epidemiology, Hokkaido University Graduate School of Medicine; 5) Sapporo Maternity-Women's Hospital.

Pregnancy-induced hypertention (PIH), major cause of maternal morbidity and mortality, is considered to be a multifactorial disease resulting from an interaction among several factors including endothelial injury. Tumor necrosis factor-alpha (TNF-A) fundamentally effects on the endothelial cells by several pathways, such as oxidant/anti-oxidant balances, changing patterns of prostaglandin production and expression of cell surface components. Some recent studies have reported that an association between a common variants in promoter region (G-308A) of the TNF-A gene and PIH in some racial population, however, it has been yet confirmed. In the present study, we genotyped and analyzed the variant and other genetic and environment risk factors in order to confirm the association in the Japanese subjects. One hundred forty Japanese patients with PIH including preeclampsia, protein uric type of PIH, were matched with 280 Japanese normal pregnant controls according to age and parity. Informed consent for the study was obtained from every subject. Genotypings of variants including G-308A of the TNF-A gene were carried out using PCR-RFLP methods after extraction of genomic DNA from 1.0 ml of whole blood samples. Differences were statistically analyzed by the chi-square test (degree of freedom=1). Fisher's exact test was used when an observed number was ≤ 5. In the subgroup analysis with other genetic and environment risk factors, the values with a p<0.0125 were considered to be significant, using the Bonferroni's correction for multiple comparison in order to reduce the probability of the alpha-error. In the allelic frequencies of A-308 a mutant type of the TNF-A gene, there were no significant differences between the cases and controls (2.2% in severe PIH, 1.5% in moderate PIH and 1.7% in controls). Furthermore, no significant differences were found in the subgroup analyses using the other PIH risk factors such as maternal age, parity, prepregnancy body mass index, family history of hypertension, M235T of angiotensinogen gene, Glu298Asp of the endothelial nitoric oxide synthase gene and some lifestyle factors. The present result suggests that the G-308A variant of TNF-A gene is not associated with PIH in the Japanese women.

## 1794/T/Poster Board #343

**Epidemiologic Architecture for Genes Linked to Environment (EAGLE): Characterizing genome-wide association study variations associated with inflammation in the National Health and Nutrition Examination Surveys.** *D.C. Crawford, L. Dumitrescu, K. Brown-Gentry, K. Spencer, J.A. Canter, D. Murdock, J.L. Haines, M.D. Ritchie.* Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

C-reactive protein (CRP), one of several serum inflammatory markers, is associated with obesity and cardiovascular disease, and its causal role is controversial. Several candidate gene and now genome-wide association (GWA) studies have identified variations associated with CRP levels, some of which are also associated with clinical outcomes. While many of the candidate gene studies have been performed in diverse populations, the GWA-identified variations have yet to be tested in diverse samples. Also, some previous association studies predated the high-sensitivity (hs) CRP assay. To characterize these candidate gene and GWA-identified variants in diverse samples with hs-CRP, we, as part of the Population Architecture using Genomics and Epidemiology (PAGE) Study, are genotyping samples collected for the National Health and Nutrition Examination Surveys (NHANES), a cross-sectional survey of Americans representing three major groups: non-Hispanic **w**hites (n=3974), non-Hispanic **b**lacks (n=1334), and **M**exican-**A**mericans (n=1877). To date, four *CRP* SNPs previously associated with CRP levels have been genotyped in NHANES 1999-2002. In preceding candidate gene and GWA studies, these SNPs explained a relatively small proportion of observed trait variability: ~3% for any one SNP. Assuming a significance threshold of 0.01, NHANES 1999-2002 has 80% power to detect small effect sizes (0.3%, 0.9%, and 0.6% in **W**, **B**, and **MA**, respectively). Using linear regression and an additive genetic model, SNPs were tested for an association with CRP levels among adults adjusting for age, sex, and body mass index. Three SNPs were significantly associated with CRP levels in all three groups in the same direction: rs1205 was associated with decreased levels (**W**: $\beta$=-0.24, p<0.001; **B**: $\beta$=-0.23, p=0.0001; **MA**: $\beta$=-0.21, p<0.001), rs1417938 was associated with decreased levels (**W**: $\beta$=-0.14, p<0.0001; **B**: $\beta$=-0.18, p=0.015; **MA**: $\beta$=-0.13, p=0.003), and rs1800947 was associated with increased CRP levels (**W**: $\beta$=0.34, p<0.0001; **B**: $\beta$=0.60, p=0.007; **MA**: $\beta$=0.31, p=0.013). *CRP* rs3093058, which is rare in **W** (minor allele frequency = 0.05%), was associated with increased CRP levels in **B** ($\beta$=0.48; p<0.0001) and **MA** ($\beta$=0.59; p=0.002), consistent with previous reports. Overall, these SNPs explained 0.1% (rs1800947) to 3.7% (rs3093058) of the variability observed in CRP levels in NHANES 1999-2002.

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## 1795/T/Poster Board #344

**From association to disease risk prediction: an optimistic view from genome-wide association studies on type 1 diabetes.** *H. Zhang[1], K. Wang[1], Z. Wei[2], H. Qu[3], J. Bradfield[1], C. Kim[1], E. Frackleton[1], C. Hou[1], J.T. Glessner[1], R. Chiavacci[1], C. Stanley[4], D. Monos[5], S.F. Grant[1,6], C. Polychronakos[3], H. Hakonarson[1,6].* 1) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA 19104; 2) Department of Computer Science, New Jersey Institute of Technology, Newark, NJ 07102; 3) Departments of Pediatrics and Human Genetics, McGill University, Montreal H3H 1P3, Québec, Canada; 4) Division of Endocrinology, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104; 5) Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA 19104; 6) Division of Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104.

Genome-wide association studies (GWAS) have been fruitful in identifying previously unsuspected disease susceptibility loci for several common diseases. A remaining question is whether we can predict and quantify the individual disease risk based on genotype data, to facilitate decision making on personalized prevention and treatment. Previous studies, using a limited number of confirmed genuine susceptibility loci, typically failed to achieve satisfactory performance and convey pessimistic view on disease risk prediction. Here we propose that sophisticated machine-learning approaches on a large ensemble of markers, which contain unconfirmed disease susceptibility variants, may improve the performance of disease risk prediction. We applied a Support Vector Machine (SVM) algorithm on an Affymetrix-typed GWAS data set for type 1 diabetes (T1D) with 1,963 cases and 1,480 controls, and optimized a risk prediction model with hundreds of SNP markers. We subsequently tested this prediction model on a second Illumina-typed GWAS data set on T1D (1,008 cases, 1,000 controls) with whole-genome imputed genotypes, as well as a third Affymetrix-typed GWAS data set on T1D (1,529 cases and 1,458 controls), achieving AUC (area under ROC cuve) scores of ~0.83 in both data sets. In contrasts, poor performance was achieved when using dozens of known susceptibility loci in the SVM prediction model, or using logistic regression model for risk prediction, with AUC scores of ~0.65. Our study suggests that improved risk prediction can be achieved by using sophisticated algorithms that take into account of interaction effects on a large ensemble of SNP markers. We are optimistic that whole-genome genotype based disease risk prediction is clinically feasible and useful, at least for diseases where notable proportion of the risk has been captured by SNP arrays.

## 1796/T/Poster Board #345

**Harnessing the Information Contained Within Genome-wide Association Studies to Improve Individual Prediction of Complex Disease Risk.** *D. Evans[1], P. Visscher[2], N. Wray[2], The TASC Consortium.* 1) Soc Med, Univeristy Bristol, Bristol, United Kingdom; 2) Genetic Epidemiology and Queensland Statistical Genetics, Queensland Institute of Medical Research, Australia.

The current paradigm within genetic diagnostics is to test individuals only at loci known to affect risk of disease- yet the technology exists to genotype an individual at thousands of loci across the genome. We investigated whether information on thousands of SNPs from genome-wide association studies could be harnessed to improve discrimination of complex disease affection status. We employed genome-wide data from the Wellcome Trust Case Control Consortium to test this hypothesis. Each disease cohort together with the same set of controls were split into two samples- a "Training Set", where thousands of SNPs that might predispose to disease risk were identified, and a "Prediction Set", where the discriminatory ability of these SNPs was assessed. Genome-wide scores consisting of, for example, the total number of risk alleles an individual carries were calculated for each individual in the prediction set. Case-control status was regressed on this score and the area under the receiver operator characteristic curve (AUC) estimated. In most cases, a liberal inclusion of SNPs in the genome-wide score improved AUC compared to a more stringent selection of top SNPs, but didn't perform quite as well as selection based upon established variants. More importantly, the addition of genome-wide scores to known variant information produced an increase in discriminative accuracy being most effective in the case of bipolar disorder, coronary heart disease and type II diabetes where AUC increased by eleven, five and three units respectively. We are currently expanding these methods in a large cohort of individuals with Ankylosing Spondylitis- the only complex disease where it is currently possible to perform genetic tests with very high sensitivity and specificity (i.e. > 90%). It is likely that the diagnostic and predictive utility of genome-wide scores will further increase as the size of training sets expand.

## 1797/T/Poster Board #346

**Individual etnic admixture in a brazilian western amazonian city (Monte Negro-RO)and effects on malaria episodes.** *F.A.B. Santos[1,2], R.G.M. Ferreira[1,2], L.M. Garrido[1,2], C.E.M. Kawamata[1,2], L.M.A. Camargo[1,2], H. Krieger[1,2].* 1) Parasitologia, Universidade de São Paulo, São Paulo, São Paulo, Brazil; 2) INAGEMP - Instituto Nacional de Genética Médica Populacional.

Several studies on population ethnic admixture proportions were done in Brazil: Krieger et al, in 1965 (Ann. Hum. Genet. 19:113-125), Culpi and Salzano (Int. J. of Hum. and Med. Gen., v. 34, n. 2, p. 388-392) are some examples. There are also more studies in Brazil or other countries using classical genetic, mitochondrial and molecular markers. A previous admixture analysis estimating ethnic proportions was done in this region by Ferreira et al in 2002 (Hum. Biol 74/4:607-614) based on a gene frequencies algorithm as recommended by Krieger et al (op. cit.). Individual admixture follows the same methodology, however some adaptations were made. Numeric calculations were performed using TC 3.0 software. After individual ethnic proportions being determined, a correlation analysis was perfomed between the corrected logarithm number of malaria episodes by each individual and ancestral proportions. A weak correlation (r = -0.068, P<0.05)was obtained for Europeans and a strong significant correlation was observed for Africans (r = 0.116, P<0.05). Knowing that this result could be partly explained by negative Duffy individuals, an analysis was built in order to exclude this component. Newer results revealed that the weak European correlation disappeared (r = -0.058, P>0.05), while the strong correlation observed for Africans remained (r = 0.104, P<0.05). This results suggests that other genetics mechanisms of malaria resistance or susceptibility evolved in Africa and may be detectable in african descendents in the New World. Sponsored by CAPES,CNPq.

## 1798/T/Poster Board #347

**Genetic Mechanisms for Venous Thromboembolism (VTE).** *J. Heit[1], T. Petterson[2], S. Armasu[2], E. Jeavons[2], J. Larson[2], J. Cunningham[3], M. de Andrade[2].* 1) Internal Med/Hematology Res, Mayo Clinic College of Medicine, Rochester, MN; 2) Biomedical Statistics and Informatics, Mayo Clinic College of Medicine, Rochester, MN; 3) Laboratory Medicine and Pathology, Mayo Clinic College of Medicine.

VTE consists of deep vein thrombosis (a blood clot occluding the lumen of large veins, usually in the leg or pelvis) and its complication, pulmonary embolism (a dislodged DVT which obstructs one or more arteries to the lung). We conducted a candidate gene association study from 780 genes in four pathways (anticoagulant, procoagulant, fibrinolytic and innate immunity) relevant to the pathogenesis of VTE using a haplotype tagging algorithm that incorporated Illumina's design score for iSelect. A total of 12,313 SNPs and 2331 unique individuals (1166 VTE and 1165 controls) were available for the analysis. From those, 261 individuals were removed due to the study exclusion criteria (82), genotype issues (150), race (22), and relatedness (13). A total of 2070 individuals were used for the association analysis (49% VTE subjects; 48% males). The analyses were performed with PLINK using an additive genetic model and adjusted for age, sex, state of residence, and prior myocardial infarction or stroke (yes/no). The most significant results included two SNPs in the F5 (procoagulant Factor V) gene including the Factor V Leiden mutation (rs6025, OR=3.36, p-value=1.38x10-16), five SNPs in the ABO gene including blood group type (rs8176719, OR=1.57, p-value=2.22x10-11) and one SNP in the F2 (procoagulant prothrombin) gene (rs1799963 [prothrombin G20210A], OR=2.51, p-value=3.44x10-5). These results remained significant when the analysis was stratified by sex. We also performed an analysis stratified on F5 carrier, F2 carrier, and blood type O status (yes/no). Significant results identified for F5 non-carriers mutation were SNPs in the ABO, F2, and IL4R genes; for F2 non-carriers mutation SNPs in ABO and F2 genes, and for non-O blood type carriers SNPs in the F5 and PLSCR1 genes. In conclusion, SNPs within the F5 (including Factor V Leiden), F2 (prothrombin G20210A) and ABO genes are susceptibility variants for VTE, and the joint population-attributable risk of the most significant risk alleles from F5, ABO and F2 was 0.21. The F2 and ABO variants appear to act independently of Factor V Leiden status.

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## 1799/T/Poster Board #348

**Association between 5' region of *NFKBIA* and TNFα receptors in Hispanic Americans: The IRAS Family Study.** *M.R. Miller[1], W. Zhang[1], C.D. Langefeld[2], D.W. Bowden[2], S.M. Haffner[3], J.I. Rotter[4], Y.I. Chen[4], J.M. Norris[1], T.E. Fingerlin[1].* 1) Colorado School of Public Health, University of Colorado Denver, Aurora, CO; 2) Wake Forest University Health Sciences, Winston-Salem, NC; 3) University of Texas Health Sciences Center at San Antonio, San Antonio, TX; 4) Cedars-Sinai Medical Center, Los Angeles, CA.

The IKKβ/NF-κB pathway is known to play an important role in inflammatory response. We have shown a relationship between SNPs in the gene that codes for IκBα (*NFKBIA*) with both insulin resistance and diabetes status. SNPs in this gene have also been implicated in diseases with a strong inflammatory component, such as carotid artery disease (CAD), Crohn's disease (CD), and multiple sclerosis (MS). Based upon these prior findings, we also investigated the association of several SNPs in the *NFKBIA* gene with other markers of inflammation, Tumor Necrosis Factor-α receptors 1 and 2 (TNFr1 and TNFr2). We typed 25 SNPs spanning 20 kb including *NFKBIA* in two Hispanic samples of the IRAS Family Study: 619 individuals from 30 families in the San Luis Valley in Colorado (SLV) and 649 individuals in 60 families in San Antonio, Texas (SA). At least one typed SNP had an $r^2 > 0.90$ with all known common variants in the gene. Soluble TNFRr1 and TNFr2 were measured from stored plasma samples. Individuals with type 2 diabetes were excluded from the analysis. We tested for association between each SNP and TNFr1 or TNFr2 using a variance components measured genotype approach as implemented in SOLAR. We identified a SNP in the 5' region of the *NFKBIA* gene that was significantly associated with both TNFr1 and TNFr2, adjusting for age, gender, and admixture. SNP rs762009 (G→A) was associated with levels TNFr1 and TNFr2 in the SA sample under a recessive model ($p = 7.01 \times 10^{-4}$, and $p = 2.02 \times 10^{-3}$, respectively). Individuals in the SA sample with two copies of the A allele for rs762009 had ~8% lower TNFr1 and TNFr2 levels than individuals with at least one copy of the G allele. In the SLV sample, the association was not significant ($p = 0.871$ and $p = 0.761$), and levels of TNFr1 and TNFr2 were not changed for people with genotype AA versus AG or GG. Adjustment for BMI did not change the evidence for association in either the SA or SLV sample (SA: $p = 1.90 \times 10^{-3}$ and $p = 4.66 \times 10^{-3}$; SLV: $p = 0.700$ and $p = 0.640$). As this SNP is one of the most 5' of any of the SNPs typed, studies to refine the apparent association at rs762009 with levels of TNFr1 and TNFr2 are ongoing. These results further support the role that IκBα may play in inflammatory response and may have important consequences for inflammatory related diseases such as obesity, insulin resistance, and cardiovascular disease.

## 1800/T/Poster Board #349

**Evidence of age-dependent genetic effects on tooth decay: heritability estimates suggest genes influencing dental caries differ between primary and permanent dentitions.** *J.R. Shaffer[1], X. Wang[2,3,4], R.J. Weyant[3,5], K.T. Cuenco[2,3,4], R. Crout[6], D.W. McNeil[7], M.L. Marazita[1,2,3,4,8].* 1) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA,15261; 2) Center for Craniofacial and Dental Genetics, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, 15219; 3) Center for Oral Health Research in Appalachia, University of Pittsburgh, Pittsburgh, PA, 15261 and West Virginia University, Morgantown, WV, 26506; 4) Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, 15261; 5) Department of Dental Public Health and Information Management, University of Pittsburgh, School of Dental Medicine, Pittsburgh, PA,15261; 6) Department of Periodontics, School of Dentistry, West Virginia University, Morgantown, WV, 26506; 7) Practice and Rural Health, West Virginia University, Morgantown, WV, 26506; 8) Clinical and Translational Sciences Institute, and Department of Psychiatry, School of Medicine, University of Pittsburgh, Pittsburgh, PA, 15261.

Dental caries (tooth decay) is a common disease, affecting children and adults in both the primary and permanent dentitions. Although it is well established that genetic factors are important for this disease, the degree to which genes contribute to the development of dental caries, and whether these genes differ between primary and permanent dentitions, is largely unknown. Using family-based likelihood methods, we assessed the heritability of caries scores for both children and adults in over 2300 participants from 701 families. We found that caries scores for primary dentition were highly heritable with genes accounting for 39% to 66% of variation (p-values < 0.01). The heritability of caries scores in permanent dentition was also substantial (15% to 55%; p-values < 0.01), although lower than analogous phenotypes in the primary dentition. Assessment of the genetic correlation between primary and permanent caries scores indicated that 26% of the covariation in these traits was due to common genetic factors (p-value < 0.01). Therefore, dental caries are likely to be partly attributable to different suites of genes and/or genes with different effects in primary vs. permanent teeth. Sex and age explained much of the phenotypic variation in permanent, but not primary, dentition. Furthermore, adding pre-cavitated white-spot lesions to the caries scores contributed substantially to the heritability of dental caries. In summary, our results show that dental caries are heritable, and that genes affecting susceptibility to dental caries in primary dentition may differ from those in permanent teeth. Moreover, metrics for quantifying caries that incorporate white-spot lesions may be better suited for genetic studies of the causes of tooth decay. In conclusion, dental caries may serve as an example phenotype in which genes that influence trait variation during childhood development may (partly) differ from those during adulthood. NIH grant #R01-DE014899.

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## 1801/T/Poster Board #350

**Kinship Analysis of Inflammatory Bowel Disease.** *M.S. Williams[1], G.M. Wood[2], G. Mineau[3], R. Pimentel[4], K. Thompson[5], S.L. Guthery[5].* 1) Director, Intermountain Healthcare Clinical Genetics Inst, Salt Lake City, UT; 2) Intermountain Healthcare Clinical Genetics Inst, Salt Lake City, UT; 3) Department of Oncological Sciences and Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; 4) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; 5) Department of Pediatrics Division of Pediatric Gastroenterology, Hepatology, and Nutrition, University of Utah and Primary Children's Medical Center, Salt Lake City, UT.

The inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are debilitating disorders without a cure. Several genetic risk factors have been identified, but even in aggregate, do not account for the observed heritability. Alternative study designs, such as extended pedigree studies, would compliment genome wide association studies in identifying new risk factors for IBD. To assess the utility of the Utah Population Database (UPDB) as a resource for IBD gene mapping studies, we determined the IBD risk to family members of affected individuals, and disease distribution within pedigrees. Cases were identified from the Intermountain Healthcare (IH) and University of Utah Health Sciences Center (UUHSC) electronic data warehouses (EDW) using ICD-9 codes 555.x or 556.x. for CD and UC respectively. Cases were probabilistically linked into UPDB. Multiple controls, matched on birth year, sex, and birth state were randomly selected for each of the patients. Relative risks (RR) for classes of kinship were computed using Conditional Logistic regression. Results. 4935 CD cases, 5448 UC cases, and 10,830 IBD cases (these represent patients who had encounters with 555.x, 556.x or both, hence are counted twice. These individuals were included in analyses for both CD and UC as well as IBD) were present in UPDB. The RR to a first-degree relative of a CD-affected proband was 5.72 compared with 3.92 for UC. There was a statistically significant increased RR for second-degree relatives (CD 1.64; UC 1.45) and first cousins (CD 1.53, UC 1.29), but no increased risk to second cousins. The relative risks to first-degree relatives, second-degree relatives, and first cousins of an IBD-affected individual were 3.37, 1.64 and 1.29 respectively, all statistically significant. Multiple high-risk IBD kindreds were identified, including some kindreds with more than 15 times the expected number of IBD cases. In conclusion this population-based study confirms the familiality of CD, UC, and IBD, and validates the Utah Population Database for targeting high-risk IBD pedigrees for genetic studies.

## 1802/T/Poster Board #351

**Genetic variations in CNTNAP2 gene are associated with Adolescent Idiopathic Scoliosis (AIS) progression.** *J. Gu, L. Nelson, R. Chettier, K. Ward.* Axial Biotech, Inc., Salt lake City, UT.

Adolescent Idiopathic Scoliosis (AIS) is a complex, familial disorder characterized by a lateral deformity of the spine, affecting school age children between 9 and 15 globally, which etiology remains elusive. Our genome wide association studies and linkage analysis in affected families have implicated contactin associated protein-like 2 (CNTNAP2), a neuronal specific gene, involved in the etiology of AIS. CNTNAP2 is a member of the neurexin superfamily, which functions as cell adhesion molecules and receptors in the myelinated axons of the central nervous system (CNS). This gene is one of the largest genes in the human genome and encompasses almost 1.5% of chromosome 7. Recent studies have reported CNTNAP2 mutations in a number of neurological disorders, including focal epilepsy, Gilles de la Tourette syndrome, autism spectrum disorder and nonsyndromic deafness. The purpose of this study is to identify genetic variants in CNTNAP2 associated with AIS progression. Genomic DNA samples from 188 severe AIS patients and 92 controls were used for bi-directional sequencing of exonic regions of CNTNAP2. Results were analyzed using Sequencher 4.9 program (Gene Code). Many known SNPs as well as novel genetic variants were identified. Interestingly, we found a known in-del rs35167289 [OR= 1.5 (0.89-2.52)], which happens to be in LD with a novel in-del [OR= 0.58 (0.3-1.1)], located 3 base pairs downstream of rs35167289. There was no evidence of deviation from HWE (p>0.01) among severe AIS patients and control population. In addition, SNP rs34738210 [OR= 1.3 (0.76-2.2)] was also found associated with the severe AIS phenotype. Currently, we are pursuing haplotype analysis on the in-del as well as the SNP. Our results provide first evidence of CNTNAP2, a neuronal specific cell adhesion molecule, involved in AIS etiology and progression. Since CNTNAP2 plays an important role in axon differentiation during CNS development, this study facilitates the understanding of the CNS function in the onset of AIS.

## 1803/T/Poster Board #352

**Genome-wide association study identified novel susceptibility loci for type 2 diabetes and related traits in a cohort of African Americans.** *G. Chen, A. Adeyemo, A. Doumatey, J. Zhou, S. Daniel, H. Huang.* CRGGH/NHGRI, NIH, Bethesda, MD.

We sought for genetic variants underlying type 2 diabetes (T2D) and related traits (fasting glucose and insulin, Insulin Resistance -IR, Beta-cell Function -BCF, and triglyceride/HDL ratio - TG/HDL) by conducting a genome wide association study in African Americans (AAs), a population group that are disproportionately affected by diabetes and related complications. A total of 2024 persons were genotyped using the Affymetrix 6.0 fixed SNPs array. 1082 unrelated T2Ds and controls were selected for T2D and TG/HDL traits, 927 unrelated non T2Ds for glucose, insulin, IR, BCF, and Homa traits analysis. After quality filters, the analysis focuses on experimentally determined 806,047 autosomal SNPs. We then search for novel variants for T2D and attempted to replicate previously reported GWAS findings for 47 SNPs. We identify novel associations in or near several genes including the CADPS (rs833662), KLHDC6 (rs13066860), GARNL4 (rs1079530), SLC10A6 (rs10050311), SC4MOL (rs17046216), PPTG2/1 (rs1895320), GPC6 (rs9524298) and CTDP1 (rs3786228) with T2D and related traits at a p value < 5x10-6. Interestingly, we replicated 15 of 47 SNPs previously reported to be associated with T2D and related traits at a p values < 0.05 in this study of African Americans.

## 1804/T/Poster Board #353

**Epidemiologic Architecture for Genes Linked to Environment (EAGLE): Characterizing genome-wide association study variations associated with lipid traits in the National Health and Nutrition Examination Surveys.** *L. Dumitrescu, K. Brown-Gentry, K. Spencer, D. Murdock, J.A. Canter, J.L. Haines, M.D. Ritchie, D.C. Crawford.* Department of Molecular Physiology and Biophysics, Center for Human Genetic Research, Vanderbilt University, Nashville, TN 37232.

Low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels are well known independent risk factors for cardiovascular disease. Lipid-associated SNPs are being discovered in genome-wide association (GWA) studies in samples of European descent, but little data exist in diverse cohorts. To characterize these GWA-identified variants, we, as part of the Population Architecture using Genomics and Epidemiology (PAGE)Study, are genotyping samples collected for the National Health and Nutrition Examination Surveys (NHANES), a cross-sectional survey of Americans representing at least three major groups: non-Hispanic whites (n=3,974), non-Hispanic blacks (n=1,334), and Mexican-Americans (n=1,877). To date, 13 SNPs previously associated with lipids have been genotyped in NHANES 1999-2002. In preceding GWA studies, these SNPs explained a relatively small proportion of trait variance, with a maximum of about 5%. Assuming a significance threshold of 0.01, NHANES 1999-2002 has 80% power to detect small effect sizes (0.3%, 0.9%, and 0.6% in W, B, and MA, respectively). Using linear regression, SNPs were tested for an association with transformed lipid traits adjusting for age, sex, and body mass index on fasting adults not on lipid-lowering medication. Among all 13 SNPs examined, only *PCSK9* rs11591147 was significantly associated with its corresponding lipid trait, LDL-C, in all three groups ($\beta$=-0.112, p=0.0090 in W; $\beta$=-0.757, p=0.0152 in B; and $\beta$=-0.165, p=0.0371 in MA). This nonsynonymous (R46L) variant explained ~0.6% of the variability in LDL-C in NHANES and was associated in the same direction as previous findings. In contrast, seven SNPs replicated in W but were not associated in B and/or MA. For example, among the five SNPs tested for association with HDL-C, *LIPC* rs1800588 and *LPL* rs328 were associated with increased levels in W ($\beta$=0.037; p=0.0002 and $\beta$=0.027; p=0.0373, respectively) and in MA ($\beta$=0.031; p=0.0027 and $\beta$=0.069; p=0.0014, respectively), but not in B ($\beta$=0.003; p=0.8380 and $\beta$=0.039; p=0.1735, respectively). This finding suggests that either population differences exist for these SNPs or that the causal SNP has not yet been identified. Overall, these post-GWAS data demonstrate the importance of characterizing replicated associations using large, diverse, population-based cohorts such as NHANES to help direct the next generation of genetic association studies.

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## 1805/T/Poster Board #354

**A multi-stage analysis of *CIITA*, *DRB1*1501* and multiple sclerosis (MS).** *P.G. Bronson[1], P.P. Ramsay[1], S. Caillier[2], S.L. Hauser[2], J.R. Oksenberg[2], L.F. Barcellos[1], International Multiple Sclerosis Genetics Consortium.* 1) Department of Epidemiology, Barcellos Lab, University of California, Berkeley, CA, USA; 2) Department of Neurology, University of California, San Francisco, CA, USA.

An association between major histocompatibility complex (MHC) genes, particularly those in the human leukocyte antigen (HLA) class II region, and multiple sclerosis (MS) is well established. The primary genetic risk factor within this region is the *HLA-DRB1*1501* allele. The MHC class II transactivator gene (*CIITA*) recently emerged as the most important transcription factor regulating gene required for HLA class II MHC-restricted antigen presentation. A multi-stage investigation of *CIITA*, *DRB1*1501* and MS was undertaken. A total of 23 *CIITA* SNPs were initially genotyped in 1,343 MS cases and 1,379 independent controls with >90% European ancestry (EA) (N= 2,722). All individuals were characterized for the *DRB1*1501* allele. Allelic tests were conducted with Fisher's exact test in PLINK. Global haplotype tests of 5 haplotype blocks (encompassing 15 SNPs) were conducted by estimating haplotype probabilities and using score statistics in HAPLOSTATS. The strongest association between *CIITA* and MS was observed for the +1614G/C missense mutation (rs4774) (OR=1.19, 95% CI=1.05 to 1.33, P=4.8x10-3), and was restricted primarily to *DRB1*1501*+ cases (OR=1.59, 95% CI=1.29 to 1.96, P=1x10-4). Second, the rs4774 SNP was genotyped in 894 independent EA families (676 trios and 491 DSPs) and tested for association using the pedigree disequilibrium test. Rs4774*C* was associated with increased MS risk in the *DRB1*1501*+ families (P=0.025). Third, rs4774 was tested for association in the combined case-control and family sample with the allelic likelihood ratio test in UNPHASED. Rs4774*C* was over-represented in *DRB1*1501*+ MS patients (24.4%) compared to *DRB1*1501*+ controls (16.2%) (OR=1.67, 95% CI=1.31 to 2.14, P=2.5x10-5). The presence of both *DRB1*1501* and rs4774*C*, compared to their absence, was strongly associated with MS in the combined cases (55%) vs controls (20.5%) (OR=4.76, 95% CI 3.82 to 5.96, P<2x10-16), and interaction between rs4774 and *DRB1*1501* was present and statistically significant (OR=1.41, 95% CI=1.12 to 1.78, P=3.7x10-3). The previously reported -168A/G promoter polymorphism (rs3087465) was not associated with MS. Our results provide for the first time, evidence for association between rs4774 and MS, particularly in the presence of the MS-associated *DRB1*1501* allele, and also for effect modification of the *DRB1*1501* allele by variation at *CIITA* (rs4774).

## 1806/T/Poster Board #355

**Incorporating causality into genetic models: insights about familial aggregation.** *A.M. Madsen[1], S.E. Hodge[2,3], R. Ottman[1,4,5].* 1) Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY 10032; 2) New York State Psychiatric Institute, New York, NY 10032; 3) Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, NY 10032; 4) G.H. Sergievsky Center, Columbia University, New York, NY 10032; 5) Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, NY 10032.

**Background:** Most genetic models start from mathematical assumptions rather than causal assumptions, i.e., from a hypothetical model of causation. This has the disadvantage that the validity of genetic models for answering questions of causation is unproven. For example, Risch uses multiplicative and additive penetrance models to derive expected patterns of increased recurrence risk among siblings ($\lambda_S$) or other relatives ($\lambda_R$) of affected individuals, compared with the general population, and the $\lambda_R$-1 ratio between relatives with increasing degrees of relationship to a proband (e.g., parents to grandparents). Determining whether these patterns correspond to causal models of gene-gene interaction (GGI) or genetic heterogeneity (GenHet) requires a comparison with a model based on causal assumptions. Here we assume a sufficient-component cause model (SCCM) of disease causation to determine patterns of familial aggregation resulting from two-locus GGI and GenHet under the SCCM framework. **Methods:** From a SCCM we derive a two-locus penetrance matrix that depends on: allele frequency, proportion-susceptible, phenocopy frequency, genetic heterogeneity, and gene-gene interaction. From this we determine disease prevalence in the population and in relatives of affected individuals, using the IBS distribution. We calculate expected $\lambda_R$ and $\lambda_R$-1 ratios for representative combinations of parameter frequencies in the SCCM framework. **Results:** The $\lambda_R$-1 ratio is slightly less than 2 when two genes cause disease independently. Increasing the number of independently-acting variants further decreases the $\lambda_R$-1 ratio. Under our assumptions, a $\lambda_R$-1 ratio < 2 indicates causal GenHet, whereas $\lambda_R$ -1 ratios > 2 indicate causal GGI. But, we find the $\lambda_R$ -1 ratio decreases with decreasing $\lambda_S$, so a $\lambda_S$<2 and $\lambda_R$-1 ratio ≈ 2 does not imply lack of interaction. When $\lambda_S$ > 2, interaction is unlikely if the $\lambda_R$-1 ratio ≈2. **Conclusion:** Patterns of familial aggregation can help distinguish whether disease is caused by gene-gene interaction or by genetic heterogeneity, but under some conditions the familial patterns of diseases caused by GGI, GenHet and a single locus are indistinguishable.

## 1807/T/Poster Board #356

**Susceptibility of premature infants to retinopathy through mutant endothelial nitric oxide synthase VNTR genotypes.** *J.G. Aryama[1], K. Yanamandra[1], D. Napper[1], H. Chen[1], S.A. Ursin[1], A. Pramanik[1], J.A. Bocchini Jr.[1], R. Dhanireddy[2].* 1) Pediatrics, LSU Health Sciences Center, Shreveport, LA; 2) Dept Pediatrics, UT Health Sciences Center, Memphis, TN.

Very low birth weight infants (VLBW) have immature retina and are susceptible to Retinopathy of prematurity (ROP). Problems associated with the immature vasculature lead to disorganized vasculature leading to scarring of retinal tissue and detachment in extreme cases. Several researchers have put forward various genetic markers in the etiology of ROP. Earlier, we have reported an association of mutant vascular endothelial growth factor (VEGF) genotypes with ROP in premature infants . We are presently studying the role of endothelial nitric oxide (eNO) synthase (eNOS) genes in the etiology of ROP. Endothelial nitric oxide serves as a vasodilator, relaxes smooth muscle, prevents platelet aggregation, and facilitates improved blood flow, vascular tonicity. Reduced nitric oxide levels result in vasoconstriction and weak tone leading to decreased blood flow and hypoxia, hypertension and thrombosis. Mutant eNOS genotypes result in reduced nitric oxide levels by decreasing the enzyme activity. Presently, we are investigating the role of eNOS polymorphism in infants with ROP. We have collected peripheral blood specimens consecutively from 144 premature infants from our NICU facility. We have studied VNTR 27bp repeat (*a/b*) polymorphism in the intron 4 region of eNOS gene by microplate PCR genotyping. Common allele *b* carries 5 repeats and rare variant allele *a* carries 4 repeats. The present data revealed a higher frequency of allele *a* in the ROP patients as compared to that in the control infants (0.25 vs. 0.14 with an odds ratio of 2.03). The data suggest that allele *a* may be a risk factor in the etiology of ROP. Previous reports have shown that allele *a* results in the decreased production of nitric oxide than the common *b* allele. Thus, we strongly believe that allele *a* through the insufficient levels of nitric oxide in the premature infants may lead to genesis of retinopathy and supplementation of either inhaled nitric oxide or L-arginine could reduce the risk of retinal detachment in severe cases in these infants. Clinical data, genotype frequencies in various ethnicities and their significance will be presented.

## 1808/T/Poster Board #357

**Comparison of genotype frequencies in Toll-like receptor genes in Ugandans, South Africans, and African HapMap populations.** *A.R. Baker[1], A.K. Randhawa[2], M.S. Shey[3], M. de Kock[3], G. Kaplan[4,6], M. Adams[5,6], W.A. Hanekom[3,6], W.H. Boom[6], T.R. Hawn[2,6], C.M. Stein[1,6].* 1) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland OH, USA 44106; 2) Department of Medicine, University of Washington, Seattle, WA, USA 98195; 3) South African Tuberculosis Vaccine Initiative, Institute for Infectious Diseases and Molecular Medicine, University of Cape Town, South Africa; 4) Laboratory of Mycobacterial Immunity and Pathogenesis, Public Health Research Institute, Newark, NJ, USA 07103; 5) Department of Genetics, Case Western Reserve University, Cleveland OH, USA 44106; 6) Tuberculosis Research Unit, Case Western Reserve University, Cleveland OH; Clinical Epidemiology Unit, Makerere University School of Medicine, Kampala, Uganda.

It has been suggested by several recent studies that susceptibility to tuberculosis (TB), a continual global health threat, is related to genes in the toll-like receptor (TLR) pathway. Single nucleotide polymorphism (SNP) data for TLR genes are available through the HapMap Project, but very little is known about how these data relate to other global populations, particularly African populations, which have an older population history, possibly subject to selective pressure. To examine the similarity in genotype frequency and linkage disequilibrium structure between HapMap populations and our study populations, we conducted full-exon resequencing of TLR genes in unrelated individuals from Uganda and South Africa. The TLR genes of interest included TLR2, TLR4, TLR6, and an adaptor-like protein involved in TLR4 signal transduction, toll/interleukin 1 receptor domain-containing adaptor protein (TIRAP). The 48 Ugandan individuals were all of Black African descent; of the South African individuals, 8 were Black Africans and 33 were Mixed Race. 7 South Africans were of Caucasian descent, however, these individuals were not analyzed in depth as Caucasians were not anticipated to be enrolled in future studies. In comparing genotype frequencies, the Ugandan population tended to show fewer differences from the Kenyan populations than the Yoruba population; most differences in the Ugandan population were seen in TLR6 where the Ugandan frequencies were dramatically different. Interestingly, the South African Blacks were very similar to both the Yoruba and Kenyan populations. The South African mixed population showed the fewest differences from the Yoruba and Southwest US African ancestry population. Comparisons of linkage disequilibrium patterns are ongoing. These results suggest that HapMap data provide a good starting point for the selection of SNPs, however, follow-up analyses using resequencing may also be required.

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## 1809/T/Poster Board #358

**On the construction of a Naïve Bayes classifier from Genome Wide Association data.** *R.W. Davies, A.F.R. Stewart, L. Chen, R. Roberts, G.A. Wells.* Univ Ottawa Heart Inst, Ottawa, ON, Canada.

Objective: We sought to develop a classification algorithm using whole genome DNA microarray data from cardiovascular Genome Wide Association (GWA) studies. Methods: We adopted a Naïve Bayes framework which assumes independence between the SNPs involved in the construction of the classifier, and constructed classifiers based on four inputs: the probability of being a case; an nSNPx3 matrix of probabilities of being a case given a genotype at a SNP; an nSNPx1 vector of the probability that a SNP is associated with disease; and a threshold for association, which can be scaled to generate Receiver Operating Characteristic curves. We considered the probability of being a case given a SNP was linear (additive) or independent (exact) with respect to genotype, and based the probability of association on p-values (Cochran-Armitage Test for Trend, Fisher's Exact Test) for the respective inheritance model corrected for multiple testing using stringent (Bonferonni style (Hochberg)), lenient (False Discovery Rate (FDR)) or no correction. We therefore considered 6 classifiers in total. Results: Two cardiovascular GWAs were looked at, the Ottawa Heart Genomics Study (OHGS) (training set) and the Wellcome Trust Case Control Consortium (WTCCC) Coronary Artery Disease (CAD) dataset (test set). OHGS consists of 1542 cases and 1455 controls genotyped on either Affymetrix 5.0 or 6.0 arrays. 482,251 SNPs common to both platforms were analyzed. Quality Control (QC) was synchronized with the WTCCC approach with the additional removal of MAF<1%. An $r^2$ filter was then used to select a subset of SNPs from OHGS which tagged the QC SNPs with minimum $r^2$=0.8, leaving 237,602 OHGS SNPs; WTCCC SNPs were taken as those which passed WTCCC QC and OHGS $r^2$. The constructed Naïve Bayes classifiers gave similar results; the following values are for the additive FDR model. Cross Validation (CV) gave sensitivity and specificity of (0.68,0.68). However, results seemed to be inflated by having run genotype calling algorithms by plate with plates being uniformly cases or controls; removing this bias by running CV by plate gave (0.64,0.65). Results on the WTCCC dataset were more modest, (0.55,0.51). Conclusions: The results on the test set were less than stellar. Next steps would be to better accommodate the independence assumption using haplotypes and to further evaluate the effects of by-plate genotype bias.

## 1810/T/Poster Board #359

**Worldwide distribution of MYH9 risk haplotype for HIV-associated nephropathy, focal segmental glomerulosclerosis, and hypertension-associated ESRD in human populations.** *T. Oleksyk[1], G. Nelson[2], J. Kopp[3], C. Winkler[2].* 1) University of Puerto Rico, Puerto Rico; 2) SAIC, NCI-Frederick, Frederick, MD; 3) NIDDK, NIH, Bethesda, MD.

*MYH9* was shown to be main effect gene for focal segmental glomerulosclerosis (FSGS) and HIV-associated nephropathy (HIVAN) (OR≈5-7, p<10-8) and a risk factor for hypertension-associated end stage renal disease (OR≈3, p<10-8). Four risk alleles (rs4821480, rs2032487, rs4821481 and rs3752462) defined the risk haplotype (E1) that was extremely common in Americans with African ancestry (AA) (≈60%) and rare in European Americans (EA) (<4%), thus explaining a major USA health disparity. The protective haplotype (E2) was most frequent in EA (69%) and less frequent in AA (21%). The worldwide distribution, selective, and demographic factors maintaining *MYH9* risk alleles in human populations remain relatively unexplored. We reconstructed *MYH9* haplotypes involved in kidney disease by combining the data available for the HapMap and the Human Genetic Diversity Panel (HGDP) and by genotyping SNPs defining the risk haplotype in the HGDP. We analyzed the continental distribution of renal disease haplotypes and examined the extent of linkage disequilibrium for four tagging SNPs among world populations. There are substantial differences between the human populations in frequencies of risk and protective E haplotypes indicated by high FST values among continental populations, suggestive of natural selection. The risk E1 haplotype for non-diabetic kidney disease is very frequent in Sub-Saharan Africa (69%) and much less frequent outside Africa. European and Middle East feature the protective haplotype (E2) (62% and 52%, respectively) while populations in South and East Asia as well as Oceania are dominated by neutral haplotypes, with a near-complete loss of the risk E1 haplotype. The E1 risk haplotype in Africa and E2 protective haplotype in Europe both show high FST, high frequencies and extended length compared to each other, specifically in Yorubans (iHs=2.7). The Yoruban haplotypes are unique among African populations, since the extent of LD in this population is greater than in the neighboring populations within Africa. The increased risk of kidney disease in African-ancestry populations relative to their non-African counterparts is nearly completely explained by the high frequency of the *MYH9* E1 haplotype in African populations and its relative infrequency outside of Africa. There are suggestions that the E1 risk and the protective E2 haplotypes may have been maintained by balancing selection but this warrants further investigation.

## 1811/T/Poster Board #360

**A method to estimate the sharing of eQTLs between tissues, with application to skin and lymphoblastoid cells.** *J. Ding[1], J.E. Gudjonsson[2], L. Liang[1], P.E. Stuart[2], W. Chen[1], R.P. Nair[2], J.T. Elder[1], G.R. Abecasis[2].* 1) Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI; 2) Department of Dermatology, University of Michigan, Ann Arbor, MI.

Gene transcript levels can serve as an intermediate phenotype that bridges genotypes and more complex organismal phenotypes, including common diseases. Genome-wide association studies of gene expression in several human tissues have identified thousands of genetic loci impacting the expression of specific transcripts. Each of these loci is called an expression quantitative trait locus (eQTL). Although it is expected that many eQTLs will be tissue specific, the exact proportion of eQTLs that are tissue specific or shared between tissues remains unknown.

A simple measure of the tissue specificity of eQTLs can be obtained by examining the overlap of eQTL lists from two different tissues. Unfortunately, this naïve approach will always underestimate the true proportion of overlapping signals. We have developed a more accurate method. Our multi-step procedure first generates a list of potential eQTLs and then uses unbiased estimates for QTL effect sizes to estimate the expected number of replicating eQTLs for a specific sample size. The proportion of overlapping eQTLs can then be interpreted in this context. When applied to compare cis-eQTLs detected in an analysis of 57 skin biopsies and in a panel of ~400 lymphoblastoid cell lines, our method shows that 70-80% of eQTLs are shared between tissues, a much larger proportion than the naïve estimate of 30-40%.

Our results provide guidance to researchers contrasting eQTL results across tissues and a specific means to accurately estimate the proportion of overlapping eQTLs between tissues.

## 1812/T/Poster Board #361

**Genome-wide association study of leprosy defines HLA and previously unidentified loci.** *F. Vannberg[1], S. Wong[1], S. Gochhait[2], D. Malhotra[2], R. Bamezai[2], A. Hill[1].* 1) Wellcome Trust Human Genetics, Univ Oxford, Oxford, United Kingdom; 2) National Centre of Applied Human Genetics, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India.

Genome-wide association studies (GWAS) have been successful at identifying common variants associated with common diseases. Using GWAS methodology we here report a screen for leprosy susceptibility in an Indian cohort based in New Delhi. The highest ranking SNP in this screen was rs9270650, p=6.44x10-10, OR=2.43, which tags an HLA-DRB allele previously known to associate with leprosy. We also identify novel loci with SNPs that rank in the top 99th percentile of associated SNPs, including the loci 3q21.3, 4p14 and 15q21.1, with each loci having multiple SNPs associated with leprosy in the range of 1.17x10-6 to 1.85x10-7. Replication efforts are now underway in additional leprosy case control populations to attempt to confirm these findings. In addition, ex-vivo PBMCs from 96 healthy individuals were stimulated with LPS and tested for TNF protein expression (pQTL). We demonstrate an overlap between the 3p21.3 loci in the leprosy association screen and TNF production pQTL, suggesting that SNPs that cause differential TNF production may promote differential susceptibility to infectious disease.

## 1813/T/Poster Board #362

**A mixture model for the analysis of allelic expression imbalance.** *R. Xiao, M. Boehnke, L. Scott.* Dept Biostatistics, Univ Michigan, Ann Arbor, MI.

Genetic polymorphisms that regulate gene expression account for major phenotypic diversity in human, and may also predispose to complex diseases and determine variability in quantitative traits. Polymorphisms affecting gene expression in cis will cause differentiated expression levels depending on which of the two alleles of the gene are present. Such an allelic expression imbalance (AEI) can be detected in individuals heterozygous for the transcribed SNP. The use of AEI is complementary to testing for SNP-gene expression association and has the advantage of testing both alleles within the same environment in each individual. To detect the association of the SNP with the AEI, we propose a mixture model and corresponding expectation-maximum (EM) algorithm that is robust to the linkage disequilibrium (LD) structure between the rSNP and the transcribed SNP.

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## 1814/T/Poster Board #363

**Multifactor Dimensionality Reduction 2.1: Open-Source Genetic Analysis Software for Embracing the Complexity of Common Human Diseases.** *P. Andrews, J.H. Moore.* Department of Genetics, Dartmouth College, Lebanon, NH.

Multifactor dimensionality reduction (MDR) was designed as a nonparametric and genetic model-free approach to identifying, characterizing and interpreting gene-gene interactions in genetic and epidemiologic studies of common human diseases. The kernel of the MDR algorithm uses constructive induction to combine two or more polymorphisms into a single predictor that captures interaction effects. This general approach has been validated in numerous simulation studies and has been applied to a wide-range of different human diseases including asthma and allergy, autoimmune diseases, cancer, cardiovascular diseases, diabetes and metabolic syndrome, Mendelian diseases, pharmacogenetics and psychiatric diseases, for example. We describe here version 2.1 of the open-source MDR software package that has been made freely available to the genetic epidemiology and bioinformatics communities since February of 2005. Over this time period MDR has been downloaded more than 20,000 times. This new version of MDR has been significantly updated to allow users to load and analyze genome-wide associations study (GWAS) data. Improved data loading procedures and memory management techniques make it possible to carry out an MDR analysis on a GWAS data set. We expect this new version of MDR will open the door to routine epistasis analysis with GWAS data when combined with stochastic search methods such as the included estimation of distribution algorithm (EDA) that has the important ability to use expert knowledge in the form of prior statistical evidence (e.g. LOD scores, ReliefF) or biological evidence (e.g. chromosomal location, KEGG pathway, Gene Ontology) to probabilistically select polymorphisms for consideration in an MDR model.

## 1815/T/Poster Board #364

**Computationally efficient ReliefF-based algorithms for detecting epistasis in genome-wide association studies.** *C.S. Greene, J. Kiralis, J.H. Moore*. Department of Genetics, Dartmouth College, Lebanon, NH.

The detection of epistasis in genome-wide association study (GWAS) data is an enormous computational challenge due the combinatorial nature of the problem. We have previously shown that the ReliefF family of algorithms is able to detect epistasis in GWAS data without the need for a combinatorial algorithm. We previously developed an improved Relief algorithm called Spatially Uniform ReliefF (SURF) that significantly increases the power to detect interacting attributes in this domain. Here we provide a novel alternative to the nearest neighbor approach that instead uses all instances and weights genetic variants differently depending on whether each instance (i.e. subject) is close to or far away from the instance in question. We show using simulated genome-wide data that this new algorithm (SURF*) significantly outperforms ReliefF and SURF for genetic analysis in the presence of gene-gene interactions across a wide range of heritabilities (0.025 to 0.4) and sample sizes (800 to 3200). For example, the power of ReliefF, SURF and SURF* to rank two interacting genetic variants in the top 95% of 1000 total variants is 5%, 10% and 80%, respectively, for an epistatic model with a heritability of 0.2 and a sample size of 1600 subjects. This study provides for the first time a computationally efficient algorithm that can detect epistatic loci in genome-wide data in a non-combinatorial manner.

## 1816/T/Poster Board #365

**Discovery of Causal Relationships between Gene Expressions by Local Causal Feature Estimation.** *E. Kang[1], I. Shpitser[2], E. Eskin[1].* 1) UCLA, Los Angeles, CA; 2) Havard University, Cambridge, MA.

The availability of high throughput microarray data has encouraged several genetical genomics studies where researchers have identified genomic locations correlated with expression quantitative traits in the form of linkages or associations. Various statistical approaches have been applied to these results to tease out the underlying biological networks that govern how genes regulate and interact with each other. Extracting causal relationships from these networks is a challenging but important step to understanding the complex diseases and phenotypes under perturbation. Our goal of the project is to discover the presence and absence of causal relationships between gene expressions of yeast data and gene expressions collected from various tissues of F2 mice data. Causal discovery is challenging in our case because our domain has many thousands of variables, while our number of samples is very limited. In particular, most conventional conditional independence tests are not reliable in the small sample case, since conditioning severely reduces the power of the test. In addition, sequential conditional independence test with few samples results in complex cascading of errors in traditional constraint-based methods, and as a result we cannot infer independence with high confidence. In this project, we develop new types of causal discovery algorithm that does not suffer from these problems. The key idea behind our algorithm is that we represent a causal graph by set of “causal features” which define the causal graph and predict these features from data independently. Our approach is flexible and robust in that it allows us to predict the presence or absence of “causal features” which gets enough support from given data so that we can only identify the confident region of causal graph. We apply our algorithm to discover the causal relationships between gene expressions of yeast data and gene expressions collected from various tissues of F2 mice data.

## 1817/T/Poster Board #366

**A computational evolution system for open-ended automated learning of complex genetic relationships.** *J.H. Moore, D. Hill, C.S. Greene.* Department of Genetics, Dartmouth College, Lebanon, NH.

The failure of genome-wide association studies to reveal the genetic architecture of common diseases suggests that it is time that we embrace, rather than ignore, the complexity of the genotype-to-phenotype mapping relationship that is characterized by epistasis, plastic reaction norms, heterogeneity and other phenomena such as epigenetics. The extreme complexity of the problem suggests that simple linear models and other approaches that assume simplicity are unlikely to capture the full spectrum of genetic effects. To this end, we have developed an open-ended computational evolution system (CES) that makes no assumptions about the underlying genetic model and can learn through evolution by natural selection how to solve a particular genetic modeling problem. This is accomplished by providing the basic mathematical building blocks (e.g. +, -, *, /, LOG, <, >, =, AND, OR, NOT etc.) for models that can take any shape or form and the basic building blocks for algorithmic functions (e.g. ADD, DELETE, COPY, etc.) that can manipulate genetic models in a manner that is dependent on expert statistical and biological knowledge or prior modeling experience. We have previously demonstrated that our CES approach has excellent power to detect epistatic relationships in genome-wide data across a wide-range of heritabilities and sample sizes (Moore et al. 2008, 2009). We have also previously shown that this system can learn to utilize one of many sources of expert knowledge thus providing an important clue as to how the system solves the problem (Greene et al. 2009). Here, we introduce an additional layer to our CES approach that introduces noise into the training data (5%, 10%, 15% and 20%) to drive the discovery process toward models that are more likely to generalize. We show using simulated epistatic relationships in genome-wide data that the CES leads to significantly smaller models ($P<0.001$) thus reducing false-positives and overfitting while maintaining a power of 97% to 100%. These results are important because they show how introduced noise in the data can yield more parsimonious models and reduce overfitting without the need for computationally expensive cross-validation. This study is an important step towards a paradigm of genetic analysis that makes few assumptions about a genetic architecture that is very complex.

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## 1818/T/Poster Board #367

**Expert Knowledge from Protein - Protein Interaction Databases to Guide Genome-Wide Genetic Analysis of Common Human Diseases.**
*K. Pattin, J. Gui, J. Moore.* Gen, Dartmouth, Lebanon, NH.

With the recent availability of high-throughput technology, the feasibility to map millions of single-nucleotide polymorphisms (SNPs) has allowed us to begin to explore the depths to which genomic variation contributes to disease susceptibility. Discovering epistatic, or gene-gene, interactions in high dimensional datasets is a problem due to the computational complexity that results from the analysis of all possible combinations of SNPs. Since one of the strongest demonstrations of the functional relationship between genes is protein-protein interactions, thus we may be able to exploit the expert knowledge from protein-protein (PPI) interaction databases to facilitate the analysis of genome-wide studies. The PPI database STRING integrates a number of PPI databases as well as PubMed abstracts to comprise a large PPI interaction network where each interaction is given a confidence score based on the evidence provided for that interaction. We use this confidence score to develop and evaluate metrics by which we can prioritize SNPs in pseudo-artificial bladder cancer datasets derived from a real bladder cancer data set. Our data sets were simulated to have two functional SNP interactions where the SNPs represent a diversity of interaction scenarios and a range of confidence scores from lower to higher including zero confidence interactions according to the confidence score in STRING. We evaluate a total of 5 metrics to see which metric(s) allow us to reduce gene list of the data set significantly while retaining the two functional SNPs. We observed that there was correlation between confidence score and the gene list size for all metrics, yet no consistent trend showing that all higher confidence scores were included in increasingly smaller subsets. The metric MAX-SUM is a measure of genes prioritized by their maximum confidence score and then subsequently by the sum of the confidence scores of all interactions per gene. We find that by Wilcoxon comparison, MAX -SUM reduced the data sets significantly more than metrics AVE, MAX, and MAX-AVE (p=0.03, 0.05, 0.03). Comparison to metric SUM showed no significance. While overall this metric was most effective at reducing the gene list in a majority of scenarios, other metrics were more effective for certain interaction scenarios. We plan to implement these metrics in a bioinformatics tool that will allow us to preprocess genome-wide data on the basis of expert knowledge from protein-protein interactions.

## 1819/T/Poster Board #368

**No evidence that gene-gene interaction contributes substantially to the missing heritability of adult height, a model complex trait.**
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Genome-wide association (GWA) studies have resulted in the identification of >200 variants involved in complex traits and diseases. Despite this success, these variants typically only explain a small proportion of the heritability for many of these traits. Adult height is an ideal model trait to start to locate the "dark matter" of human genome variation involved in complex traits. Height is highly heritable (>80%) and accurately measured, but common variants to date explain <5% of the population variance of the trait. In this study we used GWAS data from ~10,500 individuals genotyped on the Affymetrix 500K chip and replication data from 4 additional datasets (total replication N ~16,000) to test the hypothesis that pairwise non-additive interactions between SNPs contribute to the missing heritability of adult height. We used 3 different approaches to test for interaction (Y= b0+b1.A+b2.B+b3.AB+e; Test of b3 = 0). First, we assessed 44 previously robustly associated height loci against all other SNPs in the genome. Second, we tested for interaction in the subset of SNPs with a main effect P<0.01. From these analyses, we took forward any SNPs with an interaction P<1x10-5 into replication. Third, we exhaustively tested all possible SNP pair combinations across the genome using a full 8 d.f model. From this analysis we took forward any SNPs with a full model P<1x10-8 and an interaction P<1x10-5 into replication. No more significant interactions were observed than would be expected by chance. For example, for the analysis of the 44 robustly associated variants against the rest of the genome we observed 157 SNP pairs with a P<1x10-5, when we would have expected 134 by chance. Similarly, for the analysis of SNPs showing some evidence of main effects, the respective numbers were 121 observed vs. 131 expected. Of the 401 SNP pairs that were taken forward into replication, none showed strong evidence of replication (top $P_{Replication}$ = 0.01; top $P_{Overall}$ = 1x10-6; N~26,000). Using height as a model polygenic trait, this study does not provide any evidence that gene-gene interaction explains a large proportion of the missing heritability in complex trait genetics.

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## 1820/T/Poster Board #369

**A learning classifier system approach to detecting and modeling genetic heterogeneity in the presence of epistasis.** *R. Urbanowicz, J.H. Moore.* Department of Genetics, Dartmouth College, Lebanon, NH.

Genetic heterogeneity (GH) refers to the presence of different underlying genetic risk factors that result in the appearance of the same or similar disease phenotype. While some methods have been suggested to “side-step” this problem, (i.e. clustering and data stratification) these methods often rely on arbitrary cutoffs and lead to an inherent loss of power. The complexity of underlying GH makes the expectation of an accurate and comprehensive single model unrealistic. To address this problem we propose the development, application and evaluation of Learning Classifier Systems (LCS). LCS combine machine learning with evolutionary computing and other heuristics to produce an adaptive system that simultaneously learns to solve different parts of a particular problem. The solution evolved by an LCS is represented as a population of independent rules or models which are utilized collectively to make decisions or predictions. We selected LCS for study here due to their inherent focus on multiple models that each explain different subsets of the data but collectively form a solution to the problem. Three LCS algorithms of differing architectures (XCS, MCS, and GALE) were implemented, evaluated and compared using simulated genetic heterogeneity data in the presence of epistasis. We specifically compared (1) the power to correctly detect predictive polymorphisms, (2) classification accuracy on testing data, (3) computational time, and (4) model generality. This analysis indicated than an LCS is able to achieve better than 80% power to detect all of the attributes involved in the underlying GH model. Additionally, XCS was demonstrated to significantly outperform the other tested LCS methods ($p < 0.001$). The results of this study demonstrate the potential of utilizing an LCS algorithm to address the problem of GH and highlights architectural components of the algorithm best suited to the GH problem domain.

## 1821/T/Poster Board #370

**Expression analysis confirms candidate genes found in a genome wide interaction analysis with HLA-DRB1 shared epitope alleles in Rheumatoid Arthritis.** *M. Ronninger[1], E. Lundström[1], M. Seddighzadeh[1], L. Klareskog[1], L. Alfredsson[2], L. Padyukov[1].* 1) Dept of Medicine, Karolinska Inst & Hosp, Stockholm, Sweden; 2) Dept of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

**Background** Several risk factors were previously found to interact with HLA-DRB1 shared epitope (SE) alleles, which is the best known risk factor in development of RA, in the Swedish EIRA study of ACPA-positive rheumatoid arthritis (RA). Both previously known (PTPN22 and C5) as well as novel RA risk genes are pending independent replication and functional confirmation. **Objectives** We hypothesize that polymorphisms connected through interaction with the HLA-DRB1 SE may have an effect on expression regulation that can lead to a change in the phenotype. **Methods** For the top ten best scoring candidates that interact with HLA-DRB1 SE alleles, we performed dense fine mapping in a cohort of ca 4000 RA cases and controls (EIRA study). We also measured mRNA expression in PBMC from 88 RA cases and controls with known genotypes by RT-qPCR. **Results** All tested genes were found to be significantly up-regulated in individuals with RA. For the gene MAP2K4 we detected an association of the mRNA expression with genotypes. A detailed study of the MAP2K4 loci demonstrated an additional variation close to the third exon in strong association with MAP2K4 expression ($p=0.004$). **Conclusions** Our data confirm previously known and reveals several new candidate genes for RA. The strategy to use the SE risk factor for finding new disease markers is a novel approach, which indicates new pathways for the disease development. We present an integration of genetic association studies with expression analysis for specific genes providing possible directions for future functional experiment in the study of RA.

## 1822/T/Poster Board #371

**A survey of epistatic effects among four candidate genes in myocardial fatty acid metabolism in hypertensive heart disease.** *L. de las Fuentes[1], J.M. Juang[1], S. Climer[1,2], V.G. Davila-Roman[1], C.C. Gu[2].* 1) Department of Medicine, Cardiovascular Division; 2) Division of Biostatistics, Washington University, St. Louis, MO.

Alterations in myocardial metabolism are implicated in hypertensive heart disease (HHD) traits in humans and in animals. Collective actions of multiple genetic factors play an important role in modulating complex traits such as HHD. In the present study, SNPs (single-nucleotide polymorphisms) covering four candidate genes involved in fatty acid metabolism and transport were tested for epistatic interactions associated with HHD-related traits. A latent HHD trait was extracted from race/sex-adjusted residuals of measured phenotypes (i.e., ultrasound indices of LV structure/function and carotid artery intima media thickness) using independent component analysis; and was used to represent the HHD trait in subsequent analyses. SNPs in PPARGC1A, PPARA, PPARG, and CD36 were tested in Caucasian (CA, n= 611) and African-American (AA, n=229) adults. Individual SNP associations were tested by race-specific linear regression analysis; and pair-wise interactions were assessed using the epistasis model implemented by PLINK (v. 1.05). In both ethnic groups, epistatic effects involving PPARGC1A SNPs were common, representing 94% and 95% of the cross-gene SNP-SNP interactions in CA and AA, respectively. Interactions between PPARGC1A and PPARA SNPs were most frequent in CA, whereas those between PPARGC1A and CD36 SNPs were most common in AA. These results are consistent with the role of PPARGC1A as a coactivator of PPARA and PPARG which in turn regulate the expression of fatty acid metabolism and transport genes including CD36. In fact, further analyses of networks formed by significant SNP-SNP interactions identified prominent pivotal nodes (SNPs) connecting clusters of interacting variants in determining HHD. In CA, we identified 5 pivotal nodes (3 PPARGC1A and 2 PPAR$\alpha$) in a network of 24 interacting SNPs; in AA, 6 nodes (4 PPARGC1A, 1 PPARG, and 1 CD36 SNPs) were identified in a network of 31 interacting SNPs. Notably, none of the pivotal SNPs in AA or CA, and only one of the other interacting SNPs in AA, was marginally associated with the HHD phenotype. Therefore, an evaluation of epistatic effects may identify meaningful associations that would go unrecognized in analyses restricted to main effects.

## 1823/T/Poster Board #372

**On the simulation of realistic samples with complex human diseases.** *B. Peng, Y. Xu, C.I. Amos.* Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX.

Simulated datasets with known disease predisposing loci have been widely used to evaluate the power of statistical methods to detect genes that are responsible for human genetic diseases. In response to the rapid adoption of genome-wide association studies, a few simulation methods have been proposed to simulate realistic samples with high-density markers over long chromosomal regions. To evaluate the performance of these methods, we used four simulation methods, GWAsimulator, Hapgen, HapSample, and a forward-time approach to simulate a large number of datasets using markers with different densities over different chromosomal regions. The quality of simulated datasets was assessed in terms of their resemblance to a reference HapMap population, using criteria such as per-locus allele frequency, short and long range linkage disequilibrium, heterozygosity and distribution of pairwise differences between sequences. Among other properties, samples simulated by these methods have varying levels of linkage disequilibrium which lead to varying effect sizes of disease predisposing loci. For example, under identical simulation conditions, four case-control samples simulated by GWAsimulator, Hapgen, HapSample and the forward-time approach have, respectively, 57, 133, 87 and 85 significant markers (single-locus $\chi^2$ tests at the 0.05 significance level) out of 1000 simulated markers. While HapSample is recommended for simple simulations due to its overall performance, the forward-time method remains the most flexible choice for more complex simulations. We extended these methods to simulate complex human diseases with multiple interacting genetic and environmental factors and gave an example using lung cancer as a model disease.

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## 1824/T/Poster Board #373

**Genome-wide interaction analysis of coronary artery disease.** *Y. Liao[1], H. Dong[1], G. Peng[2], Y. Zhu[2], M. Xiong[1].* 1) Biostatistics, University of Texas School of Public Health, Houston, TX; 2) School of Life Science , Fudan University, Shanghai, China.

Numerous studies have been carried out to try to better understand the genetic predisposition for cardiovascular disease. Although it is widely believed that multifactorial diseases such as cardiovascular disease is the result from effects of many genes which working alone or interact with other genes, most genetic studies have been focused on identifying of cardiovascular disease susceptibility genes and usually ignore the effects of gene-gene interactions in the analysis. We argue that interaction holds a key to dissecting the genetic architecture of complex diseases. Although genetic interactions on a genomic scale have been investigated and genetic interaction networks have been constructed for yeast, genome-wide studies of genetic interactions in humans have not been conducted. To construct genome-wide interaction networks, minimum requirement is to assess the pair-wise interaction between SNPs. However, the number of combinations of SNPs to be evaluated will be extremely large. An exhaustive evaluation of all combinations of more than two SNPs is impossible even if we use a large supercomputer. The current study applies a novel linkage disequilibrium based statistic for testing all possible pair-wise interactions between A total of 53,394 SNPs among 501 biochemical pathways assembled from BioCarta and KEGG PATHWAY database using data from Wellcome Trust Case Control Consortium (WTCCC) Coronary Artery Disease (CAD) studies. WTCCC dataset consisted a total of 469,612 SNPs typed in 1926 CAD cases and 2938 controls. A total of 8,644 interactions are found to be significant with p-values less than $3.5\times10^{-11}$ and formed several interaction networks. Among the 8,644 significant interactions, 111 contain at least one SNP that is in the previously identified cardiovascular disease susceptibility genes. The SNP with the largest number of interaction is rs3785579, which is in CACNG1 (calcium channel, voltage-dependent, gamma subunit 1) gene and has 57 interactions with known cardiovascular disease susceptibility genes. This SNP is also found to be significant from the single SNP association test. The SNP with the second largest number of interaction is rs642298, which is in IL3RA (interleukin 3 receptor, alpha) gene and has 16 interactions with known cardiovascular disease susceptibility genes. This SNP shows marginal significance in the single SNP association test ($P=1.84\times10^{-6}$).

## 1825/T/Poster Board #374

**Studying gene-by-environment interaction using case-control-parent tetrads.** *D.M. Umbach, M. Shi, C.R. Weinberg.* Biostatistics Branch, NIEHS, Research Triangle Park, NC.

Several proposed methods for examining haplotype-by-exposure interactions use data from affected individuals and their parents. Unfortunately, that design cannot assess the effects of exposure on risk, limiting the interpretability of identified interactions. We propose a new design that supplements the case-parents design with an unaffected sibling who is not genotyped but provides exposure data. If, in the population at large, inheritance is Mendelian and haplotypes do not influence propensity for exposure, then this case-control-parent tetrad structure permits study of haplotype effects, exposure effects and haplotype-by-exposure interactions. We describe omnibus permutation tests of no haplotype-by-exposure interaction. We base our test statistics on covariances between exposure and a score that reflects transmission. We consider two different permutation schemes; both involve interchanging exposures of sibs and one involves additional permutations among families within strata defined by sums of unphased parental genotypes. Our test can accommodate many SNPs, e.g., representing a multi-gene functional pathway, and can handle sporadically missing SNP genotypes and missing individuals. It is robust to exposure-involved population stratification and requires no distributional assumptions about the exposures under study. We characterize our test's performance through simulations. Preliminary findings are somewhat unexpected. In many scenarios, the tetrad design provides much better power than does the triad design for detecting haplotype-by-exposure interaction. We describe application of this tetrad design to the Two Sister Study, an ongoing study of families affected by breast cancer under age 50.

## 1826/T/Poster Board #375

**African ancestry-tobacco interaction accelerates lung function decline among African Americans.** *M.C. Aldrich[1], S. Kritchevsky[2], B. Meibohm[3], T. Leak[4], T. Harris[5], R. Kumar[6], E. Ziv[1], E.G. Burchard[1] for the Health ABC Study.* 1) Department of Medicine, UCSF, San Francisco, CA; 2) Sticht Center on Aging, Wake Forest School of Medicine, Winston-Salem, NC; 3) College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN; 4) Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 5) Laboratory of Epidemiology, Demography and Biometry, Intramural Research Program, National Institute on Aging, Bethesda, MD; 6) Children's Memorial Hospital, Department of Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, IL.

African Americans have significantly lower lung function compared to Caucasians and Mexican Americans. However, African Americans are a genetically heterogeneous admixed population with contributions from several ancestral populations of continental origin. Moreover, while tobacco smoke exposure is an established risk factor for decreased lung function, African Americans have lower clearance of cotinine from cigarette smoke compared to Caucasians. To understand disease mechanisms, it is crucial to examine genetic factors influencing racial/ethnic differences in smoking-related diseases. We sought to identify whether African genetic ancestry modifies the association between tobacco smoke exposure and decreased lung function among 936 African Americans (68-81 years) enrolled in the Health, Aging and Body Composition Study. Genetic ancestry was estimated using 1229 ancestry informative markers and the Bayesian software program STRUCTURE. Only pulmonary function tests meeting American Thoracic Society criteria for acceptability and reproducibility were included in analyses. Multivariable linear regression analyses of forced expiratory volume in one second ($FEV_1$) revealed a greater decrease in $FEV_1$ per pack-year smoking among African Americans having greater than the median (80.9%) African ancestry (-6.5 ml $FEV_1$/ smoking pack-year) compared to African Americans having less than the median African ancestry (-5.0 ml in $FEV_1$/ smoking pack-year). There was a significant interaction between African genetic ancestry and smoking (p-value = 0.03). Significant African ancestry*smoking interactions were also observed for the pulmonary function measures, forced vital capacity (FVC) and $FEV_1$/FVC (p-value = 0.02 for each measure). These results suggest individuals with greater African ancestry may have greater risk of smoking-related lung function decline. Efforts examining interactions between admixture and smoking on rate of lung function decline are ongoing. These results highlight the importance of studying admixed populations for understanding disease. This research was supported by the Intramural Research Program of the NIH, National Institute on Aging, National Heart, Lung, and Blood Institute (R01 HL74104), contracts N01-AG-6-2101, N01-AG-6-2103, N01-AG-6-2106 and by the Flight Attendants Medical Research Institute.

## 1827/T/Poster Board #376

**A Likelihood Based Approach to Detecting Gene-Gene Interactions for Nuclear Families.** *X. Cui, HS. Chen.* Mathematical Sci, Michigan Technological Univ, Houghton, MI.

Common complex human diseases are the result of interactions of several genes instead of the effect of a single gene. To detect gene-gene interactions, we propose a novel likelihood based combinatorial method which modifies and extends the MDR method (Multifactor-Dimensionality Reduction, by Ritchie et al. 2001). Our approach can deal with nuclear families of any size, and improve computational efficiency by incorporating likelihood scores. In the simulations, a variety of different models have been investigated. Under these models we demonstrate that our method has correct type I errors and uniformly better powers than the other method, PGMDR (Lou et al., 2008). We also compared our method with the MDR-PDT (Martin et al., 2006). Under some circumstances our method performed better, and in other cases, both methods performed equally well. In summary, to detecting gene-gene interactions, we propose a novel likelihood based method, which can deal with nuclear family of any size and gain better or equal power than the other two popular methods.

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## 1828/T/Poster Board #377

**SAFARI: A software package for genome-wide interaction studies.** *B. Ding[1], H. Källberg[1], L. Padyukov[2], L. Klareskog[2], L. Alfredsson[1].* 1) Ins. of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 2) Rheumatology Unit, Department of Medicine, Karolinska Institutet, Sweden.

The most common human diseases have a complex etiology involving both genetic and environmental factors. Genome-wide association studies (GWAS) has recently become a popular method for identifying susceptibility loci. However, for most diseases the genetic variants discovered so far account for only a small portion of the genetic influence, indicating that there still are many genes or loci with small effects yet to be discovered. There exists the possibility that a gene's effect is more easily detected within a framework that accommodates gene-environment interactions. This pinpoints the need of developing a computation tool for detecting gene-environment interactions in a genome-wide fashion. We developed a software package SAFARI (statistical assessment of factors related to interaction) that incorporates concepts of both statistical interaction referring to departure from the underlying form of a statistical model and biologic interaction defined as departure from additivity of the disease rates. In the program, statistical interaction is estimated by inclusion of an interaction term in a logistic regression model while the estimation of biological interaction is based on the attributable proportion due to interaction (AP) as described by Rothman (2002). We have applied this software to our Swedish Epidemiologic Investigation of Rheumatoid Arthristis (EIRA) GWAS data (Illumina 317K) and successfully identified several novel disease susceptibility loci that are modified by the environmental factor smoking. SAFARI has two packages: SAS package (coded in SAS 9.1) for SAS users and R (coded in R 2.7.2) for R users. A copy of the software is available upon request.

## 1829/T/Poster Board #378

**Gene-environment interaction testing in family-based association studies with phenotypically ascertained samples: A causal inference approach.** *D.W. Fardo[1], D.L. DeMeo[2], E.K. Silverman[2], S. Vansteelandt[3].* 1) Biostatistics, UK College Pub Hlth, Lexington, KY; 2) Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 3) Applied Mathematics and Computer Science, Ghent University, Gent, Belgium.

It is well accepted that the interplay of genetic and environmental factors can form an important part of the biological foundation of common diseases. Detecting, and more importantly understanding, these gene-environment (G x E) interactions, thus, plays a crucial role in solving the puzzle of disease-phenotypic variation.

The class of family-based association tests (FBATs; Laird et al, 2000) provides strategies for testing main genetic effects that are robust to undetected/unaccounted for population substructure. Conditioning on parental/founder genotypes (or the corresponding sufficient statistics when parental genotypes are missing; Rabinowitz and Laird, 2000) insulates these testing strategies from the bias due to ancestry-driven confounding. However, once a main genetic effect must be estimated, as in the case of testing for G x E and gene-gene (G x G) interactions, ascertainment conditions for sample recruitment must appropriately be taken into account.

The calculus of directed acyclic graphs (DAGs), specifically rules of d-separation (Pearl, 2000; Robins, 2001), helps identify estimating equations that can properly incorporate ascertainment criteria. We employ the concept of principal stratification (Frangakis and Rubin, 2002) and G-estimation techniques (Robins et al, 1992) to estimate main genetic effects consistently and are able, then, to test for gene-environment interactions.The resulting test maintains robustness to population stratification, avoids assumptions on the phenotypic and allele frequency distributions and accounts for sample ascertainment. We assess the performance of this test empirically through extensive simulation studies. To illustrate the approach in practice, we also apply these new techniques to a study of chronic obstructive pulmonary disease (Silverman et al, 1998).

## 1830/T/Poster Board #379

**Detecting Gene-Gene Interactions via Optimally Weighted Markers.** *J. He[1], K. Wang[2], A. Edmondson[3], A. Rader[3,4], C. Li[5], M. Li[1].* 1) Department of Biostatistics and Epidemiology, University of Pennsylvania; 2) Center for Applied Genomics, Children's Hospital of Philadelphia; 3) Institute for Translational Medicine and Therapeutics, University of Pennsylvania School of Medicine; 4) Cardiovascular Institute, University of Pennsylvania School of Medicine; 5) Department of Biostatistics, Vanderbilt University.

Gene-gene interactions play an important role in complex human diseases. Detection of gene-gene interactions has long been a challenge due to their complexity. The standard method that aims at detecting pairwise interactions between genetic markers may be inadequate since it does not model linkage disequilibrium (LD) among markers in the same gene and may lose power due to a large number of pairwise comparisons. To improve power, we describe a gene-based interaction test in which information of markers within a gene is optimally combined. A unique feature of our method is its ability to incorporate LD information provided by a reference dataset such as the HapMap. We analytically derive the optimal weight for both quantitative and binary traits. We then use principal components to summarize information for each gene, and test the interactions between the principal components of two genes under consideration. To evaluate the performance of our method, we conducted extensive simulations under various settings. We compared our method with several approaches, including the maximum pairwise interactions approach, which reports the maximum test statistic among all pairwise interaction test statistics, and a straightforward principal components analysis (PCA) based approach without optimal weighting. Our results show that when a single locus in the first gene interacts with a single locus in the second gene, the maximum pairwise interactions approach is the most powerful, followed by our method, but when there are two loci in the first gene interact with two loci in the second gene, then our method yields the greatest power, followed by the PCA-based approach. In general, our method performs better than the other two approaches under more complicated interaction mechanism, for example, when each of the two genes involve several disease loci and when not all disease loci are directly genotyped. In addition to simulations, we also applied our method to a HDL dataset which consists of 1,231 individuals genotyped using the ITMAT/Broad/CARe vascular disease 50K SNP array. Our method detected significant interactions between genes CETP and BCAT1. Given the complexity of human disease etiology, our method should prove useful in unraveling disease susceptibility genes for complex human diseases.

## 1831/T/Poster Board #380

**A general framework for studying genetic effects and gene-environment interactions with missing data.** *Y.J. Hu, D.Y. Lin, D. Zeng.* Dept Biostatistics, Univ North Carolina, Chapel Hill, NC.

Missing data arise in genetic association studies when genotypes are unknown or when haplotypes are of direct interest. We provide a general likelihood-based framework for estimating genetic effects and gene-environment interactions with such missing data. We allow genetic and environmental variables to be correlated while leaving the distribution of environmental variables completely unspecified. We consider three major study designs --- cross-sectional, case-control, and cohort designs --- and construct appropriate likelihood functions for all common phenotypes (e.g., case-control status, quantitative traits, and potentially censored ages at onset of disease). The likelihood functions involve both finite- and infinite-dimensional parameters. The maximum likelihood estimators are shown to be consistent, asymptotically normal, and asymptotically efficient. EM algorithms are developed to implement the corresponding inference procedures. Extensive simulation studies demonstrate that the proposed inferential and numerical methods perform well in practical settings. Illustration with a genome-wide association study of lung cancer is provided.

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## 1832/T/Poster Board #381

**Association of BFSP2 for age-related cortical cataract is modulated by cigarette smoking.** *G. Jun[1], B.E.K. Klein[2], R. Klein[2], T. Joshi[1], K.E. Lee[2], T.D. Spector[3], T. Andrew[3], C.J. Hammond[3], R.C. Elston[1], S.K. Iyengar[1,4,5].* 1) Epidemiology and Biostatistics, Case Western Reserve University School of Medicine, Cleveland, OH; 2) Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI; 3) King's College London, St Thomas' Hospital Campus, Twin Research & Genetic Epidemiology Unit, London, UK; 4) Genetics, Case Western Reserve University School of Medicine, Cleveland, OH; 5) Ophthalmology, Case Western Reserve University School of Medicine, Cleveland, OH.

Age-related cortical cataract is a multifactorial disease influenced by both genetic and environmental risk factors. We studied two family-based cohorts (twin study: 185 individuals from 172 families, population-based study: 1401 individuals in 494 families) for age-related cortical cataract. We examined four variants in the gene for beaded filament structural protein 2 (BFSP2) because it is known to be associated with juvenile cataract. We also examined gene-environment interactions between SNPs in BFSP2 and two known environmental risk factors: cigarette smoking and alcohol intake. A quantitative measure, the percent of the lens affected, was adjusted for covariates and transformed before the analysis. Under the additive model, rs2078262 was the most significant in the twin cohort ($P = 7 \times 10^{-9}$), but was not significant in the population-based cohort or in a meta-analysis of both. However, rs9289447 was significant using a regression model with the interaction term (INT) between the SNP and cigarette smoking (COV) in both datasets. The joint model (JOINT) including the interaction, the SNP, and the smoking covariate was the best model for the cortical cataract trait in the population-based cohort (SNP = 0.190, COV = $3 \times 10^{-5}$, INT = $2 \times 10^{-5}$, JOINT = $4 \times 10^{-8}$) and the twin cohort (P values: SNP = 0.024, COV = 0.218, INT = $1 \times 10^{-5}$, JOINT = $6 \times 10^{-6}$); the combined P value of the joint model was $3 \times 10^{-6}$. We have thus demonstrated that development of age-related cortical cataract is modulated by exposure to cigarette smoking in later life and genetic predisposition at the BFSP2 gene.

## 1833/T/Poster Board #382

**Gene Set Analysis based on Gene Interactions for Microarray Expression Data.** *J. Li[1], J. Zhang[2], H. Deng[2,3,4].* 1) Dept Info Med Person Hlth, Univ Missouri-Kansas City, Kansas City, MO; 2) Depts Orthopedic Surgery and Basic Medical Science, Univ Missouri-Kansas City, Kansas City, MO; 3) 3. The Key Laboratory of Biomedical Information Engineering of Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, Shaanxi 710049, P. R. China; 4) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P. R. China.

Gene set analysis is an important tool in analyzing microarray data of complex human diseases. It allows the inclusion of knowledge from established gene sets, such as gene pathways, and potentially improves the power of detecting differentially expressed genes, even when the expression changes of individual genes are modest. However, conventional methods of gene set analysis ignore the information of gene interactions which may contribute to variation of complex human diseases. In this study, we propose a method that uses knowledge of predefined gene sets (e.g. gene pathways) and identifies those gene sets, in which gene interactions are enriched and associated with the phenotype of interest. The utility of our method is demonstrated through analyses on two publicly available microarray datasets. The results show that our method identifies several sets of genes that show strong gene interaction enrichments that are associated with the studied phenotypes. The enriched gene interactions identified by our method may provide clues to new gene regulation pattern which are not already accounted for through known pathway identification or classical gene set analyses. In summary, our method can give researchers the ability to extract potentially disease-related genes from microarray data, and thus is helpful to delineate the sophisticated knowledge of relevant molecular pathways of disease pathogenesis. Our method can be a useful complement to classical gene set analysis which only considers single genes in a gene set.

## 1834/T/Poster Board #383

**Contribution of smoking and known susceptibility variants to the increased risk of urinary bladder neoplasms in individuals with a familial cancer history.** *J. Lorenzo Bermejo[1,2], J. Lascorz[2], K. Hemminki[2,3].* 1) Institute of Medical Biometry , University Hospital Heidelberg, Heidelberg, Germany; 2) Division of Molecular Genetic Epidemiology, German Cancer Research Center, Heidelberg, Germany; 3) Center for Primary Health Care Research, University of Malmö, Malmö, Sweden.

The aim of our study was to quantify the contribution of smoking and known genetic susceptibility variants to the increased risk of urinary bladder neoplasms in individuals with a familial cancer history. Gene variants, genetic parameters and reference values for smoking were retrieved from meta-analyses and from genome-wide association studies. This information was combined using epidemiological models and simulation.

Under the investigated domain of parameter values, the contribution of smoking to the clustering of lung and bladder cancers in families was limited to a familial relative risk (FRR) of 1.11. Daughters of women affected by lung cancer showed a FRR=1.81 of bladder cancer. In other words, the FRR explained by smoking represented 0.11/0.81=16.6% of the familial excess. Brothers of laryngeal cancer patients showed a FRR=2.93 and the proportion attributable to smoking was 5.2%. The contribution of smoking to the clustering of two cases of bladder cancer in the same family was 8.3% for sons of affected fathers and 3.5% for mother-daughter pairs.

The attributable risks of known common susceptibility variants are extensively narrowed by their conferred penetrances (genotype relative risks (GRRs) under 1.5). For example, the increased risk of stomach cancer (GRR=1.3) and bladder cancer (GRR=1.4) in *GSTM1* null genotypes, assuming a phenotype frequency of 56% and a recessive penetrance model, translated into FRR=1.03. Brothers of men affected by stomach cancer showed a FRR=2.00 of bladder tumours. Thus, *GSTM1* contributed with a 3% to the familial excess. If known risk alleles in *XRCC3*, *NAT2*, *GSTM1*, *GSTP1* and *NQO1* interacted multiplicatively on bladder cancer susceptibility, they would result in FRR=1.09. The contribution of these five variants to the aggregation of two cases of bladder cancer in one single family was 15% for sons of affected fathers and 6.3% for mother-daughter pairs.

Our results suggest that, in addition to gender and smoking history, future association studies may benefit from familial histories of other malignancies than bladder cancer. Even under the assumption that smoking and known susceptibility variants interact multiplicatively, the aetiology of a large proportion of familial cases remains still unknown.

## 1835/T/Poster Board #384

**A Bayesian hierarchical model with gene and environment interaction in a matched case-control metabolic syndrome study.** *S. Wang[1,2], C. Hsiao[1,3], L. Chuang[4], P. Liu[1,2], K. Chien[2,5], S. Lin[6], W. Chen[1,2,3].* 1) Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan; 2) Genetic Epidemiology Core Laboratory, Division of Genomic Medicine, Research Center for Medical Excellence, National Taiwan University, Taipei, Taiwan; 3) Department of Public Health, College of Public Health, National Taiwan University, Taipei, Taiwan; 4) Department of Internal Medicine, College of Medicine and National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan; 5) Institute of Preventive Medicine, College of Public Health, National Taiwan University, Taipei, Taiwan; 6) Bureau of Health, Taoyuan County Government, Taoyuan, Taiwan.

Genes, environment, and the interaction between them are known to play important roles in metabolic syndrome. Environmental influences include individual-level effects, such as personal characteristics and life styles, and group-level effects, usually called contextual effects. To explore the interaction between genes and contextual factors, it requires a formulation of hierarchical statistical models. In this study, we adopted a Bayesian hierarchical mixed-effects model for a matched case-control study, where each selected case was matched with one to two controls by sex, age, education level, ethnicity, and a contextual covariate. There were 326 matched pairs, a total of 738 persons. Five SNPs located on genes LEP (H1328082 and G2548A), APM1 (A-10066G and G-7950T), and PPARG (Pro12Ala) were genotyped. Meanwhile, availability in exercise facility in an area was treated as the contextual covariate. We evaluated the gene and environment interactions by examining if the allelic effect differs among various contextual levels. The magnitude of the corresponding variability indicates the heterogeneity of this allelic effect among areas. Under non-informative Gaussian priors on the pair and individual specific random effects and inverted Gamma priors on variance components, we performed the statistical analysis based on posterior samples obtained from the software WinBUGS version 1.4.3. For the SNP Pro12Ala, the posterior means of effects on the logit scale were 1.3, -0.7, and -0.1, respectively, for the three levels in exercise facility availability, indicating a substantial difference among areas. In addition, the posterior mean and median of the corresponding variance were 1.3 and 1.1, indicating the gene effect could be modified by the contextual factor. With regard to other SNPs, their effects on metabolic syndrome were similar across areas, all with small posterior variances. We also carried out the sensitivity analysis to examine the effect of prior specifications and the results were robust. In summary, this Bayesian hierarchical mixed-effect model provides an alternative approach to inference on the interaction between genes and a contextual variable.

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## 1836/T/Poster Board #385

**A Comparison of Sample Size and Power in Case-only Association Studies of Gene-Environment Interaction.** *G.M. Clarke, A.P. Morris.* Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom.

Assuming continuous environmental and categorical genotype variables, the authors compare six case-only tests of association for gene-environment interaction. Novel tests modelling the environmental exposure as response, a non dominant coding of the genetic exposure as response, as well as the traditional test with its dominant coding of the genetic exposure as response, are included. The authors show that tests imposing the same genotypic pattern of inheritance perform similarly regardless of whether genotype is modelled as response or predictor. These novel tests with genetic exposure as response are advantageous as they are robust to non-normally distributed environmental exposures. Dominance deviance, deviation from additivity in the main or interaction effects, is key to test performance: when zero or modest, tests that search for a trend with increasing risk alleles, are optimal; when large, tests for genotypic effects are optimal. However, the authors show that when testing at a proxy locus, common in genome-wide association studies, a large dominance deviance is rare and so genotypic, as opposed to trend, tests are not generally recommended. Traditional tests assuming a dominant pattern of inheritance can suffer from serious losses of power in the presence of any recessive, or modest dominant, effects.

## 1837/T/Poster Board #386

**Insulin resistance related expression quantitative trait network: Application of linkage analysis to directed genetic network construction.** *K. Kim[1], J. Min[2], G. Kwon[3], J. Sung[1], S. Cho[1].* 1) School of Public Health, Seoul National University; 2) Institute of Health and Environment, Seoul National University; 3) Department of Preventive Medicine, School of Public Health, Seoul National University.

**Introduction** Insulin resistance is a knwon cause of endocrine-metabolic disorders, and the pathologic or epidemiologic characteristics were continually investigated, including the genetics. In this study, we suggest a novel, intuitional method to construct the expression quantitative trait (eQT) network related to insulin resistance syndrome using LOD scores and the peak loci for eQTs based on gene-gene interaction concept. **Materials and Methods** Data were from 15th Genetic Analysis Workshop, Problem 1. The data included 194 individuals in 14 three-generation families. Phenotypes were expression levels of 3554 genes in lymphoblastic cells. Of those, we selected 50 eQTs related to insulin resistance. Genotype data were available for 2884 SNP markers across the genome. Variance component linkage analysis was performed to explore the expression loci of each of the eQTs. The linkage peak loci were investigated, and the “support zone” was defined within the bounds to 0.5 LOD score from the peak. In order to explore gene-gene interactions, we investigated the relationships between all possible pairs among 50 genes. If one gene was located within the “support zone” of the peak loci for the eQT of another gene, the relationship was considered as a potential “directed causal pathway” from the former to the latter gene. Merlin 1.1.2 software was used for the analysis. **Results** We identified linkage peaks with >2.0 LOD scores for 13 eQTs, and 6 of them showed >2.5 LOD scores. SNP markers under the linkage peaks or within the support zone were searched in the database to identify the genes at the loci. 8 genes were identified on the peak, and 31 genes were identified in the support zone. 9 genes were included among the selected 50 eQTs. Gene network was constructed with those eQTs. 2 groups of gene network were formed, each around IRS2 and UGCGL2 genes; IRS2 with 3 genes, ASCL3, IDE, and IGFBP2; UGCGL2 with 4 genes, APOL3, APOL6, LEPR, and UGP2. **Conclusion** This study supported the evidence of gene networks among the genes related to insulin resistance syndrome. Use of linkage analysis enabled construction of causal directed networks. The methodology of our study showed that characterizing and locating eQTs can provide an effective way to construct genetic network model.

## 1838/T/Poster Board #387

**ReliefF Methods for Gene-Gene Interactions Including Covariate Information: Application to Antibody Response/Adverse Events Following Anthrax Vaccine Adsorbed Vaccination.** *N. Pajewski[1], V. Srinivasasainagendra[1], S. Parker[1], R. Kaslow[1], H. Tiwari[1], B. McKinney[2].* 1) University of Alabama at Birmingham, Birmingham, AL; 2) University of Tulsa, Tulsa, OK.

Interactive effects, both gene by gene and gene by environment, may play a role in the development of complex traits. Due to the high-dimensional nature of current genetic studies, comprehensive yet powerful searches for these effects require computationally efficient approaches. Machine learning methods, such as ReliefF, Multifactor Dimension Reduction, and different variations of neural networks have been popularized as one such set of approaches. However, the need to account for covariate information with these methods is an issue that has not been fully explored. As an example, our work was motivated by a Centers for Disease Control sponsored clinical trial investigating immunogenicity and reactogenicity of a reduced-dose schedule and intramuscular injection of Anthrax Vaccine Adsorbed (AVA). We aimed to identify variations within 154 candidate immune response genes (densely genotyped with 7800 SNPs) that might influence anti-PA IgG concentrations and/or local/systemic adverse events within the AVA trial population. The population included a ethnically diverse group (Caucasians, African-Americans, and others), recruited from 6 clinical sites, distributed widely by age (18 to 61 years) and equally by sex, who were randomized to reduced dose schedules or to intramuscular versus subcutaneous injections. Because machine learning algorithms are not cast within the usual linear model statistical framework, most do not directly offer an option to adjust for phenotypic effects attributable to covariates such as those mentioned above. Herein, we focus on ReliefF, which is a filter capable of reducing genome-wide datasets while respecting the structure of any gene-gene interactions inherent in the data. Using data from the AVA trial and simulations based on Phase 3 of the International HapMap Project, we first illustrate the deleterious impact of ignoring this covariate information upon applying the ReliefF interaction filter. In particular, we illustrate that ReliefF is just as susceptible to spurious results from population stratification as the usual practice of single marker hypothesis testing. We then introduce a modified ReliefF filter that incorporates stratification/group information, thus allowing for powerful detection of interactive effects while alleviating covariate induced confounding.

## 1839/T/Poster Board #388

**Finding that Elusive Gene-Environment or Gene-Gene Interaction: Prioritizing SNPs for Quantitative Trait Interaction Testing.** *G. Pare[1,2], N.R. Cook[2], P.M. Ridker[2], D.I. Chasman[2].* 1) McMaster University, Hamilton, ON, Canada; 2) Brigham and Women’s Hospital, Boston, MA, USA.

Whole genome association studies have identified many common genetic determinants of complex traits. However, few gene-gene and gene-environment interactions have been identified and validated. One of the main obstacles to interaction testing stems from multiple hypothesis testing. In this report, we show that under plausible interaction scenarios, the variance of a quantitative trait is expected to differ between the three possible genotypes of a biallelic SNP. Leveraging this observation with Levene’s test of equality of variance, we propose a novel method to prioritize SNPs for subsequent gene-gene and gene-environment testing. Prioritization is independent of subsequent interaction testing and substantially reduces the burden of multiple hypothesis testing. We first use simulations to demonstrate that our method has increased power over conventional ones. Then, using data from our ongoing genome scan (n=20,628) of two inflammatory markers, C-reactive protein and soluble ICAM-1, we successfully apply our method to identify two novel interactions which replicate (combined P-values of $4.2 \times 10^{-10}$ and $4.4 \times 10^{-8}$, respectively). Both C-reactive protein and soluble ICAM-1 are plasma markers used in the prediction of cardiovascular disease and diabetes. The first interaction involves the leptin receptor SNP rs12753193 and body mass index in the prediction of C-reactive protein. The second interaction involves the PNPLA3 SNP rs738409 and body mass index in the prediction of soluble ICAM-1. This second interaction is particularly interesting because 1) it would not have been identified using conventional methods and 2) the PNPLA3 SNP rs738409 has recently been associated with fatty liver disease, a phenotype tightly linked to both insulin resistance and inflammation.

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## 1840/T/Poster Board #389

**Risk of tuberculosis (TB) among close contacts to TB patients: role of SLC11A1 and Interleukin-10 promoter polymorphisms.** *M. Reichler[1], C.C. Luo[1], B. Chen[1], E. Sigman[1], F. Maruri[2], T. Sterling[3] for the TB Epidemiologic Studies Consortium.* 1) Div TB Elimination, Centers Disease Control, Atlanta, GA; 2) Tennessee Department of Health, Nashville, TN; 3) Vanderbilt University, Nashville, TN.

**Background:** Single nucleotide polymorphisms (SNPs) in the SLC11A1 gene have been implicated in susceptibility to TB, possibly through regulation of the immunosuppressive cytokine interleukin-10 (IL-10). **Methods:** We enrolled US-born contacts with latent TB infection or active TB disease with >180 hours of exposure to smear+ TB patients at 9 sites. Blood was collected and genotyped for SLC11A1 gene SNP 3'UTR and IL-10 gene SNPs -592C/A, -819C/T and -1082A/G and epidemiologic data collected by contact interview. Pair-wise analysis was conducted for gene SNPs singly and in combination using chi2 tests with homozygous wild genotype as the referent group. This analysis is limited to contacts of Black race/ethnicity. **Results:** Among 663 contacts, 17 had active TB and 646 had a positive tuberculin skin test (TST+). Compared with TST+ contacts, contacts with active TB were more likely to have the homozygous mutant genotype for SLC11A1 3'UTR in combination with the homozygous mutant genotype for IL-10 -1082A/G (OR=9.09, P<.001), IL-10 -819C/T (OR=10.05, P<.001), or IL-10 -592C/A (OR=15.50, P<.001), but equally likely to have the homozygous mutant genotype for each individual SNP (OR=2.13, P=.33 for SLC11A1 3'UTR; OR=1.24, P=.75 for IL-10 -592C/A; OR=1.17, P=.81 for IL-10 -819C/T; OR=1.96, P=.20 for IL-10 -1082A/G). **Conclusions:** In this U.S. population, active TB in contacts after exposure to *M. tuberculosis* was associated with homozygous mutant genotype for SLC11A1 in combination with homozygous mutant genotype for three IL-10 SNPs. These findings suggest that the risk of active TB after M. tuberculosis exposure may be modulated by gene combinations rather than single genes, and that synergy between SLC11A1 3'UTR and IL-10 gene SNPs may exist.

## 1841/T/Poster Board #390

**Test for gene-gene interaction from case-control data: A study using a combination of non-parametric methods.** *A. Sengupta Chatterjee[1,2], C.C Chang[1], L.B Lian[4], C.S.J Fann[1,3].* 1) Department of Biostatistics, Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Department of Life Sciences, Institute of Biomedical Informatics, National Yang-Ming University,Taipei, Taiwan; 3) Department of Life Sciences, Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan; 4) Department of Mathematics, National Changhua University of Education, Changhua,Taiwan.

A major goal of genetic epidemiology is to determine the genetic architecture of common human diseases. Identification and characterization of susceptibility genes influencing such diseases remains a computational challenge since the effect of any single genetic variant on a complex disease may be dependent on other genetic variants through gene-gene interactions. To address this situation, statisticians have developed novel methods over the years. Presently, no single method is recognized as the best in this direction. Hence a combination of methods with the aim of balancing their specific strengths may be an optimal approach for obtaining significant results, minimizing the probability of producing false positives. Nonparametric methods have gained increased interest for their ability to handle high dimensional data. In this study we propose to include three non-parametric approaches to detect gene-gene interactions, on case-control datasets, namely (a) GINI criteria (used in CART decision trees) ,(b) a naïve Bayesian approach (A sum of absolute differences in probability of finding cases and controls) and (c) an entropy based method (based on Shannon's information entropy). We use each of these methods to score and rank 2-way Single Nucleotide Polymorphism (SNP) interactions, and subsequently obtain a summary score based on the above three scores to rank the SNP combinations. To evaluate the power we simulate 100 datasets, each for 6 different two locus epistasis models. Each dataset consists of 200 SNP markers with 900 cases and 1000 controls. Our approach produces high power (81%) to detect two-way SNP interactions for SNPs with main-effects (false positive = 1%) and shows moderate power (39%) to detect interactions for SNPs without main-effects (false positive = 3.5%). We also compare our results with Multifactor Dimensionality Reduction (MDR) method to obtain comparable results in terms of power (78% for SNPs with main-effects and 28% for SNPs without main-effects) with slightly better type I error rates than MDR. Although both the methods show less power for SNPs without main-effects, our method seems to perform slightly better than MDR for both, SNPs with and without main-effects.

## 1842/T/Poster Board #391

**RAPID detection of pairwise epistatic interactions in Genome wide association.** *V. Bafna[1], D. Brinza[1], G. Tesler[3], D. O' Connor[2].* 1) Computer Science & Engineering, UC San Diego, La Jolla, CA; 2) Department of Medicine, UC San Diego, La Jolla, CA; 3) Department of Mathematics, La Jolla, CA.

Significant challenges remain in GWA studies. In complex disorders, many independently evolving loci might interact to confer disease susceptibility, with only a modest effect at each locus. Detecting interactions in GWAS on large populations is computationally challenging. A test involving all pairs of m markers, with a case-control population of n individuals, involves nm^2 computations. For GWAS (n=1000,m=1M), this implies 10^(15) computations. Further, a straightforward (bonferroni-like) correction for the multiple tests results in significant loss of sensitivity. Our algorithm, RAPID, scales linearly with m SNPs, with no loss of sensitivity. The algorithm is based on a two-stage, iltering model. In the first stage (the filter stage), we discard a vast majority of the locus pairs, while retaining the truly interacting pairs. The filtering stage is guarenteed to be (a) fast; (b) efficient (Most candidate pairs are discarded), and (c) sensitive (truly interacting pairs are admitted). The second stage scores the remaining candidate pairs using standard tests of association. Previous filtering strategies (Marchini et al.) are based on the assumption that interacting pairs of loci should also show a marginal effect at each locus. Empirical results on simulated models of interaction show that there is a clear loss of power by employing these filters. By contrast, our method provides explicit guarantees on power of detecting interactions. There are two key ideas in RAPID. The first is a mapping of loci to a high-dimensional Euclidean metric so that statistical correlation between two loci corresponds to distance in the Euclidean space. The second is the use of a randomized technique, Locality Sensitive Hashing (LSH), in which the loci are independently projected to random hyperplanes while preserving distance between loci. Simulations show that RAPID outperforms previous approaches in speed and power. We also applied our method to the WTCCC hypertension data-set, which is particularly challenging case for GWAS, as a number of genes of modest effect define disease etiology. In a fast (10 hrs. on a PC) computation designed to uncover the most significant interactions, we identified ~30 pairs with un-adjusted p-values ranging from 10^{-10} to 10^{-11}. The gene X gene interactions reveal insights into this complex disease.

## 1843/T/Poster Board #392

**A Powerful Genome-Wide Association Study of Multiple SNPs considering Interlocus Interactions.** *R. Nakamichi, T. Tsunoda.* Center for Genomic Medicine, RIKEN, Yokohama, Kanagawa, Japan.

By the success of International HapMap Project, half million of tag SNPs covering whole genome were selected, and genome-wide association study (GWAS) to identify disease-related genes is realized. In the case of multi factorial disease, pathogenic factors are commonly observed among the population, and the contribution of each factor is relatively small. Therefore, case/control study is the mainstream to analyze multi factorial disease. Since the detection power is not enough because of small sample number, the current study is mainly focused on the analysis between a trait and a single locus. However even a binary trait, to which case/control analysis is applicable, is often controlled by multiple genes, and is difficult to analyze each locus separately, especially when the interaction exists between genes. Simple chi-squared analysis is not enough to analyze these situations. Therefore, we need more powerful statistical model. When considering gene interactions mentioned above, multiple comparison is an important problem. Bonferroni correction is too conservative when linkage disequilibrium is observed among target SNPs. Permutation test is another approach which can achieve practical correction when linkage disequilibrium exists, however is computationally heavy. For this problem, rapid association test (RAT) was proposed. It uses importance sampling to approximate permutation test, and succeeded in drastic reduction of calculation in the association study on the basis of simple chi-squared test. In this study, we extend RAT to multiple SNP model and discuss feasibility of GWAS considering gene interaction. We implemented extended RAT as fast permutation test based on logistic regression and likelihood ratio test to analyze a pair of SNPs associated to disease by interaction. We performed simulations for GWAS reflecting actual linkage disequilibrium under 2SNP interaction model, and we showed that extended RAT can effectively analyze a set of two SNPs with interaction within practical calculation time. Actually, GWAS is performed as case/control study using a few thousands of samples and limited in the single SNP analysis because of detection power. In the future, sample size may increase to tens of thousands, and we can expect to perform the analysis of combination of two or more SNPs because of increase of detection power. And then, we expect our extended RAT will effectively contribute to analyze huge data at that time.

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## 1844/T/Poster Board #393

**Gene-gene interaction on selected pathways.** *S. Eyheramendy[1], S. Sober[2], E. Org[2], M. Laan[2].* 1) Statistics Department, Pontificia Univ Catolica de Chile, Santiago, Chile; 2) Department of Biotechnology, Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia.

In genetic association studies of complex diseases, the discovery of gene-gene interactions is of great interest because most complex disorders are thought to be caused by numerous genes and environmental factors, a subset of which may act synergistically. Gene-gene interactions are hard to detect because there are thousands of genes and millions of SNPs that might be interacting in an unknown manner. Statistically, it is hard to detect epistasis because of the sparseness of data in higher dimensions, which results in that exponentially larger datasets are needed to reliably estimate the interacting models. We address this difficulty by grouping SNPs according to the pathway that they belong to and then do a gene-gene interaction analysis within (and also between) pathways for a few selected pathways. We select a handful of pathways among the ones that show the highest level of association with the phenotype. In this way we decrease the dimensionality problem by reducing the number of pair-wise tests that need to be performed while at the same time attempting not to loose valuable genetic functionality information. Hence, this approach generates a list of candidate interactions that can be empirically tested. We use the KORA 500k Affymetrix genotype data to illustrate this methodology on blood pressure traits. Our study shows that poulation genetic data can be used to perform genome-wide searches for gene-gene interactions, generating interesting gene pairs that can be further probed for their contribution to phenotypic variation in comptex traits.

## 1845/T/Poster Board #394

**A model for family-based case-control studies of genetic imprinting.** *T. Liu[1], R.L. Wu[2].* 1) Genome Inst Singapore, Singapore, Singapore; 2) Departments of Public Health Sciences and Statistics, Pennsylvania State University.

Genetic imprinting has been recognized to play an important role in the formation and pathogenesis of human diseases. In this study, we present a statistical model for testing the effect of genetic imprinting (or parent-of-origin) on a human disease in case-control studies with family structure. For each subject sampled from a case and control population, we not only genotype its own single nucleotide polymprohisms (SNPs), but also collect its parents' genotypes. By tracing the transmission pattern of alleles from parental to offspring generation, the model allows the characterization of genetic imprinting effects based on chi-squared tests of a contingency table. The model is expanded to test the interactions between imprinting effects and additive, dominant, and epistatic effects in a complex web of genetic interactions. Statistical properties of the model are investigated and its practical usefulness is validated by a real data analysis.

## 1846/T/Poster Board #395

**Characterization of Gene Interaction and Assessment of LD Matrix Measures for the Analysis of Biological Pathway Association.** *D.R. Crosslin[1], X. Qin[2], E.R. Hauser[2].* 1) Department of Biostatistics, University of Washington, Seattle, WA; 2) Center for Human Genetics, Duke University Medical Center, Durham, NC.

Complex diseases will have multiple functional sites, and it will be invaluable to understand this LD interaction between those sites (epistasis) in addition to the haplotype-LD effects. We investigated the statistical properties of a class of matrix-based statistics to assess epistasis. These statistical methods include three LD contrast tests (Zaykin et al. 2006, Wang et al. 2007) and partial least squares regression (Wang et al. 2008). To estimate Type 1 error rates and power, we simulated multiple two-variant disease models using the SIMLA software package. SIMLA allows for the joint action of up to two disease genes in the simulated data, with all possible multiplicative interaction effects between them. Our goal was to detect an interaction between multiple disease-causing variants by means of their linkage disequilibrium (LD) patterns with other markers. While the disease variants were not included in the correlation matrices assessed, we measured the effects the marginal disease effect size in terms of relative risk (RR) (1.0, 1.5 and 3.0), haplotype LD, disease prevalence and minor allele frequencies (MAF) have on cross-locus interaction (epistasis) that we specified though RR (1.0, 3.0 and 10.0). We evaluated 3 categories of null epistasis models: 1. No marginal effects and no LD; 2. Marginal effects and no LD; and 3. LD and no marginal effects. We evaluated 3 categories of alternative models: 1. No epistasis in the presence of marginal effects and LD; 2. Epistasis in the presence of LD and no marginal effects; and 3. Epistasis in the presence of marginal effects and LD. We simulated the data using a multiplicative disease model. We calculated the correlation between the two disease variants to evaluate the underlying evidence for the interaction disease models. In the setting of strong allele effects and strong interaction, the correlation between the two disease genes was weak (r=0.2). In a complex system with multiple correlations (both marginal and interaction), it was difficult to determine the source of a significant result. Despite these complications, the partial least squares approach maintained adequate power to detect the epistatic effects; however, for many of the analyses we often could not separate interaction from a strong marginal effect. While we did not exhaust the entire parameter space of possible models, we do provide guidance on the effects that population parameters have on cross-locus interaction.

## 1847/T/Poster Board #396

**Investigating the interactive effects of RHD and ABO blood group loci on human fitness.** *W.K. Meyer[1], G.H. Perry[1], M. Caliskan[1], R.S. Smith[2], C. Lee[2,3], R.R. Hudson[4], C. Ober[1,5], M. Przeworski[1,4].* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Pathology, Brigham & Women's Hospital, Boston, MA; 3) Harvard Medical School, Boston, MA; 4) Department of Ecology & Evolution, University of Chicago, Chicago, IL; 5) Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL.

We considered the functional interaction between the RHD and ABO blood group systems in an evolutionary framework. The RHD negative phenotype is recessive, most often the result of homozygous deletion of the entire *RHD* gene. Prior to the availability of the clinical preventive treatment Rho(D) immune globulin in 1968, some pregnancies involving RHD positive children of RHD negative mothers resulted in miscarriage, stillbirth, or infant mortality due to hemolytic disease of the newborn (HDN). Following an initial "sensitizing" pregnancy of a RHD positive child, which exposed the mother to the RHD antigen at birth and initiated anti-RHD production, subsequent RHD positive offspring could develop HDN. Studies estimate that RHD sensitization was prevented in 55 to 90% of susceptible pregnancies if the child also carried a foreign ABO antigen, as determined by the *ABO* gene. The *RHD* and *ABO* genes are thus involved in an indirect biological interaction resulting in differential selective pressures according to maternal and child genotypes. We investigated the selective effects of different alleles of these genes in a sample of 841 Hutterites, a founder population of European descent. We hypothesized that there might be a statistical association between *RHD* and *ABO* genotypes in the Hutterites due to differential survival and reproductive potential among two-locus genotypes. We used a chi-square test of homogeneity among genotypes to provide preliminary evidence of such an association. Because the Hutterites are related through a common 1650 person, 13 generation pedigree, we assessed the significance of the association by allele dropping down this pedigree. The chi-square statistic calculated from the true data was marginally significant ($p<0.1$) when evaluated against 10,000 allele-dropping simulations. We also compared number of offspring and inter-birth interval among families with different genotype combinations before the use of Rho(D) immune globulin. These analyses reveal selection against RHD positive children of negative mothers, but with incomplete penetrance. Modeling the effects of *RHD* and *ABO* on fitness indicates that, given plausible parameters, this interaction could have played a role in maintaining appreciable frequencies of *RHD* deletion alleles. We are now extending this work by developing a statistical framework to identify other genetic interactions of potential evolutionary and biological importance.

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## 1848/T/Poster Board #397

**Selecting multiple epistatic models using MB-MDR.** *T. Cattaert[1], V. De Wit[1], J.M. Mahachie John[2], K. Van Steen[1].* 1) Montefiore Institute - Bioinformatics, University of Liege, Liège, Belgium; 2) Department of Oto-rhino-laryngology, Ghent University, Ghent, Belgium.

When searching for epistasis, parametric regression approaches have severe limitations when there are many independent variables compared to the number of observed outcome events. Alternatively, the non-parametric Multifactor Dimensionality Reduction method[3] can be applied. It handles the dimensionality problem by pooling multi-locus genotypes into two groups of risk: high and low. This method and its numerous extensions are all extremely computer-intensive. Best models are evaluated on the basis of cross-validation and prediction accuracy measures, and only one such best model is proposed and its significance assessed through permutations.

We recently proposed FAM-MDR,[2] a novel unified MDR strategy for genetic interaction association analysis that can handle both unrelateds and families of any structure, different outcome types (e.g. categorical, continuous or survival type), and allows adjustment for lower order effects or confounding factors. By relying on the flexible Model-Based MDR,[1] a semi-parametric MDR method to detect epistasis in unrelated individuals, we obtain a less computationally intensive method. Association tests are applied to identify multi-locus risk cells and on the final one-dimensional construct.

In the present research, we focus on allowing multiple clusters of markers to be proposed as showing significant association with the outcome under investigation. In this light, improvement of the identification of high and low multi-locus risk cells is discussed also. Optimal definitions may substantially improve the power of our method and may dramatically impact computation time. This epistasis detection method will be evaluated and validated via a simulation study, by computing power and type I error under a variety of scenarios, including heterogeneity.

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## 1849/T/Poster Board #398

**Allelic based Gene-Gene Interaction in longitudinal data.** *J. Jung.* Med & Molec Gen, Indiana Univ Sch Med, Indianapolis, IN.

In longitudinal data where repeated measures are present, there is lack of statistical methods to identify genetic association through interaction caused by multiple single nucleotide polymorphisms (SNPs) within a gene as well as by SNPs at unlinked genes contributing to risk of a disease. A novel statistical approach is proposed to detect the gene-gene interactions at the allelic level under semiparametric framework. With a new allelic score inferred from the observed genotypes at two or more unlinked SNPs, we derive profiled score statistics which are unbiased and asymptotically efficient using profile likelihood function. By testing for the association, the interaction can be assessed both in cases where the SNP association can be detected and cannot be detected as a main effect in single SNP approach. The analytical power and type I error rates over 6, two-way interaction models are investigated based on simulation study. Simulation studies demonstrate that (1) the profiled score statistic follows chi-square distribution on three degrees of freedom in two unlinked genes and (2) the allelic based method provides higher power than a genotypic based methods.

## 1850/T/Poster Board #399

**Detection of SNP-SNP Interactions in Case-Parent Trios.** *I. Ruczinski[1], Q. Li[1], TA. Louis[1], MD. Fallin[2], AE. Pulver[3].* 1) Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 2) Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) Department of Psychiatry and Behavioral Sciences, Johns Hopkins Bloomberg School of Medicine, Baltimore, MD.

Statistical approaches to detect higher order SNP-SNP (and possibly SNP-environment) interactions are critical in genetic association studies, as susceptibility to complex disease is likely related to the interaction of multiple SNPs (and environmental factors). We present a novel method to detect SNP-SNP interactions in trios with affected probands. The interactions are assessed in a regression framework, and become part of the model search space. The approach accounts for the linkage disequilibrium (LD) structure in the genotype data, and accommodates missing genotypes via haplotype-based imputation. We present a case study using case-parent trios from a study of schizophrenia and schizo-affective disorder, which revealed a genotype-phenotype association that includes an allele without marginal effect. We also introduce the open source software, which includes an efficient algorithm to simulate case-parent trios where genetic risk is determined by epistatic interactions.

## 1851/T/Poster Board #400

**TSLP Polymorphisms are Associated with Asthma in a Sex-Specific Fashion.** *G. Hunninghake[1, 2, 4], H. Kim[2, 4], M. Soto-Quiros[5], J. Lasky-Su[1, 4, 6], L. Avila[5], N. Rafaels[7], K. Barnes[7], J. Wilk[8], G. O'Connor[8], W. Gauderman[9], J. Baurley[9], F. Gilliland[9], S. Sharma[1, 2, 4], B. Himes[1, 3, 10], E. Israel[2, 4], B. Raby[1-4], A. Bush[11], A. Choi[2, 4], S. Weiss[1, 2, 4], J. Celedon[1-4].* 1) Channing Laboratory, Boston, MA; 2) Division of Pulmonary and Critical Care Medicine. Brigham and Women's Hospital, Boston, MA; 3) Center for Genomic Medicine, Boston, MA; 4) Harvard Medical School, Boston, MA; 5) Division of Pediatric Pulmonology, Hospital Nacional de Niños, San José, Costa Rica; 6) Department of Biostatistics, Harvard School of Public Health, Boston, MA; 7) Division of Allergy and Clinical Immunology, Department of Medicine, Johns Hopkins University, Baltimore, MD; 8) The National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, MA, Boston University School of Medicine, Boston, MA; 9) Department of Preventive Medicine, University of Southern California, Los Angeles, CA; 10) Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA; 11) Paediatric Respirology, Imperial School of Medicine at National Heart and Lung Institute, Royal Brompton Hospital, London, United Kingdom.

Rationale: We recently demonstrated a sex-specific association between single nucleotide polymorphisms (SNP) in thymic stromal lymphopoietin (TSLP) and IgE. There has been no previous study of association between TSLP and asthma. Methods: We conducted regular and sex-stratified analyses of association between SNPs in TSLP and asthma in families of asthmatic children in Costa Rica. Significant findings were replicated in white and African-American participants in the Childhood Asthma Management Program, in African Americans in the Genomic Research on Asthma in the African Diaspora study, in a population of mixed ethnicity from the Children's Health Study, and in white subjects from the Framingham Heart Study (FHS). Results: The minor alleles of two SNPs in TSLP (rs1837253 and rs2289276) were inversely associated with asthma in a combined analysis of all subjects (p values of 5x10-5 and 7x10-5, respectively). In all studies, the direction of association was consistent and the combined result was significant after adjustment for multiple testing. In a sex-stratified analysis, the minor allele (T) of SNP rs1837253 was significantly associated with a reduced risk of asthma in all males (p= 3x10-6) but not in females. Alternately, the minor allele (T) of SNP rs2289276 was significantly associated with a reduced risk of asthma in all females (p= 2x10-4). Findings for SNP rs2289276 were consistent in girls but not in adult females in the FHS. Conclusions: TSLP variants are associated with asthma in a sex-specific fashion.

## 1852/T/Poster Board #401

**Quantifying epistasis between two sets of signaling pathway genes by canonical correlation analysis.** *B.H. Yip, C.S. Tang, S.S. Cherny, P.K. Tam, E.S. Elly, M.M. Garcia-Barcelo, P.C. Sham.* Psychiatry, Hong Kong University, Hong Kong.

This paper focuses on three aspects related to the conceptualization and application of canonical correlation analysis (CCA) as a statistical model in genetic study: 1) partial canonical correlation analysis to control the effect of population stratification, 2) epistasis analysis for case control study and 3) backward or stepwise canonical analysis to provide more insight into the dynamics of the complex genetic network. We applied these methods to simulated data and to a genetic pathway study of Hirschsprung disease. The results suggest the DLL3 gene from the NOTCH pathways interacted with the PTCH1 gene from the SHH pathway. The first canonical correlation is 0.444 (p-value from the Wilks Lamda test is 8.40E-06) among the cases and 0.157 (p-value 0.236) among the control. The difference between the correlations is significant (p-value 0.00011), after Fisher z transformation.

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## 1853/T/Poster Board #402

**Penetrance of *CDKN2A* mutations estimated using population-based Australian families.** *M. Jenkins[1], A.E. Cust[1], D. Schmidt[1], E. Makalic[1], E.A. Holland[2], H. Schmid[2], R.F. Kefford[2], G.G. Giles[3], B.K. Armstrong[4], J.F. Aitken[5], G.J. Mann[2], J.L. Hopper[1], the Australian Melanoma Family Study.* 1) Centre for MEGA Epidemiology, University of Melbourne, Melbourne, Australia; 2) Westmead Institute for Cancer Research and Melanoma Institute of Australia, University of Sydney at Westmead Millennium Institute, Sydney, Australia; 3) Cancer Epidemiology Centre, The Cancer Council Victoria, Melbourne, Australia; 4) School of Public Health, University of Sydney, Sydney, Australia; 5) Viertel Centre for Research in Cancer Control, The Cancer Council Queensland, Brisbane, Australia.

**Background**: Germline mutations in the CDKN2A gene increase risk of melanoma. For example, previous estimates of the cumulative risk (i.e. penetrance) of melanoma for *CDKN2A* mutation carriers were 30% by age 50 (95% CI: 12 to 62%) and 67% (95% CI: 31 to 96%) by age 80 when using multiple-case families, and 14% (95% CI = 8 to 22%) by age 50 and 28% (95% CI: 18 to 40%) by age 80 when using population-based families limited to first-degree relatives with unverified melanoma histories. **Methods**: The Australian Melanoma Family Study is a population-based case-control-family study which included probands with incident, histopathologically-confirmed, first-primary cutaneous melanoma diagnosed before age 40 recruited from Brisbane, Sydney and Melbourne and their first- and second-degree relatives. Of the 595 probands with DNA samples, we identified 12 with pathogenic or suspected pathogenic *CDKN2A* mutations for whom there were data for first- and second-degree relatives. The hazard ratio (HR) of melanoma incidence for carriers relative to that for the general population, and hence penetrance, were estimated using a modified segregation analysis that incorporates both genotyped and ungenotyped relatives and conditions on ascertainment to produce unbiased estimates. **Results**: Based on reports of melanoma for relatives, the HR was greater for females than males (HR = 34.2 and 9.9, respectively; p=0.02). There were no statistically significant differences in HR by age group or city of recruitment, although the power of these subgroup analyses was limited by small numbers. Combining males and females, the estimated penetrance of melanoma for *CDKN2A* mutation carriers was 18% (95% CI: 11 to 27%) by age 50, and 60% (95% CI: 43 to 77%) by age 80. For females, the penetrance estimates were 29% (95% CI: 17 to 48%) by age 50, and 70% (95% CI: 47 to 90%) by age 80, and for males they were 9% (95% CI: 4 to 20%) by age 50, and 43% (95% CI: 22 to 73%) by age 80. Restricting the reports of melanoma to the 50% that could be confirmed through official records resulted in an approximate halving of the HRs from 19.5 to 8.4 for males and females combined. **Conclusion**: Our population-based estimates of melanoma risk to age 80, which apply to the *CDKN2A* mutations identified in early-onset cases of melanoma, appear to be higher than previous population-based estimates from studying the families of cases unselected for age at onset, and were higher for females than males.

## 1854/T/Poster Board #403

**Risks of cancer for *MLH1* and *MSH2* mutation carriers.** *J.G. Dowty[1], A.K. Win[1], D. Buchanan[2], S.N. Thibodeau[3], G. Casey[4], N. Lindor[5], S. Gallinger[6], L. LeMarchand[7], R. Haile[4], P. Newcomb[8], J.L. Hopper[1], M.A. Jenkins[1], the Colon Cancer Family Registry.* 1) Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne, Parkville, Victoria, Australia; 2) Familial Cancer Laboratory, Queensland Institute of Medical Research, Brisbane, Queensland, Australia; 3) Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota; 4) Department of Preventive Medicine, University of Southern California, Los Angeles, California; 5) Departments of Medical Genetics, Mayo Clinic, Rochester, Minnesota; 6) Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada; 7) University of Hawaii Cancer Research Center, Honolulu, Hawaii; 8) Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle, Washington.

**Background** Germline mutations in the DNA mismatch repair genes MLH1 and MSH2 are associated with a substantially increased risk of some cancers. Due to the rarity of these mutations, previous studies have been underpowered to provide precise estimates of age- or gene-specific risks. **Methods** We identified 307 families of MLH1 (n=133) and MSH2 (n=174) mutation carriers (probands) from the Colon Cancer Family Registry (163 from Australasia, 93 from USA and 51 from Canada). These were ascertained either because they had a family history of cancer (n=262) or from cancer registries independently of family history (n=45). MLH1 and MSH2 mutation status, sex, age and history of cancer, polypectomy and hysterectomy were sought from 13,226 probands and their relatives. Hazard ratios (HR) of cancer incidence for mutation carriers relative to those for the general population, and hence age-specific cumulative risks (penetrance), were estimated using a modified segregation analysis that incorporated both genotyped and ungenotyped relatives and conditioned on ascertainment to produce unbiased estimates. **Results** The HR for colorectal cancer did not differ by gene (p=0.9). Under a log-linear model, the HR for males was 141 at age 40 yrs and decreased with age by 8% per yr; for females, the HR was 43 at age 40 yrs and decreased with age 6% per yr. For mutation carriers from USA, the cumulative risks of colorectal cancer to age 70 yrs were estimated to be 67% (95%CI 55-79) for males and 30% (95%CI 20-45) for females. For females, the HR for endometrial cancer was higher for MSH2 mutation carriers than for MLH1 mutation carriers (16.8 versus 2.8; p=0.01). The cumulative risk to age 70 yrs was estimated to be 25% (95% 12-49) for MSH2 mutation carriers and 5% (95%CI 2-13) for MLH1 mutation carriers. Male and female MLH1 and MSH2 mutation carriers were at increased risk of other Lynch cancers (HR = 7.3 for males and 12.6 for females). The cumulative risks to age 70 yrs were estimated to be 15% (95%CI 7% - 30%) for males and 24% (95%CI 15-38) for females. There was no evidence that mutation carriers were at increased risk of non-Lynch cancers (p=0.8). **Conclusions** This international study shows that MLH1 and MSH2 germline mutations are associated with the same multiplicative increase in colorectal cancer risk, which is greater for males and greater at younger ages. MSH2 mutations are associated with a greater endometrial cancer risk than MLH1 mutations.

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## 1855/T/Poster Board #404

**Disease associated polymorphisms and all cause mortality in a population cohort.** *D.S. Cross, C.A. McCarty.* Center for Human Genetics, Marshfield Clinic Research Foundation, Marshfield, Wi.

**Background**: While there have been many studies to determine alleles associated with disease, there have been few studies to determine if the alleles are associated with mortality in a population. Here we used the Personalized Medicine Research Project cohort, a population of approximately 20,000 adult individuals in 19 zip codes surrounding Marshfield Wisconsin, that have agreed to provide DNA, serum and plasma with a linked dynamic medical record for medical research. The average age at enrollment was 48, with 57% of the cohort female and 98% Caucasian. We genotyped 36 polymorphisms in the entire population to determine if disease associated polymorphisms were associated with mortality in the population. **Methods**: 36 polymorphisms with at least 1 association with a disease in two independent populations and at least 20% minor allele frequency in a Caucasian population were chosen to investigate the association of disease polymorphisms with death. All cause mortality was determined by known deaths in the population between the time of enrollment and 2008. We also stratified the population by sex to determine if there were differences in alleles associated with death by gender. **Results**: Four polymorphisms were associated with mortality in the population at the 0.05 level, rs1042031 (APOB), rs7493 (PON2), rs6313 (HTR2A), and rs688 (LDLR). Of these rs688 and rs1042031 were associated with mortality only in males when stratified by sex, and rs6313 was associated with mortality in both males and females. For rs6313 (HTR2A) there was a strong linear trend in both women alone (Mantel-Heanszel $\chi 2 = 0.0037$) and men and women combined Mantel-Heanszel $\chi 2 = 0.0053$). **Conclusions**: We provide suggestive evidence that disease associated polymorphisms may also be associated with all cause mortality, particularly alleles that are associated with cardiovascular disease such as rs1042031 and rs688 in males. In females there was a greater tendency toward mortality in a linear fashion with a greater number of T alleles for rs6313 (HTR2A). Interestingly, the C allele of this polymorphism has been associated with extraversion personality traits and the TT allele has been associated with increased tendency toward depression and suicide.

## 1856/T/Poster Board #405

**Evidence of polymorphisms of *Xpo1* as a biomarker for susceptibility to anti-tuberculosis drug-induced hepatotoxicity in Japanese patients with tuberculosis.** *T. Mawatari[1], A. Nakaura[1], N. Higuchi[2], S. kondo[1], K. Tsukamoto[1].* 1) Dept Pharmacotherapeutics, Nagasaki Univ Grad Sch, Nagasaki, Japan; 2) Dept Pharmacy, Nagasaki Univ Hosp.

**Objective**: Tuberculosis (TB) is a re-emerging infectious disease. TB care involves serious problems including the occurrence of adverse effects of anti-TB drugs, especially hepatotoxicity. Recent studies suggest that hepatotoxicity is induced by reactive oxygen species derived from isoniazid metabolites. Thus, we investigated whether polymorphisms of the exportin 1 gene (*Xpo1*), which regulates transcription of antioxidant enzymes, are associated with anti-TB drug-induced hepatotoxicity in Japanese patients with pulmonary TB.

**Methods**: This study subjects comprised 100 Japanese patients with pulmonary TB treated at least with isoniazid and rifampicin-containing regimen for six or nine months. The three tag single-nucleotide polymorphisms (SNPs) in *Xpo1* were detected by PCR-restriction fragment length polymorphism or PCR-direct DNA sequencing method. Subsequently, haplotypes were constructed from two tag SNPs, which were located within a common linkage disequilibrium block. The frequencies of alleles, genotypes, haplotypes, and diplotypes were compared between TB patients with and without hepatotoxicity by a chi-square test or a logistic regression analysis using SPSS 17.

**Results**: The frequency of an A/A genotype of an intron SNP site in *Xpo1* in TB patients with hepatotoxicity was significantly higher in comparison to that in patients without it [$P = 0.030$, odds ratio (OR) = 3.20]. Moreover, the frequencies of a Hap 3 haplotype and its homozygous Hap 3/Hap 3 diplotype in TB patients with hepatotoxicity were significantly increased as compared with those in patients without it [$P = 0.016$, OR = 3.08 and $P = 0.019$, OR = 16.2, respectively].

**Conclusion**: The present study is the first report to demonstrate the association between *Xpo1* polymorphisms and susceptibility to anti-TB drug-induced hepatotoxicity. Therefore, having the Hap 3/Hap 3 diplotype in *Xpo1* may be useful as a new biomarker for identifying high-risk TB patients with anti-TB drug-induced hepatotoxicity.

## 1857/T/Poster Board #406

**COMPARATIVE STUDY OF AMELIORATIVE ACTION OF CURCUMIN AND KALMEGH ON MERCURIC CHLORIDE INDUCED GENOTOXICITY.** *M.V. Rao, T.A. Patel.* Human Genetics Division, Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad, India - 380009.

Heavy metals, particularly through their wide commercial use, pose environmental problems which may ultimately threaten human health. Mercury, one of the heavy metals, has xenobiotic and deleterious effects on public health for many decades. Mercury is released into the environment through various natural geological processes, such as volatilization of rocks, dissolution, and volcanic eruption as well as due to some anthropogenic activities like combustion of fossil fuels, incineration of waste, mining and industrial discharge. Curcumin and kalmegh are very well known antioxidants and have been used in medicine as natural remedies for the various health problems. The objective of the present study was to determine the protective effect of curcumin and kalmegh against mercuric chloride induced genotoxicity in human peripheral blood lymphocyte cultures (PBLC). Cultures were treated with two different doses of mercuric chloride 2.6 $\mu$M and 10.5 $\mu$M for two different time exposures and genotoxic indices including chromosomal aberrations, sister chromatid exchanges and proliferative index were scored and their percent frequencies were also calculated. Curcumin (3.8 $\mu$M) and kalmegh (0.4 $\mu$M) of plant products were used alone and in combination along with the higher dose of mercuric chloride. Results of the present study showed that the mercuric chloride treatment gradually increased these genotoxic indices. Supplementation of these herbal products reduced the metal exerted genotoxicity in cultures. In conclusion both herbal products viz., curcumin and kalmegh diminished mercuric chloride induced increase in genotoxic endpoints revealing their ameliorative property.

## 1858/T/Poster Board #407

**Gene-dosage effect of GSTM1 contributes to development of Rheumatoid Arthritis.** *E. Lundström[1], T. Hartshorne[2], K. Li[2], L. Alfredsson[3], L. Klareskog[1], L. Padyukov[1].* 1) Medicine, Rheumatology Unit, Stockholm, Stockholm, Sweden; 2) Applied Biosystems, Foster City, CA, USA; 3) Stockholm Center for Public Health and Environmental Medicine, Cardiovascular Epidemiology Unit, Stockholm, Sweden.

Objective. Copy number polymorphisms (CNPs) are important contributors to the diversity in human genome and the functional consequences of CNPs are not well studied. Glutathione-S-transferases (GSTs) have an important role in tobacco smoke detoxification and approximately 50% of individuals in most human populations completely lack the gene GSTM1 due to common CNP. Our goal was to investigate the impact of GSTM1 CNP in rheumatoid arthritis (RA) and its interaction with smoking and HLA-DRB1 shared epitope (SE) status, which are the two best known risk factors in development of RA. Methods. qPCR was performed using TaqMan copy number variation assays (Applied Biosystems) for 2426 incident cases and 1257 controls from the Swedish EIRA study. To quantify GSTM1 number, the software CopyCaller v0.99 was used. DAS28 was assessed for 1426 cases. Odds ratio (OR) together with 95% confidence intervals (CI) was calculated and used as a measure for risk of developing disease. Results. No association between RA and GSTM1 CNP could be found when analyzing whole study population. However, we found that the presence of GSTM1 appears to be a significant risk factor for autoantibody positive RA in females 60 years or older in absence of smoking and SE alleles (OR: 5.33 95% CI: 1.26-22.57) compared to females not carrying GSTM1, never smokers and without the SE. The presence of GSTM1 does not influence the baseline activity of RA and this CNP does not interact with SE alleles. Conclusion. For the first time in a case-control study we assessed the exact number of GSTM1 gene copies in relation to development and severity of RA. Our data suggest that presence of GSTM1 is a risk factor for a certain subgroup with probable gender effect in development of this disease.

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## 1859/T/Poster Board #408

**Relation of Genetic Variants in Fatty Acid Binding Protein-4 (FABP4) and Clinical Diabetes Risk in the Women's Health Initiative Observational Study.** *K.K. Chan[1,7], Y. Song[2], Y. Hsu[3], N.Y. You[1,7], L. Tinker[4], S. Liu[1,5,6,7].* 1) Program on Genomics and Nutrition, Department of Epidemiology, University of California-Los Angeles, Los Angeles, CA; 2) Divison of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02215; 3) Molecular and Integrative Physiological Science Program, Department of Environmental Health, Harvard School of Public Health, Boston, MA 02215; 4) Fred Hutchinson Cancer Research Center, Seattle, WA 98109; 5) Department of Medcine, David Geffen School of Medicine, UCLA, Los Angeles, CA 90095; 6) Johnson Comprehensive Cancer Center, David Geffen School of Medicine, UCLA, Los Angeles, CA 90095; 7) Center for Metabolic Disease Prevention, UCLA.

Adipocypte fatty acid binding protein (FABP4/aP2) may play a central role in energy metabolism and inflammation. In animal models, genetic defects of the FABP4 gene partially protected against the development of obesity-related insulin resistance, dyslipidemia, and atherosclerosis. However, it is unclear whether common genetic variation in FABP4 gene contributes to risk of type 2 diabetes (T2D) or diabetes-related metabolic traits in humans. We comprehensively assess the genetic associations of variants in the FABP4 gene with T2D risk and diabetes-associated biomarkers in a nested case-control study of 1,529 cases and 2,147 controls from postmenopausal women aged 50-79 years enrolled in the Women's Health Initiative Observational Study (WHI-OS). We selected and genotyped a total of 11 haplotype-tagging single nucleotide polymorphisms (tSNPs) spanning 41.3 kb across FABP4 in all samples. None of the SNPs and their derived haplotypes showed significant association with T2D risk. Neither were there any significant associations between SNPs and plasma levels of inflammatory and endothelial biomarkers (including CRP, TNF, IL-6, E-selectin, and ICAM-1). Among African American women, several SNPs were significantly associated with lower levels of vascular cell adhesion molecule-1 (VCAM-1), especially among those with incident T2D. On average, plasma levels of VCAM-1 were significantly lower among carriers of each minor allele at rs1486004(C/T) (-1.08 ng/mL; P=0.01), rs7017115(A/G) (-1.07 ng/mL, P= 0.02), and rs2290201(C/T) (-1.12 ng/mL, P=0.002) as compared with the homozygotes of the common allele, respectively. After adjusting for multiple testing, carriers of the rs2290201 minor allele remained significant association with decreasing levels of plasma VCAM-1 in these women (p=0.02). In conclusion, our finding from a multiethnic cohort of postmenopausal women did not support the notion that common genetic variants in the FABP4 gene may confer increased risk of T2D, although the observed significant association between reduced VCAM-1 levels and FABP4 genotypes in African American women requires further confirmation.

## 1860/T/Poster Board #409

**Variants in Neuropeptide Y Receptor 1 and 5 Are Associated with Nutrient-Specific Food Intake and Are Under Recent Selection in Europeans.** *C.C. Elbers[1,2], C.G.F. de Kovel[1], Y.T. van der Schouw[2], J.R. Meijboom[1], F. Bauer[1,2], D.E. Grobbee[2], G. Trynka[4], J.V. van Vliet-Ostaptchouk[3], C. Wijmenga[1,4], N.C. Onland-Moret[1,2].* 1) Complex Genetics Section, Department of Biomedical Genetics, University Medical Center Utrecht, Utrecht, Netherlands; 2) Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, the Netherlands; 3) Department of Pathology and Laboratory Medicine, University Medical Center Groningen, University of Groningen, the Netherlands; 4) Department of Genetics, University Medical Center Groningen, University of Groningen, the Netherlands.

ABSTRACT There is a large variation in caloric intake and macronutrient preference between individuals and between ethnic groups, and these food intake patterns show a strong heritability. The transition to new food sources during the agriculture revolution around 11,000 years ago probably created selective pressure and shaped the genome of modern humans. One major player in energy homeostasis is the appetite-stimulating hormone neuropeptide Y, in which the stimulatory capacity may be mediated by the neuropeptide Y receptors 1, 2 and 5 (NPY1R, NPY2R and NPY5R). We assess association between variants in the NPY1R, NPY2R and NPY5R genes and nutrient intake in a cross-sectional, single-center study of 400 men aged 40 to 80 years, and we examine whether genomic regions containing these genes show signatures of recent selection in 270 HapMap individuals (90 Africans, 90 Asians, and 90 Caucasians) and in 846 Dutch bloodbank controls. Our results show that derived alleles in NPY1R and NPY5R are associated with lower carbohydrate intake, mainly because of a lower consumption of mono- and disaccharides. We also show that carriers of these derived alleles, on average, consume meals with a lower glycemic index and glycemic load and have higher alcohol consumption. One of these variants shows the hallmark of recent selection in Europe. Our data suggest that lower carbohydrate intake, consuming meals with a low glycemic index and glycemic load, and/or higher alcohol consumption, gave a survival advantage in Europeans since the agricultural revolution. This advantage could lie in overall health benefits, because lower carbohydrate intake, consuming meals with a low GI and GL, and/or higher alcohol consumption, are known to be associated with a lower risk of chronic diseases.

## 1861/T/Poster Board #410

**Novel mutations in the *ABCA4* gene in French-Canadian patients affected by Stargardt disease.** *M-K. Gauthier[1], E. Deilhes[1], S. Dubois[1], P. Belleau[1], R. Arseneault[1], M-A. Rodrigue[1], M. Malenfant[2], Y. Tardif[2], G. Lalonde[2], P. Turcotte[2], V. Raymond[1,2].* 1) Biology of Sensory Systems, Laboratory of Genetics and Genomics, CREMOG, Laval University Hospital (CHUL) Research Center, Québec City, PQ, Canada; 2) Department of Ophthalmology, Laval University, Québec City, PQ, Canada.

Among 250 forms of hereditary blindness, 15 are severe monogenic macular dystrophies. The most prevalent is Stargardt disease, usually beginning between the age of 7 and 12. This autosomal disease can be either recessive (AR) or dominant, and is associated with *ABCA4*, *ELOV4* or *CNGB3*;*ABCA4* being the sole gene for AR Stargardt. We initiated a program to determine the prevalence of mutations causing Stargardt disease in the French-Canadian population. Here, we present our first study on 13 Stargardt families. Ophthalmologic examinations were performed on 22 patients. Six microsatellite markers overlapping *ABCA4* (6,9 Mb) were genotyped to establish disease haplotypes. Mutational screening was performed in the AR kindreds by sequencing all of *ABCA4* 50 exons and splicing junctions. Splicing mutations were predicted using *Automated Splicing Mutations* software and confirmed by RT-PCR. Among the 10 families which showed AR inheritance, 18 subjects were diagnosed with Stargardt. These patients harbored 25 disease haplotypes. Mutational screening of *ABCA4* revealed 12 non-synonymous amino-acid (AA) changes and one splicing mutation. Five of these AA changes, N96H, R537C, R943Q, G1961E and L2027F, have been reported as mutations. Three families, harboring the same disease haplotype, carried G1961E, the most frequent mutation worldwide. The other 6 AA changes, R212H, H423R, L1250P, A1773E, N1861I and S2255I, have been previously categorized either as disease-causing mutations or as non-disease associated polymorphisms. Interestingly, we found 2 novel mutations. These were the F56S AA change and the intron IVS37-2ΔA deletion. The F56S AA variation causing a major AA change within a mutational hot spot, we considered it as a mutation. The IVS37-2ΔA deletion was also considered a mutation as it caused the complete and/or partial deletion of exon 38, generating a troncated protein. Mutations in the 12 remaining disease haplotypes are still to be identified. In conclusion, we report 2 novel *ABCA4* mutations. Our screening also revealed at least 1 *ABCA4* mutation in 10 of our 18 Stargardt patients. In 3 of these patients, both disease-causing alleles were identified, whereas only 1 was observed in 7 patients. Founder mutations have been frequently observed in the French-Canadian population. However, the present study does not support major founder effects in *ABCA4* in our population, probably due to the vast number of exons favoring DNA rearrangements in the gene.

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## 1862/T/Poster Board #411

**Multiple genetic factors (*MC1R*, *GSTT1*, *XPD*) modify melanoma risk in melanoma-prone families segregating *CDKN2A* mutations.** *V. Chaudru[1], C. Balanan[2], H. Mohamdi[1], C. Marian[3], E. Corda[1], K. Laud[4], M. Barrois[2], M-F. Avril[5], B. Bressac-de-Paillerets[2], F. Demenais[1].* 1) INSERM U946, CEPH, Paris, France; 2) Service de Genetique and FRE2939 CNRS, Institut Gustave Roussy, Villejuif, France; 3) Cancer Genetics and Epidemiology Division, Georgetown University Medical Center, Washington DC; 4) INSERM U830, Institut Curie, Paris, France; 5) AP-HP Hopital Cochin, Universite Rene Descartes Paris 5, Paris, France.

**Objectives:** The effect of *CDKN2A*, the major high-risk melanoma susceptibility gene, has been shown to be modified by melanoma-associated phenotypes and variants of *MC1R* gene. Genes implicated in DNA repair (such as *ERCC2/XPD*) or in detoxification of metabolites after UV exposure (such as Glutathione S-transferase (*GST*s)) are also candidates as modifiers of *CDKN2A* penetrance. Few case-controls studies have investigated the effect of *GST*s or *XPD* on melanoma risk, and have led to controversial results while these genes have not yet been studied in families segregating *CDKN2A* mutations. Our aim was to study potential genetic modifiers of *CDKN2A*, namely *GSTT1*, *GSTM1*, *GSTP1* and *XPD*, in the presence of *MC1R* gene variants in 34 French melanoma-prone pedigrees with *CDKN2A* mutations.

**Methods:** Logistic regression analyses, adjusted for age, sex, *CDKN2A* and *MC1R*, and taking into account correlations among family members, were applied to 212 subjects genotyped for all investigated genes. These analyses were also conducted by stratifying on melanoma-associated phenotypes.

**Results:** We found a significant protective effect of the null *GSTT1* allele (OR=0.26 [0.12-0.56]), confirming a previous result obtained in a subset of our families. A borderline significant protective effect of the null *GSTM1* allele was also detected (OR=0.45 [0.20-1.03]). The effect of *GSTP1* (p.I105V, p.A114V) or *XPD* (p.K751Q, p.D312N) variants on melanoma risk did not reach significance. However, stratifying on melanoma-associated phenotypes led to an increase in melanoma risk in blond/red haired subjects carrying at least one p.K751Q (OR=8.76 [1.97-38.94]) or p.D312N (OR= 7.67 [1.90-30.96]) variant of *XPD*. Multiple logistic regression conducted in the whole sample showed that the factors modulating independently the melanoma risk associated with *CDKN2A* mutations were: *MC1R* and dysplastic nevi (increasing the risk) and *GSTT1* (decreasing the risk). In red/blond hair subjects, the genetic modifiers of *CDKN2A* penetrance included *MC1R*, *GSTT1* and *XPD*.

**Conclusions:** This study shows the multiplicity of genetic pathways predisposing to melanoma.

## 1863/T/Poster Board #412

**The role of reduced folate as a risk factor for nondisjunction in chromosome 21.** *N. Hollis, L. Bean, A. Locke, S. Sherman.* Human Genetics, Emory University, Atlanta, GA.

The folate pathway is important for DNA synthesis, protein synthesis and cellular methylation. It has been hypothesized that a decrease in folate and subsequent increase in homocysteine may lead to DNA hypomethylation during meiosis. In turn, this may result in chromosome instability and abnormal chromosome segregation during oocyte formation. Supporting evidence comes from studies that show an increased frequency of variants in genes known to reduce folate metabolism, such as C677T MTHFR, or increased levels of homocysteine among case mothers who had a child with trisomy 21 compared to control mothers who had a child without trisomy 21. However, other studies have failed to find such associations. We tested this hypothesis further by comparing folate supplementation prior to conception (i.e., during resumption of meiosis I) among case mothers (n=1084) and control mothers (n=987). Dietary supplementation of folate can help maintain normal homocysteine levels and remethylation. We used logistic regression to examine the use of preconception folate supplementation adjusting for race/ethnicity, education, smoking, and maternal age. There was no significant difference in folate use between case and controls. We have just completed genotyping 21 SNPS in four genes of the folate pathway including MTHFR, MTR, MTRR, and CBS to further test this hypothesis. We will include these variables in our model and examine the interaction of these folate pathway variants with folate intake around conception to better understand the influence of reduced folate on nondisjunction. We will also incorporate our data on recombination along the nondisjoined chromosomes 21. For example, we can test the hypothesis that reduced folate may increase hypomethylation of centromeric DNA and increase the risk for a pericentromeric recombinant event, a known predisposing factor for abnormal chromosome segregation.

## 1864/T/Poster Board #413

**Genomewide Linkage and Association Study of Onset Age of LRRK2-related Parkinson's Disease.** *J.C. Latourelle[1], N. Pankratz[2], A.E. Hendricks[3], J.B. Wilk[1], A.L. DeStefano[1,3], T. Foroud[2], R.H. Myers[1], the PSG -PROGENI and GenePD Investigators, Coordinators and Molecular Genetic Laboratories.* 1) Boston University School of Medicine, Department of Neurology,Boston, MA; 2) Indiana University, Indianapolis, IN; 3) Boston University School of Public Health, Department of Biostatistics, Boston, MA.

Mutations in the leucine-rich repeat kinase 2 gene (LRRK2) located at 12q12, are the most common known genetic causes of Parkinson's Diseases (PD). The most frequently seen LRRK2 mutation, Gly2019Ser (G2019S), is associated with approximately 5 to 6% of familial PD cases and 1 to 2% of idiopathic cases. Studies of LRRK2 mutation carriers have shown incomplete and age-dependent penetrance of the disease. In addition, greater lifetime penetrance has been seen in unascertained parents of PD affected siblings compared to parents of sporadically ascertained LRRK2 cases, suggesting that inherited susceptibility factors may modify the penetrance of LRRK2 mutations. In this study, genotyping from an Illumina HumanCNV370Duo array was used to examine linkage and association to age of onset of PD in a sample of 99 LRRK2 PD cases from 59 families. The sample consisted of 54 men and 45 women, with an average age of onset of 62.6 years (StDev = 9.8, range: 41-88). Linkage analysis was performed using two methods: (1) non-parametric quantitative trait linkage (QTL) and (2) a robust score statistic based on variance components (RSS). Association to onset age for each SNP was tested under an additive mode of inheritance, using a linear mixed effects model to account for the familial relationships. The top LOD score observed for the QTL method was 2.43 and for the robust score statistic method was 1.94, both located in the region 1q32.1, within the gene neuron navigator 1 (NAV1). Moderate linkage peaks were also identified at 16q12.1 for both methods (QTL LOD=1.58, RSS LOD=1.10). In the association analyses, no SNPs reached a genomewide level of significance. Restricting the SNP association results and controlling for multiple comparisons only under the linkage peaks, also revealed no statistically significant SNP associations. The top association observed was at 19q12 between the hypothetical genes LOC148189 and LOC148145, with a p-value of 4x10-6. Additional association analyses that account for covariates and population substructure will also be presented. Further investigation of regions identified by linkage or association may provide insight into understanding the penetrance and progression of LRRK2 related PD.

## 1865/T/Poster Board #414

**Frequencies of C282Y and H63D Alleles in the HFE Gene among Various Ethnic Jewish Groups in Israel: A Change of Concept Required.** *O. Reish[1], N. Shefer-Kaufmann[2], D. Chapman Shimshoni[1], P. Renbaum[3], A. Orr-Urtreger[2], H. Steiner[3], E. Levy-Lahad[3], G. Altarescu[3].* 1) Genetic Institute, Assaf Harofeh Med Ctr, Zerifin, Israel; 2) Genetic Institute, Sourasky Medical Center, Tel Aviv, Israel; 3) Shaare Zedek Medical Center, Jerusalem, Israel.

Background: Hereditary Hemochromatosis (HHC) is a leading cause for iron overload due to increased absorption of dietary iron from the GI tract resulting in multiorgan dysfunction. Ninety percent of HHC patients are homozygous for the HFE-C282Y, 5-7% are compound heterozygotes for the C282Y and HFE-H63D and less than 2% are H63D homozygotes. Allele frequencies of these 2 mutations are highly variable among different populations in the world. There is a lack of knowledge regarding affected patients with HHC in the variable ethnic Jewish groups and allele frequency has not been well established except for the Ashkenazi group. Aim: We ascertained 3 HHC non related families of North African Jews with the C282Y/C282Y genotype, which led to frequency evaluation of C282Y and H63D alleles among the different ethnic Jewish groups in Israel, focusing on the North African one. Methods: Participants were healthy individuals from the relevant ancestries. The genotype of C282Y and H63D alleles was assessed using RFLP . Data was collected from 3 Israeli Medical centers, Assaf Harofeh, Sourasky and Shaare Zedek. This cohort included individuals from North African, Oriental, Yemenite and Sephardic Jews. Results: 586 chromosomes derived from North African individuals were evaluated: 6 alleles carried the C282Y genotype and 81 the H63D, leading to allele frequencies of 1.02% and 13.92% respectively. From the Oriental, Yemenite and Sephardic groups, 304, 94 and 118 chromosomes were evaluated. Allele frequency of C282Y was 0 in these groups but H63D frequency was 11.6%, 14.9% and 9.3% respectively. Discussion & conclusions: The detection of 3 families with HHC along with carrier frequencies of 1/50 and 1/8 for C282Y and H63D respectively, represents an under-detection of this disorder among the North African Jews in Israel even though penetrance is estimated at 1-4%. As to the Oriental, Yemenite and Sephardic groups, although no C282Y heterozygotes were detected, the H63D carrier frequency was relatively high. An aggravation of the abnormal iron metabolism has been associated with the co-inheritance of H63D genotype with other iron metabolism modifier conditions, such as the beta-thalassemia trait. As both conditions are relatively frequent in Oriental and Yemenite ethnic groups, further medical attention should be given when detected, and evaluation of the H63D allele should be considered to enable further medical attention and possible preventive care.

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## 1866/T/Poster Board #415

**Meta analysis of *filaggrin* polymorphisms in eczema and asthma: robust risk factors in atopic disease.** *E. Rodríguez[1], HJ. Baurecht[2,3], E. Herberich[3], S. Wagenpfeil[3], S.J. Brown[4,5], H.J. Cordell[5], A.D. Irvine[6,7], S. Weidinger[1,8].* 1) Division of Environmental Dermatology and Allergy, Helmholtz Zentrum Munich and ZAUM-Center for Allergy and Environment, Technische Universität München, Munich, Germany; 2) Department of Epidemiology, Helmholtz Zentrum Munich, Neuherberg, Germany; 3) Institute for Medical Statistics and Epidemiology IMSE, Technische Universität München, Munich, Germany; 4) Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom; 5) Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, United Kingdom; 6) Department of Paediatric Dermatology, Our Lady's Children's Hospital, Crumlin, Dublin, Ireland; 7) Department of Clinical Medicine, Trinity College Dublin, Ireland; 8) Department of Dermatology and Allergy Biederstein, Technische Universität München, Munich, Germany.

The discovery of null mutations in the gene encoding the key epidermal protein filaggrin (*FLG*) as a major risk factor for eczema and related asthma represents a milestone towards the understanding of an important genetic mechanism in these complex diseases. However, studies published to date demonstrate differences concerning study design and strength of associations. In addition, studies on the impact of *FLG* in asthma have reported apparently conflicting results. The aim of this study was to provide a general and overall measure of effect size based on all available association studies on *FLG* in eczema and asthma respectively. We also carried out a sensitivity analysis by grouping the studies based on characteristics like definition of eczema, study size and power and source of controls. We conducted a meta-analysis of 24 studies on common *FLG* mutations and eczema involving 5791 cases, 26454 controls and 1951 eczema families as well as 17 studies involving 3138 cases, 17164 controls and 4 family studies including 1511 affected offspring on asthma. In both case-control and family studies strong associations with eczema were observed. Case-control studies were heterogeneous and odds ratios (ORs) ranged from 1.69 to 11.56 with an overall OR of 3.39 (95%CI=2.73-4.23), while family studies showed more homogeneous results with an overall OR of 2.03 (95%CI=1.77-2.34). Random effects meta-analysis after combining both study types showed that overall *FLG* haploinsufficiency increases the odds of eczema by more than 3-fold. *FLG* mutations are also significantly associated with asthma with an overall OR of 1.62 (95%CI=1.36-1.93). However, while strong effects for the compound phenotype "asthma + eczema" with an OR of 3.45 (95%CI= 2.74-4.34) were observed, there appears to be no significant association with asthma in the absence of eczema. This meta-analysis summarizes the strong evidence for a high risk for eczema conferred by *FLG* null mutations, one of the largest ever observed in the genetics of complex diseases. The results of this extensive analysis refine the risk profiles of *FLG* null alleles and suggest an association with both more severe and dermatologist diagnosed disease. The results also clearly indicate that *FLG* loss-of-function mutations are a robust risk factor in determining genetic predisposition to asthma, and suggest that FLG deficiency might help define the endophenotype of asthma linked with eczema.

## 1867/T/Poster Board #416

**Performance of gene signature-based predictions: effect of gene selection, gene numbers, and sample size.** *L.-Y. Wang, W.-C. Lee.* Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan.

With the advance of microarray technology, gene signature-based prediction is becoming a hot topic. Researchers try to make use of gene signature to predict the outcome of treatment or certain cancers. The prediction performance varies mainly due to the heterogeneity among studies, such as sample size, gene numbers, and different selection algorithms. It's very interesting to know how these factors influence the prediction performance. We used three microarray data of cancers. One is Golub's leukemia dataset with 7129 genes and 72 subjects. The other is Armstrong's leukemia dataset with 12582 genes and 44 subjects. The last one is Alon's colon cancer dataset with 2000 genes and 62 subjects. We then did some experiments on these datasets. For example, we added noise genes to the pre-existent genes and gradually curtail sample sizes of the training datasets. Simple linear regression and univariate testing with significance level $\alpha$ is used to examine the effect of different selection criteria. All genes, no matter significant or not, are ordered by means of their $p$ values. The prediction performance increases as the gene number increases, and quickly reaches its peak (e.g., AUC =0.95 or 0.98). Later, the performance decreases due to the harm of noise genes. However, as the gene number gets larger (e.g., >2000 genes, depending on individual dataset), the prediction performance starts rising again and finally achieve the same performance as the initial peak. We found that the reduction of the sample size in the training datasets will postpone the initial peak time and decrease the corresponding prediction performance. The prediction model seems to be very resistant to the considerable number of new added noise genes because the highest performance is still >0.95 when all genes are put into the model. Also, we showed an optimal gene selection strategy depending on signal-to-noise ratio and signal strength given a fixed sample size. There exists a no-selecting zone where any gene selection procedure within the zone will only jeopardize prediction performance. We conclude that the sample size in the training dataset is influential to the initial peak prediction performance. The gene selection in some occasions may not benefit the prediction performance. We suggest the further studies of these no selecting zones may be as important as that of gene selection procedures.

## 1868/T/Poster Board #417

**candidate gene pharmacogenetics study of mGlu 2/3 agonist LY2140023.** *W. Liu, P. Chen, J.E. Brandt, R. Njau, L. Munsie, B. Kinon, A. Downing, L. Nisenbaum.* Lilly Research Laboratories, Indianapolis, IN.

LY2140023 is the oral prodrug of LY404039, a selective agonist for metabotropic-glutamate 2/3 (mGlu) receptors. LY2140023 is currently under development for the treatment of schizophrenia. mGlu2/3 agonism by LY2140023 has the potential to introduce a novel mechanism of action for oral antipsychotics, as all other commonly prescribed medications target monoaminergic activity. The primary goal of the current pharmacogenetics effort is to identify genetic markers that are predictive of patients' response to LY2140023 that could be used in future trials to target specific subpopulations of patients. This study examined genetic variants in eight candidate genes related to the mechanism of action of LY2140023 or the atypical antipsychotic olanzapine. Single nucleotide polymorphisms (SNPs) were genotyped in DNA samples collected as part of the Phase II proof of concept trial, H8Y-MC-HBBD, which was a 28-day, randomized, double-blind, placebo controlled study of LY2140023 in the treatment of patients with schizophrenia. Statistical analysis was conducted on 193 Caucasian patients treated with either placebo, LY2140023 or olanzapine. Associations between LY2140023 response and genotypes were identified for SNPs within the HTR2A and NRG1 genes. Three SNPs (1 in NRG1, 2 in HTR2A) were statistically significantly associated with LY2140023 response at 28 days as measured by Positive and Negative Syndrome scale (PANSS) Total change from baseline. All three SNPs had unadjusted p-values less than 0.001 (FDR=0.114) at 28 days. Twenty-three SNPs had unadjusted p-values less than 0.01 (FDR<0.2) in the LY2140023 arm at 28 days and 16 of them were in HTR2A, - all in tight linkage disequilibrium based on HapMap data. Top LY2140023 HTR2A and NRG1 SNPs showed similar results in PANSS Positive, Negative, and Clinical Global Impressions-Severity analyses. In conclusion, these data suggest that a genetic association may exist between SNPs in two genes and response to LY2140023 treatment. These findings will need to be examined in additional clinical trials to gain a better understanding of the clinical utility of these genetic markers.

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## 1869/T/Poster Board #418

**Disease model distortion and effect size underestimation in association studies.** *D. Vukcevic, E. Hechter, C. Spencer, P. Donnelly.* Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom.

Most GWAS findings are consistent with the simplest disease model, in which each additional copy of the risk allele increases disease risk by the same multiplicative factor, in contrast to recessive or dominant effects. Using theoretical and empirical results we show that LD markedly distorts these effects, with the power to detect dominant or recessive effects dropping off extremely quickly. For example, power to detect departures from the simplest model decays as a function of $r^4$, where $r^2$ is the usual correlation between the causal and marker loci. This is in contrast to the the well-known result that power to detect a multiplicative effect decays as function of $r^2$. Similar results apply to the detection of interactions between distinct GWAS loci. Using replicated findings for breast cancer, Crohn's disease and type 2 diabetes, we examine the consequences of these results for prediction of individual disease risk. Disease model distortion can also account for much of the missing heritability in common diseases.

## 1870/T/Poster Board #419

**The cholesterol 7 alpha-hydroxylase gene susceptible to the severe progression of primary biliary cirrhosis in Japanese patients.** *S. Higa[1], T. Inamine[1], F. Noguchi[1], A. Kawauchi[1], H. Hashiguchi[1], S. Kondo[1], M. Nakamura[2], H. Ishibashi[2], K. Omagari[3], K. Tsukamoto[1].* 1) Dept Pharmacotherapeutics, Nagasaki Univ Grad Sch Biomed Sci, Nagasaki, Japan; 2) Clin Res Center, Natl Hosp Organi Nagasaki Med Center, Nagasaki, Japan; 3) Dept Nutr, Fac Nurs Nutr, Univ Nagasaki, Nagasaki, Japan.

**Purpose**: Primary biliary cirrhosis (PBC) is a chronic liver disease characterized by destruction of the bile ducts and inflammation, resulting in the accumulation of bile acids, thus leading to hepatic damage. Here, we focused on the cholesterol 7 alpha-hydroxylase gene (*CYP7A1*), which is a rate-limiting enzyme of bile acid synthesis, and examined an association between polymorphisms of this gene and susceptibility to the severe progression of PBC.

**Methods**: The study subjects comprised 149 unrelated Japanese patients with PBC. Patients were classified into two different stages based on the findings of liver biopsy and clinical manifestations. Jaundice stage was defined as any Scheuer's stage with persistent jaundice. While, non-jaundice stage was defined as any Scheuer's stage without persistent jaundice. The five tag single-nucleotide polymorphisms (SNPs) in *CYP7A1* were detected by PCR-restriction fragment length polymorphism or by PCR-direct DNA sequencing method. Subsequently, haplotypes were constructed from two SNPs, which showed a significant association of the severe progression of PBC. The frequencies of alleles, genotypes, haplotypes, and diplotypes were compared between subgroups of PBC patients by a chi-square test or a logistic regression analysis using SPSS 17.

**Results**: The frequency of a Hap 3 haplotype and a Hap 1/Hap 3 diplotype were significantly increased in jaundice stage in comparison to that in non-jaundice stage [$P = 0.0152$, odds ratio (OR) = 3.348 and $P = 0.0040$, OR = 8.370, respectively].

**Conclusion**: These results suggest that the abilities of bile acid biosynthesis may be accelerated in PBC patients possessing the Hap 1/Hap 3 diplotype, resulting in the accumulation of toxic bile salts, and leading to the severe progression of PBC. Thus, *CYP7A1* appears to be a genetic determinant of the progression of PBC in Japanese patients. Genetic polymorphisms of *CYP7A1* may be useful as a biomarker for identifying high-risk PBC patients.

## 1871/T/Poster Board #420

**Diplotype polymorphisms of the bile salt export pump gene are associated with susceptibility to the severe progression of primary biliary cirrhosis in Japanese patients.** *T. Inamine[1], S. Higa[1], F. Noguchi[1], A. Kawauchi[1], H. Hashiguchi[1], S. Kondo[1], M. Nakamura[2], K. Omagari[3], H. Ishibashi[2], K. Tsukamoto[1].* 1) Dept Pharmacotherapeutics, Nagasaki Univ Grad Sch Biomed Sci, Nagasaki, Japan; 2) Clin Res Center, Natl Hosp Organi Nagasaki Med Center, Nagasaki, Japan; 3) Dept Nutr, Fac Nurs Nutr, Univ Nagasaki, Nagasaki, Japan.

Purpose: Primary biliary cirrhosis (PBC) is a chronic liver disease characterized by destruction of the bile ducts and inflammation, resulting in the accumulation of bile acids, thus leading to hepatic damage. Here, we focused on the bile salt export pump gene (*BSEP, ABCB11*), whose polymorphisms and mutations are associated with intrahepatic cholestasis of pregnancy, and examined an association between polymorphisms of *ABCB11* and susceptibility to the progression of PBC.

Methods: The study subjects comprised 145 unrelated Japanese PBC patients. Patients were classified into two different stages based on the findings of liver biopsy and clinical manifestations. Jaundice stage was defined as any Scheuer's stage with persistent jaundice. While non-jaundice stage was defined as any Scheuer's stage without persistent jaundice. The twenty-two tag single nucleotide polymorphisms (SNPs) in *ABC11* were detected by PCR-restriction fragment length polymorphism or PCR-direct DNA sequencing method. Subsequently, haplotypes were constructed from two tag SNPs, which showed a significant association of the severe progression of PBC. The frequencies of alleles, genotypes, haplotypes, and diplotypes were compared between subgroups of PBC patients by a chi-square test or a logistic regression analysis using the SPSS 17 statistical program package.

Results: The frequency of a Hap3/Hap4 diplotype was significantly increased in patients in jaundice stage in comparison to that in patients in non-jaundice stage ($P = 0.038$, odds ratio = 4.831). Inversely, all of the PBC patients having another Hap2/Hap3 diplotype were observed only in non-jaundice stage, but not in jaundice stage, although statistical analysis could not be carried out.

Conclusion: Our results suggest that the diminution of the bile acid efflux activities due to *ABCB11* polymorphisms, especially having the Hap3/Hap4 diplotype, may accelerate the accumulation of bile acid, eventually leading to the severe progression of PBC. Therefore, *ABCB11* appears to be a genetic determinant of the severe progression of PBC in Japanese.

## 1872/T/Poster Board #421

**Haplotype and diplotype polymorphisms of *HNF-4α* as a genetic biomarker for susceptibility to the severe progression of primary biliary cirrhosis in Japanese patients.** *F. Noguchi[1], T. Inamine[1], S. Higa[1], A. Kawauchi[1], H. Hashiguchi[1], S. Kondo[1], M. Nakamura[2], H. Ishibashi[2], K. Omagari[3], K. Tsukamoto[1].* 1) Dept Parmacotherapeutics, Nagasaki Univ Grad Sch Biomed Sci; 2) Clin Res Center, Natl Hosp Organi Nagasaki Med Center; 3) Dept Nutr, Fac Nurs Nutr, Univ Nagasaki.

**Objective:** Primary biliary cirrhosis (PBC) is a chronic liver disease characterized by destruction of the bile ducts and inflammation, resulting in the accumulation of bile acids, thus leading to hepatic damage. Here, we focused on the hepatocyte nuclear factor-4 alpha gene (*HNF-4α*), which regulates the expression of a set of genes related to bile acid synthesis and metabolism in the liver as a transcription factor, and examined an association between polymorphisms of this gene and susceptibility to the progression of PBC.

**Methods:** The study subjects comprised 149 unrelated Japanese patients with PBC. Patients were classified into two different stages based on the findings of liver biopsy and clinical manifestations. Jaundice stage was defined as any Scheuer's stage with persistent jaundice. While, non-jaundice stage was defined as any Scheuer's stage without persistent jaundice. The six tag single-nucleotide polymorphisms (SNPs) in *HNF-4α* were detected by PCR-restriction fragment length polymorphism method. The frequencies of alleles, genotypes, haplotypes, and diplotypes were compared between subgroups of PBC patients by a chi-square test or a logistic regression analysis using the SPSS 17 statistical software package.

**Results:** The frequencies of a Hap 6 haplotype and its heterogenous Hap 2/Hap 6 diplotype, which were constructed from three tag SNPs showing a significant association of the severe progression of PBC, were significantly increased in jaundice stage as compared with those in non-jaundice stage [$P = 0.0020$, odds ratio (OR) = 11.0 and $P = 0.0096$, OR = 13.0, respectively].

**Conclusion:** The present study is the first report to demonstrate that *HNF-4α* appears to be a genetic determinant of the severe progression of PBC, and may be useful as a new DNA-based diagnostic biomarker for identifying high-risk PBC patients. Furthermore, HNF-4α may be good target molecule for the development of novel drugs in the future.

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## 1873/T/Poster Board #422

**Genetic risk score for systemic lupus erythematosus is associated with age of onset and autoantibody production.** *K. Taylor[1], S. Chung[1], R. Graham[2], W. Ortmann[2], A. Lee[3], C. Langefeld[4], C. Jacob[5], M. Alarcón-Riquelme[6], B. Tsao[7], K. Moser[6], P. Gaffney[6], J. Harley[6], M. Petri[8], S. Manzi[9], P. Gregersen[3], T. Behrens[2], L. Criswell[1].* 1) Division of Rheumatology, University of California San Francisco, San Francisco, CA; 2) Genentech, Inc., South San Francisco, CA; 3) Feinstein Institute for Medical Research, Manhasset, NY; 4) Wake Forest University Health Sciences, Winston-Salem, NC; 5) University of Southern California, Los Angeles, CA; 6) Oklahoma Medical Research Foundation, Oklahoma City, OK; 7) University of California at Los Angeles, Los Angeles, CA; 8) Johns Hopkins University School of Medicine, Baltimore, MD; 9) University of Pittsburgh, Pittsburgh, PA.

Systemic lupus erythematosus (SLE) is a genetically complex disease with heterogeneous clinical manifestations. Recent studies have greatly expanded the number of established SLE risk alleles, but the distribution of multiple risk alleles in cases versus controls and their relationship to SLE subphenotypes has not been studied. We define a genetic risk score (GRS) for SLE as the number of risk alleles from 11 established SLE susceptibility polymorphisms in 10 genes: *HLA-DRB1* (*HLA-DR3* and *HLA-DR2* alleles), *PTPN22, STAT4, IRF5, TNFAIP3, FCGR2A, ITGAM, KIAA1542, PXK,* and *BLK*. We studied the GRS of 1919 SLE cases from 9 independent Caucasian SLE case series and 4813 independent controls. We also studied associations with clinical manifestations for the cases, namely age of onset, the 11 American College of Rheumatology (ACR) classification criteria, and anti-double-stranded DNA autoantibodies (anti-dsDNA), a subset of the ACR immunologic criterion. These manifestations strongly influence disease severity and outcome. This expands our previous work by the addition of 624 cases, principal components analysis for intra-European ancestry adjustment, and the addition of a *TNFAIP3* risk allele. The mean number of SLE risk alleles was 7.77 (SD ± 2.04) for cases versus 6.70 (SD ± 1.91) for controls, linear trend in log odds ptrend=$2.5 \times 10^{-87}$. The odds ratio for SLE risk comparing 9 or greater (22%) versus 5 or fewer (23%) risk alleles was 4.33 (95% CI 3.64-5.14). After adjustment for significant terms among sex, disease duration, cohort, and ancestry, each single-allele increase in the GRS was associated with increased risk of anti-dsDNA autoantibody production (OR=1.14, p=$6 \times 10^{-7}$) and the immunologic criterion (OR=1.12, p=0.00001), decreased risk of oral ulcers (OR=0.93, p=0.002), and a 9-month earlier age of onset (p=$10^{-6}$). Furthermore the GRS was more significant than any single SNP effect for these subphenotypes. In contrast, renal disease was most significantly associated with the *HLA-DR3* tag SNP (OR=1.34, p=0.004), arthritis with *ITGAM* (OR=0.71, p=0.001), serositis with *BLK* (OR=1.23, p=0.010), the hematologic criterion with *HLA-DR3* (OR=1.22, p=0.04), and anti-nuclear autoantibodies with *IRF5* (OR=2.14, p=0.010). Other clinical manifestations, such as photosensitivity and malar rash, were not associated with any genetic predictors.

## 1874/T/Poster Board #423

**A haplotype polymorphism of *FUT1* is associated with susceptibility to pulmonary emphysema in the Japanese population.** *R. Uemura[1], M. Taniguchi[1], Y. Naka[1], T. Inamine[1], S. Kondo[1], K. Nakatomi[2], S. Kohno[2], K. Tsukamoto[1].* 1) Dept Pharmacotherapeutics, Nagasaki Univ Grad Sch Biomed Sci, Nagasaki, Japan; 2) Second Dept Int Med, Nagasaki Univ Sch Med, Nagasaki, Japan.

**Objective**: Pulmonary emphysema (PE) is a multifactorial disorder characterized by destruction of alveolar septa, loss of lung elasticity, and enlargement of alveolar airspaces. Although the precise etiology of PE remains unknown, not only environmental factors such as cigarette smoking, but also genetic factors may contribute to the pathogenesis of PE. In this study, we focused on the alpha1,2-fucosyltransferase gene (*FUT1*), which down-regulates the expression of sialyl-Lewis x in neutrophils as well as the binding of neutrophils to E-selectin in the lung epithelial cells, and eventually inhibits the transfer of neutrophils to the site of inflammation in the lungs. Thus, we examined an association between polymorphisms of *FUT1* and susceptibility to PE.

**Methods**: The study subjects comprised 72 Japanese patients with PE and 72 age- and gender-matched healthy control subjects. All participants were former or current smokers. The three tag single-nucleotide polymorphisms (SNPs) in *FUT1* were detected by PCR-restriction fragment length polymorphism method. Subsequently, haplotypes were constructed from these SNPs. The frequencies of alleles, genotypes, haplotypes, and diplotypes were compared between PE patients and control subjects by a chi-square test or a logistic regression analysis using the GraphPad Prism 4 or SPSS 17 statistical software packages.

**Results**: The frequency of a Hap 1 haplotype of *FUT1* was significantly increased in PE patients as compared with that in control subjects [$P$ = 0.013, odds ratio = 1.815].

**Conclusion**: Our results suggest the possibility that the Hap 1 haplotype polymorphism may lead to the loss-of-function of FUT1, resulting in a decrease in both the transfer of neutrophils into the lungs and the prevention of alveolar destruction due to smoking. Therefore, *FUT1* appears to be a genetic determinant of susceptibility to PE. Furthermore, the haplotype polymorphism of *FUT1* may be useful as a DNA-based diagnostic biomarker for identifying Japanese individuals at high-risk for PE.

## 1875/T/Poster Board #424

**Polymorphic Glutathione S Transferase P (GSTP) as genetic risk factor for myopia in Indians Ch.Hema Bindu Gokaraju Rangaraju Institute of Engineering and Technology, JNTU, Hyderabad, India.** *c. HemaBindu.* Biotechnology, GRIET, Hyderabad, India, India.

Purpose : Myopia or short sightedness is the most common human eye disorder affecting 30% of world population. Genetic and environmental factors are implicated in the onset of myopia. Environmental causes include mechanical factors, oxidative stress, nutritional factors etc. Oxidative stress can arise due to intrinsic or extrinsic factors. Oxidation reduction mechanism has special importance in many tissues, including lens. Oxidative damage can result in number of molecular changes that contribute to the development of myopia. Crystallins and other protein in lens fiber cells do not turn over and must serve the lens for the life time of persons. thus, lens must have efficient reducing and detoxification system. GSTP are one of the Enzyme thought to be important in the protection of the eye from oxidative damage. our aim is to investigate the association of GSTP gene polymorphism with myopia in Indian patients. Methods: GSTP gene polymorphism were detected in 320 myopic patients and 320 control individuals by using Polymerase chain reaction and genotyping was done. Results: Results were compared with 320 cases of age and sex matched controls. The frequency of GSTP val/val genotype is case of disease is (3.40%) than in control (2%). When the data on myopia was sub grouped with respect to different parameters, interesting results were obtained. Frequency of val/val genotype in case of familial is (4.10%) when compared with nonfamilial (1.9%).patients with age at onset greater than 10 years have higher Val/Val genotype frequency (3.7%) when compared with early at onset. Further myopia patients having refractive error greater than 2 diopter have higher frequency of val/val genotype (4.03%) when compared with refractive error less than 2 diopter . there is an elevation in the val/val genotype frequency in case of females (4.40%) when compared that of males(2.7%). Conclusions : These results indicates that there is an elevation of val/val genotype in myopia patients which indicate that inefficient detoxification system to combat the accumulation of free radicals might be responsible for the onset of myopia . There was no association of GSTP gene polymorphism with parental consanguinity.

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## 1876/T/Poster Board #425

**Is Familial Mediterranean Fever Prevalent and Undiagnosed in Ethiopia?** *L. Kogleck[1], C. Plaster[1], A. Tarekegn[2,1], E. Bekele[2], L. Yepiskosyan[3], A. Harutyunyan[3], N. Bradman[1], M.G. Thomas[1].* 1) Research Department of Genetics, Evolution and Environment (G.E.E), University College London, London, United Kingdom; 2) Addis Ababa University, Addis Ababa, Ethiopia; 3) Institute of Man, Yerevan, Armenia.

Familial Mediterranean Fever (FMF) is a hereditary auto-inflammatory disease prevalent in Armenian, Turkish, Arab and non-Ashkenazi Jewish populations. The gene responsible for this disorder, MEFV, has been mapped to chromosome 16p13.3 and most common mutations associated with the disease are located in exon 10. An additional less common mutation, A744S, has been reported in 2% (n=3) of Tunisian FMF patients (n=139), 2% (n=3) of Syrian patients (n=153) and 1% (n=16) of Turkish patients (n= 1201). Consequently, although computer modelling using PolyPhen predicts the A744S amino acid change to be benign, the presence of the mutation in patients, both as a simple heterozygote and as a compound heterozygote with more common exon 10 non synonymous mutations, suggests that it may be a cause of FMF. Archaeological, linguistic and genetic data suggest substantial migration into Ethiopia from the Arabian Peninsula over the past few thousand years. It is therefore reasonable to anticipate that mutations present in exon 10 of MEFV associated with the disease might be present in Ethiopians. To investigate this possibility we sequenced the whole of exon 10 in general population sample sets comprising individuals unrelated at the paternal grandfather level from five geographically separated Ethiopian ethnic groups (Amhara, Semitic speakers; Afar and Oromo, Cushitic speakers; Maale, Omotic speakers; and Anuak, Nilo-Saharan speakers, n= 187). A744S was identified in all four non Nilo-Saharan speaking groups being present in a heterozygous state in 5.4% of individuals. If, as with common exon 10 mutations, homozygous or compound heterozygous individuals are prone to FMF, as many as one in 1,500 Ethiopians may be at risk of suffering from the disease. This may be of medical importance since Ethiopians, in Ethiopia as well as in the UK, USA and Israel (all three of which have accepted many Ethiopian migrants during the past few decades), may have periodic fevers misdiagnosed and consequently receive inappropriate and potentially harmful healthcare. Clinical investigation to establish the presence or otherwise of FMF in Ethiopians is warranted.

## 1877/T/Poster Board #426

**Personalized Decomposition of the Genetic Variance Using Probabilistic Conjoint Measurement (PCM) Models.** *N.J. Markward.* Pennington Biomedical Research Center, Baton Rouge, LA.

Drawing on the intimate relationship between measurement theoretic reliability and genetic heritability, this project outlines how probabilistic conjoint measurement (PCM) models can be used to personalize decomposition of the phenotypic variance and, in turn, to extract haplotype- and individual-specific estimates of disease propensity, pharmacologic response, and environmental susceptibility. Example calculations are presented and discussed in the context of both large-scale candidate gene and genome wide association (GWA) studies.

## 1878/T/Poster Board #427

**Multiple loci associated with prostate cancer susceptibility on 8q24.** *A. Amin Al Olama[1], R.A. Eeles[2,3], Z. Kote-Jarai[2], G.G. Giles[4], G. Severi[4], M. Guy[2], J. Hopper[5], D. Leogamornlert[2], M. Tymrakiewicz[2], E. Saunders[2], J. Morrison[1], D. Easton[1].* 1) Cancer Research UK Genetic Epidemiology Unit, Department of Public Health & Primary Care, University of Cambridge, Strangeways Laboratry, Worts Couseway, Cambridge, CB1 8RN, United Kingdom; 2) The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5NG, United Kingdom; 3) The Royal Marsden NHS Foundation Trust, Downs Road, Sutton, Surrey, SM2 5PT, and Fulham Road, London SW3 6JJ, United Kingdom; 4) Cancer Epidemiology Centre, The Cancer Council Victoria, 1 Rathdowne street, Carlton VIC 3053, Australia; 5) Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne, 723 Swanston street, Carlton VIC 3053, Australia.

Previous studies have identified multiple loci associated with prostate cancer on 8q24[1-4]. To provide a comprehensive analysis of common alleles associated with prostate cancer risk, we analysed two large case-control studies for SNPs across the 1.2Mb 'desert' region on between FAM84B and C-MYC[5] : (1) 1,854 individuals with clinically detected prostate cancer diagnosed below age 60 years or with positive family history, and 1,894 population screened controls, genotyped for 322 SNPs from the Illumina 550k array (2) 3,650 cases and 3,940 controls from UK and Australia, genotyped for 427 SNPs from the Illumina 1M array[6]. We also genotyped five additional SNPs (rs13254738, rs6983561, rs16901979, rs7000448 and rs10090154) previously associated with prostate cancer risk. 39 SNPs were associated with prostate cancer at $P(trend)<10^{-5}$, eight of which were independently associated with risk. These included three establish SNPs: rs6983561 ($P=10^{-11}$); rs6983267 ($P=6x10^{-23}$) and rs10090154 ($P=7x10^{-24}$), together with 5 additional SNPs ($P=4x10^{-6}$ to $P=10^{-12}$). All SNPs were associated in both stages but the per-allele ORs effects were all higher in stage 1. The combined effects of all SNPs were consistent with a multiplicative model, and imply a twofold difference in risk between the top 1% and bottom 1% of the population based on 8q24 genotypes. [1]Amundadottir,L.T. et al. A common variant associated with prostate cancer in European and African populations. Nat Genet 38, 652-658 (2006).[2]Gudmundsson,J. et al. Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. Nat Genet 39, 631-637 (2007).[3]Haiman,C.A. et al. Multiple regions within 8q24 independently affect risk for prostate cancer. Nat Genet 39, 638-644 (2007).[4]Witte,J.S. Multiple prostate cancer risk variants on 8q24. Nat Genet 39, 579-580 (2007).[5]Ghoussaini,M. et al. Multiple loci with different cancer specificities within the 8q24 gene desert. J Natl Cancer Inst 100, 962-966 (2008).[6]Eeles,R.A. et al. Multiple newly identified loci associated with prostate cancer susceptibility. Nat Genet 40, 316-321 (2008).

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## 1879/T/Poster Board #428

**Functional dbSNP rs28493229 of the ITPKC gene is not associated with Kawasaki disease in Taiwanese children.** *H. Chi[1,3,5], F.-Y. Huang[1,4], M.-R. Chen[1,5], N.-C. Chiu[1,5], H.-C. Lee[1,4], S.-P. Lin[1,2,5,6], W.-F. Chen[2], C.-L. Lin[2], H.-W. Chan[2], H.-F. Liu[2], M. Lin[2], L.-M. Huang[3], Y.-J. Lee[1,2,4].* 1) Pediatrics, Mackay Memorial Hosp. 92, Section 2, Chung Shan North Road, Taipei 10449, Taiwan; 2) Department of Medical Research, Mackay Memorial Hospital, 45, Min-Sheng Rd., Tamshui, 25115 Taipei, Taiwan; 3) Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan; 4) Department of Pediatrics, Taipei Medical University, Taipei, Taiwan; 5) Mackay Medicine, Nursing and Management College, Taipei, Taiwan; 6) Department of Infant and Child Care, National Taipei College of Nursing, Taipei, Taiwan.

Kawasaki disease (KD) is an acute febrile illness with considerable geographic and racial variability. Vasculitis is the pathologic lesion underlying most of the clinical findings and 15-25% of untreated KD patients are complicated by the development of coronary artery lesions (CALs). The etiology and pathogenesis remain unknown and a suggestive mechanism of its onset would be a genetically determined exaggerated immune response triggered by several different unknown microbial agents. T-cell activation and cytokines play an important role in the pathogenesis of vascular endothelial cell injury by eliciting proinflammatory in the onset of KD. Inositol 1,4,5-trisphosphate (IP3) is a second messenger in various types of cells involved in the pathogenesis of KD. ITPKC is a kinase of IP3 and serves as a negative regulator of the Ca2+/ nuclear factor of activated T-cells signaling pathway in T cells. The C allele of dbSNP rs28493229 of the ITPKC gene contributes to immune hyper-reactivity that affects susceptibility to KD and leads to CALs among Japanese and US KD patients. The aim of this study was to investigate whether the single-nucleotide polymorphism (SNP) was associated with susceptibility to KD or with occurrence of CALs in Taiwanese children. We collected 168 unrelated Taiwanese children (99 boys and 69 girls) with KD including 65 patients had CALs. The mean age of the patients was 1.8 ± 1.6 years (0.1- 7.4). THE dbSNP rs28493229 was genotyped in children with KD and 1147 ethnically matched healthy controls using the TaqMan Allelic Discrimination Assay. No significant differences in genotype, allele, and carrier frequencies of the three SNPs were found between healthy controls and children with KD or those with CALs (p = 0.34 and p = 0.41, respectively). In conclusion, we did not find statistically significant association between the functional dbSNP rs28493229 of the ITPKC gene and KD or CALs in Taiwanese children. The discrepancy might be due to difference in ethnicity.

## 1880/T/Poster Board #429

**Potential clinical validity of germline genomic profiling for colorectal cancer screening and risk prediction.** *S. Hawken, J. Little.* Epidemiology & Community Med, Univ Ottawa, Ottawa, ON, Canada.

The heritability of colorectal cancer (CRC) has been estimated to be 25-35%, with 2-6% of cases attributed to specific genetic syndromes. Common, low-penetrance variants could play a substantial etiological role and in combination have potential utility in targeting individuals at elevated risk for preventive interventions, such as enhanced screening. Although mass CRC screening in average risk people aged 50+ has been implemented in several jurisdictions, uptake is low except in people with a family history of CRC. If uptake in response to a positive genetic test improved to that observed for people with elevated familial risk, then a substantial number of additional CRC cases might be detected early. We used statistical modeling in empirical and in simulated data to assess the potential value of genomic profiling in the context of CRC screening. 1. Empirical modeling in the ARCTIC case-control data: we defined a panel of 80 genetic variants associated with CRC, by conducting a systematic review of original research and meta-analyses. This *a priori* list of variants was then used to develop and test risk prediction models in a data obtained from genome-wide genotyping of 1200 CRC cases, and 1200 population-based controls. We report model building for ~30 SNPs in the panel that were covered directly or by LD. Allele frequencies ranged from 1%-50%. Univariate OR per allele ranged from 0.8 to 1.4. Counting the number of risk alleles per person, the risk increase was 8% for each additional risk allele (OR 1.08 95% CI 1.05-1.11); the OR for persons with 30+ alleles compared those with ≤20 was 2.26 (95% CI 1.27-4.04). The cross-validated area under the ROC curve was <60%, suggesting that many more risk alleles would be needed to constitute a genomic profile useful in screening. 2. Simulation work: We examined possible scenarios that could lead to a genomic test that could concentrate 80% of CRC cases in the top 50% of test scores, constrained to a maximum of 30% of CRC cases being attributable to genetic factors. For an average risk allele prevalence of 20% and per allele OR of 1.2 between 100 and 200 risk alleles with would be required. We estimate that such a genomic test could result in several thousand more cancers being detected for every one million people targeted for screening.

## 1881/T/Poster Board #430

**SNP association study of Behcet's disease to unravel the genetic susceptibility and pathoetiology.** *R. Kurata[1], H. Nakaoka[1], A. Tajima[1], T. Saito[1], K. Hosomichi[1], T. Shiina[1], A. Meguro[2], N. Mizuki[2], H. Inoko[1], I. Inoue[1].* 1) Dept. of Molecular Lifescience, Tokai University School of Medicene; 2) Dept. of Ophthalmology, Yokohama City University School of Medicine.

Behcet's disease (BD) is a chronic inflammatory disorder characterized by recurrent oral and genital ulcers, skin legions, uveitis, arthritis, and neurological manifestations. Human Leukocyte Antigen (HLA) has been analyzed as a candidate region of BD, because of the autoimmunity nature. It has already reported that *HLA-A26* and *-B51* are the strong genetic factor responsible for BD. Because patients who harbor *HLA-A26*, *-B51* and both are 48 %, 35 % and 29 %, respectively, among Japanese, *HLA-A26* and *-B51* are not the absolute causes of BD and the functional causality is not still unclear. We speculated a possibility that other susceptible genes contributing to BD exist in HLA region. We screened the entire HLA region with microsatellite markers and could narrow the susceptible region down to *HLA-A* to *-E*. Then, we carried out SNP association study in the region to identify a novel susceptible gene. We genotyped 384 Japanese patients with BD and 384 controls for 192 SNPs covering the *HLA-A* to *-E* using BeadXpress system (illumina). 19 SNPs are associated (P<0.0001) with BD, and the logistic regression analysis showed 2 SNPs were associated (P=0.0005) with BD independently of *HLA-A26* and *HLA-B51*. In conclusion, we identified the 2 SNPs as new genetic factors to BD that are independent of the previous HLA association and one of the SNP showing amino acid substitution may have a functional relevance to BD.

## 1882/T/Poster Board #431

**Coclustering genes and common diseases.** *N. Tanaka[1], M. Muramatsu[2], T. Arai[3], M. Sawabe[3].* 1) Dept Biostatistics, Harvard Sch Pub Hlth, Boston, MA; 2) Dept Molecular Epidemiology, Medical Research Institute, Tokyo Medical and Dental Univ, Tokyo, Japan; 3) Dept Pathology, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan.

Several genetic polymorphisms have been linked to more than one disease, for example, polymorphisms in the ACE, APOE, and TGF-β gene. However, most of the study are focusing on only one disease and does not shed light on disease-disease relationship. It has been well established that the proportion of people with multiple diseases may vary from 30% in the general population to over 50% in people aged 60 years and older. Interestingly, many people have both any cardiovascular diseases and any cancers before they die, whereas some people have none of any common diseases throughout their lives. We focused on this disease-disease relationship and the susceptible multiple common genetic risks, and have developed algorithms that perform simultaneous clustering on the row (diseases) and column (SNPs) dimensions to find the subgroups of genes and the subgroups of diseases. We apply the co-clustering method and demonstrate that a specific combination of genes results in a specific combination diseases using a consecutive autopsy study data.

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## 1883/T/Poster Board #432

**Multiple Subsampling of Dense SNP Data Localizes Disease Genes with Increased Precision.** *A.L. Peljto, W.C.L. Stewart, D.A. Greenberg.* Division of Statistical Genetics, Biostatistics Dept, Columbia University, New York, NY.

Genome-wide linkage studies routinely use genotypes sampled at hundreds of thousands of single nucleotide polymorphisms (SNPs) to look for disease-related genes. However, linkage disequilibrium (LD) between neighboring SNPs can distort the information about trait location. We present an efficient method for trait location estimation that accounts completely for LD and yields narrow, high-resolution candidate gene regions (CGRs). Our method is freely available, and is implemented in the software package EAGLET (Efficient Analysis of Genetic Linkage: Estimation and Testing).

Existing linkage methods either: (1) ignore LD; (2) approximate the LD-related correlation; or, (3) analyze a single, uncorrelated subset of the original dense data. By contrast, our method makes efficient use of all available data by averaging location estimates obtained from random subsamples of the original dense SNP data. We use pairwise LD estimates to ensure that the SNPs within each subsample are approximately uncorrelated; and, we use the nonparametric bootstrap procedure to construct 95% confidence intervals (i.e. CGRs) for the true trait location. To assess the performance of our method in comparison to the three existing methods, we analyzed simulated dense SNP linkage data in the presence of LD, as well as the real data of 15 families with pulmonary hypertension.

RESULTS: Based on simulated data, we show that the existing approaches to dense SNP linkage analysis (described above) can yield biased and/or inefficient estimation depending on the underlying LD pattern. With respect to mean squared error, our estimator outperforms the single random subsample approach, and is at least as good as, and usually better than, methods that ignore or approximate LD. Furthermore, using the dense SNP data from 15 hypertension families, the CGR estimated by EAGLET is 47.5% shorter than the CGR estimated by the common approach, which uses a single random subsample.

CONCLUSION: Our method completely accounts for LD, and is the best available tool for constructing high-resolution CGRs. Furthermore, it is not limited to the analysis of small families, and can be used to inform deep sequencing projects.

## 1884/T/Poster Board #433

**Model-free Linkage Analysis of QT Interval Duration in the Jackson Heart Study.** *S. Buxbaum[1], K. Lewis[2], S. Tribune[2], L. Ekunwe[1].* 1) Jackson Heart Study, Jackson State Univ, Jackson, MS; 2) Tougaloo College, Jackson, MS.

The QT interval is measured in electrocardiograms and prolonged QT intervals are associated with ventricular arrhythmias. QT interval has been shown to be heritable, with an estimate of 35% in the Framingham Heart Study and 41% in the Jackson Heart Study, a study of African Americans in Jackson, Mississippi. Findings of polygenic effects (multiple loci with small effects) have previously been reported in genome wide association studies. A complex segregation analysis in the JHS data suggested the existence of a major gene or genes. Following on this work, a model-free multipoint linkage analysis of the QT interval, adjusted for age, sex, the RR interval, body mass index (BMI), coronary heart disease, use of diuretics, hypertension, plasma potassium levels, the QRS interval, and Sokolow-Lyon voltage was conducted, using 374 autosomal microsatellite markers. Two regions with strong linkage signals for QT were found using the weighted square trait sum and difference of QT between sibs in the SIBPAL program in the S.A.G.E. package. 772 sib pairs, comprising 159 half sibs and 613 full sibs, were analyzed. One peak was on chromosome 10q23, with a nominal p-value less than 1.0 x 10-7 (LOD > 5.9) and another on chromosome 11q11, with a nominal p-value of 3.5 x 10-6 (LOD 4.4). Power transforms of QT (log QT and square root QT) were analyzed with similar results. Using a reduced model of QT adjusted for sex, age, BMI and the RR interval resulted in less missing phenotype data, but also less power. Using the reduced model, analysis of 800 sib pairs, comprising 177 half sibs and 634 full sibs, gave similar results at the same loci, although less strong: p-value = 1.31 x 10-5 (LOD 3.8) and p-value = 2.84 x 10-4 (LOD 2.4), respectively. Hence, the full regression model with additional adjustment for coronary heart disease, use of diuretics, hypertension, plasma potassium levels, QRS interval and Sokolow-Lyon voltage had more power to detect linkage. Previous genome wide association studies (GWAS) with large Caucasian samples have reported 15 loci that explain a small percentage of QT variance; however, there is no overlap with these two loci found by linkage analysis in this African American sample.

This research from the Jackson Heart Study is supported by NIH contracts N01-HC95170, N01-HC-95171, and N01-HC-95172 provided by the national Heart, Lung and Blood Institute and the National Center for Minority Health and Health Disparities.

## 1885/T/Poster Board #434

**A new strategy for linkage analysis under epistasis taking into account genetic heterogeneity.** *A. Bureau[1,2], C. Mérette[1,3], J. Croteau[1], A. Fournier[1], Y.C. Chagnon[1,3], M.-A. Roy[1,3], M. Maziade[1,3].* 1) Centre de recherche Université Laval - Robert-Giffard, Québec, Québec, Canada; 2) Département de médecine sociale et préventive, Université Laval, Québec, Québec, Canada; 3) Département de psychiatrie, Université Laval, Québec, Québec, Canada.

Epistasis, the biological interaction of multiple genes modulating their individual effects, is likely omnipresent in complex diseases, and modelling epistasis in linkage studies can help detect loci with little marginal effect and detect epistatic effects themselves. We propose a complete three-step strategy for parametric linkage analysis under epistasis and heterogeneity in extended pedigrees. 1) Loci most likely involved in epistatic interactions are pre-screened using two-locus one-marker analyses. 2) Among selected loci, linkage to each locus is evaluated conditionally on linkage information at another locus under two-locus epistatic models. Linkage statistics are maximized over a space of epistatic models to avoid misspecification of model parameters. 3) Families linked to the conditioning locus are selected to deal with heterogeneity between pairs of epistatically interacting loci and other unlinked loci. Properties of conditional linkage statistics prevent the introduction of bias. Simulations reveal important gains in power to detect a locus with weak marginal effect involved in epistatic interaction. Application of our methods to schizophrenia and bipolar disorder in Eastern Quebec kindreds suggests epistasis between three locus pairs for bipolar disorder: 8p11-16p13, 15q11-16p13 and 18q12-15q11. These results suggest that the proposed strategy is powerful for tackling complex phenotypes involving epistasis and heterogeneity.

## 1886/T/Poster Board #435

**EAGLET: A Powerful New Tool for Analyzing Dense SNP Linkage Data.** *E.N. Drill, W.C.L. Stewart, D.A. Greenberg.* Division of Statistical Genetics, Biostatistics Department, Columbia University Mailman School of Public Health, New York, NY.

Linkage disequilibrium (LD), the non-random distribution of alleles at different loci, can be a serious problem for the analysis of dense SNP linkage data when the genotypes of founders are unavailable and families are enriched for affected individuals. If ignored, LD can bias estimates of trait location, inflate the Type I error, and reduce the power to detect linkage. We developed the ALOD, a test for linkage that uses all available SNP data, and accounts completely for LD. It is based on the average of multiple lod score curves calculated from random subsamples of the original dense data, and uses pairwise estimates of LD to ensure that each subsample has approximately uncorrelated SNPs. Our test has power comparable to, but often better than, alternative approaches, and is implemented in the software package EAGLET (Efficient Analysis of Genetic Linkage: Estimation and Testing). We examine the performance of four different methods: the ALOD, the MLOD, MERLIN, and a naïve approach that ignores LD using all SNPs to calculate the Kong & Cox LOD. The MLOD is another multiple subsampling approach; however, the SNPs in each subsample are chosen on the basis of physical distance, not absence of LD. MERLIN models the alleles of highly correlated SNPs as non-recombining haplotypes (or clusters) in the founders. Each cluster is then assumed to be independent. Both MERLIN and MLOD have been shown to increase power relative to single subsampling methods.

RESULTS: Using dense SNP data simulated with LD but without linkage, we show that relative to the approach that ignores LD, the ALOD reduces bias in the evidence for linkage by at least 72% across the different simulation scenarios that we considered. For example, based on simulated data involving 100 affected sibling pairs (ASPs), with a high-LDlow-LDhigh-LD pattern in three 33 cM blocks, the percent reductions in bias for the ALOD, MLOD, and MERLIN were 76, 69, and 59, respectively. Moreover, assuming a linked dominant trait and incomplete penetrance, we simulated dense SNP data for the same number of ASPs, LD pattern, and map. The percent powers for the ALOD, MLOD, and MERLIN were 29, 19.5, and 30.5.

CONCLUSIONS: The maximum ALOD accounts well for LD, and is more powerful than the MLOD, with comparable power to MERLIN. Furthermore, it is twice as fast as MERLIN, and is not limited to the analysis of small families.

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## 1887/T/Poster Board #436

**Robust Score Statistics for QTL Linkage Analysis Using Extended Pedigrees.** *C. Kuo[1], D. Weeks[1, 2], N. Mukhopadhyay[2], S. Bhattacharjee[3], E. Feingold[1, 2].* 1) Dept Biostatistics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 3) Division of Cancer Epi and Genet, National Cancer Institute, NIH, Dept Health and Human Services, Bethesda, MD.

Score statistics for QTL linkage analysis have been proposed by many authors as an alternative to variance components and/or Haseman-Elston type methods because they have high power and can be made robust to selected samples and/or non-normal traits. But most literature exploring the properties of these statistics has focused on nuclear families. There are a number of computational complexities involved in implementing the score statistics for extended pedigrees, primarily having to do with computation of the statistic variance. In our work, we propose several different practical methods for computing this variance in general pedigrees, some of which are based only on relative pairs and some of which require working with the overall pedigree structure, which is computationally more difficult. We evaluate the performance of these different score tests using various trait distributions, ascertainment schemes, and pedigree types.

## 1888/T/Poster Board #437

**Estimation of trait parameters in human QTL mapping under different ascertainment schemes.** *I. Mukhopadhyay[1], E. Feingold[2], D.E. Weeks[2].* 1) Human Genetics Unit, Indian Statistical Institute, Kolkata, WB, India; 2) Department of Human Genetics & Biostatistics, University of PIttsburgh, Pittsburgh, PA, USA.

In almost all the regression-based statistics and the score statistics methods of QTL mapping, usually it is assumed that the trait parameters (segregation parameters) ? (=(?, ?$^2$)) are known. This is rarely so unless one has a very good idea about ? from a previous study. Through simulation, Sham et al (2002), T.Cuenco et al (2003a, 2003b) and Szatkiewicz and Feingold (2005) studied the effect of misspecification of the segregation parameters considering a few values, which differs from the true values of the parameters, for population samples and selected samples. They showed that departure from the true values of ? results in substantial loss of power. In view of this it becomes important to develop a proper estimation procedure of the segregation parameters involved in regression-based statistics and score statistics. In this work, we propose some estimation methods for ? based on population samples as well as selected samples. We discuss our proposed methods of estimation under several ascertainment schemes e.g. affected concordant sib-pair, discordant sib-pair, discordant and concordant sib-pair etc. Through simulation, we evaluated the performance of several test statistics by calculating probability of Type I error and power under several genetic models. Keeping the probability of Type I error at the desired level, the power of the statistics using our proposed method is very close to the power that can be obtained assuming the true values of the segregation parameters.

## 1889/T/Poster Board #438

**Kinship testing goes linkage.** *M. Nothnagel, M. Krawczak.* Institute of Medical Informatics and Statistics, University of Kiel, Germany.

Practical applications of kinship testing in humans so far have largely ignored linkage. This is mainly due to the fact that, until quite recently, genetic markers routinely used for identification purposes were not (closely) linked. Furthermore, over 95% of kinship cases in humans involve a disputed paternity in a trio of mother, child, and alleged father. Here, inter-marker linkage is indeed computationally irrelevant in the absence of linkage disequilibrium. However, with the introduction of a substantial number of novel markers into forensic practise, linkage has become an issue in kinship testing. Furthermore, the availability of more comprehensive markers sets has rendered increasingly more complex kinship cases tractable with fewer individuals tested, eventually involving only pairs of supposed relatives. We performed extensive simulations of genotypes for the 34 autosomal STRs widely used in forensic practise today to assess the current limits of the resolution of pair-wise kinship testing, the effects of an appropriate consideration of linkage between STRs on the ensuing likelihood ratios, and the potential gain in power from expanding the current marker set by a realistic amount. The results reveal a clear-cut difference between the power of the 34 STRs to resolve first degree and second degree relatedness. Expanding the marker set improved the chance of distinguishing second degree relatives from unrelated individuals, but at the same time intensified the need to take inter-marker linkage appropriately into account.

## 1890/T/Poster Board #439

**Deciphering The Genetic Architecture Of Alcohol Dependence: The Success Of Multivariate Phenotypes.** *S. Ghosh[1], L. Bierut[2], Collaborative Study On The Genetics Of Alcoholism.* 1) Human Genetics Unit, Indian Statistical Inst, Kolkata, India; 2) Department of Psychiatry, Washington University School of Medicine, St. Louis, MO.

Using the clinical end-point of alcoholism as a linkage phenotype in the Collaborative Study On The Genetics Of Alcoholism (COGA) project has failed to identify chromosomal regions harboring potential genes for the complex trait. This has motivated analyses of quantitative endophenotypes correlated with alcoholism, which capture greater variation within trait genotypes compared to the end-point binary trait. However, a single quantitative trait is unlikely to be a powerful surrogate of the end-point trait and it may be more prudent to use a correlated multivariate phenotype for linkage analyses. We propose along the lines of Sham et al. (2002), a linear regression formulation in which the traditional response variable, that is, some function of the multivariate phenotype and the explanatory variable, that is, the genetic similarity between sib-pairs are interchanged. The major advantage of the method is that it neither requires specification of any probability model for the quantitative trait values nor any data reduction technique like principal components. Hence, the method is more robust to violations in distributional assumptions compared to likelihood based methods. On the other hand, linkage inferences based on the proposed method are more meaningful compared to data reduction methods. Moreover, the method can incorporate both quantitative as well as qualitative traits within the multivariate phenotype vector. Based on simulations, we find that the proposed method is more powerful than analyses based on the first principal component of the correlated phenotypes and the end-point binary trait. We perform a genome-wide linkage scan of a multivariate phenotype comprising three alcohol related quantitative traits: number of drinks in a day, Beta 2 electroencephalogram and count of externalizing symptoms associated with anti-social behavior using the proposed regression approach and identify chromosomal regions on Chromosomes 1,4,5 and 15 exhibiting significant linkage. We find that these regions harbor potential genes for alcoholism like the GABRA receptor, the ADH cluster and CHRNA7. We follow up the significant linkage findings using a novel association approach based on logistic regression, which can analytically shown to be statistically equivalent to the binary TDT (Spielman et al. 1993) and have been able to obtain significant evidence of association in the regions exhibiting linkage.

## 1891/T/Poster Board #440

**Optimally Discovering cis eQTLs Across Multiple Tissues.** *C. Ye[1], B. Han[2], T. Choi[3], E. Eskin[4,5].* 1) Bioinformatics Program, University of California, San Diego, San Diego, CA; 2) Department of Computer Science, University of California, San Diego, San Diego, CA; 3) Predictive Biology, Inc., San Diego, CA; 4) Department of Computer Science, University of California, Los Angeles, CA; 5) Department of Human Genetics, University of California, Los Angeles, CA.

Recent technological improvements and cost decreases in gene chips have led to a growing accumulation of gene expression studies in different tissues across the same genetic gradient in both human and mouse. Current methods for comparing eQTLs across tissues are qualitative in nature, often reporting a set of significant eQTLs specific to one tissue or shared between several tissues based on the results of analyzing the tissues independently. This approach faces two major challenges that significantly reduce the power we have at identifying cross tissue and tissue specific eQTLs. Intuitively, one would like to use information about the similarities between tissues, for example between two homogeneous brain regions, to help detect shared eQTLs. In this work, we develop a new multiple hypothesis testing procedure, Multi-dimension Decision Procedure (MDP) that utilizes similarities between tissues in terms of shared status of test statistics to detect tissue specific and cross tissue eQTLs. We present two simple approaches for estimating our procedure in practice. Using simulated data, we show that MDP outperforms traditional eQTL approaches if there's sharing of tests between tissues. It performs as well as traditional approaches if there's no sharing. We then applied our method to one of the first systematic genetical genomics studies of different tissues from F1-backcrossed mice. We studied four datasets, including two populations collected in liver, two similar tissues (cortex and hippocampus), two different tissues (cortex and liver) and across four tissues (cortex, liver, heart and spleen). In all cases, our method outperforms traditional approaches in terms of discovering more cis-eQTLs at the same FDR cutoffs. Not only did we discover proportionally more cross tissue cis -eQTLs, we also discover more tissue specific cis-eQTLs because we directly optimize for the FDR. Using the results across four tissue types with unmatched individuals, we present one of the first cross tissue cis-eQTL maps identifying genomic loci with tissue specific or cross tissue effects on gene expression. We hypothesize that with denser genotyping data, we can apply our method to construct denser cross tissue eQTL maps to identify regulatory components. Combined with additional phenotype data, we can begin to understand how tissue specificity affect the way regulatory components interact to give rise to complex phenotypes.

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## 1892/T/Poster Board #441

**How common is hypophosphatasia?** *M.E. Nunes[1], J-L. Serre[2], E. Mornet[2,3].* 1) Dept Genetics/Pediatrics, Kaiser Permanente, San Diego, CA; 2) Universite de Versailles-Saint Quentin en Yvelines, Versailles, France; 3) Laboratoire SESEP, Centre Hospitalier de Versailles, Le Chesnay, France.

Hypophosphatasia is characterized by defective bone mineralization with highly variable clinical expression, caused by mutations in the tissue-nonspecific alkaline phosphatase (*TNSALP*) gene. To date, 204 novel mutations have been reported. 44 of these represent insertions, deletions, splice site, and nonsense mutations resulting in haploinsufficiency and predicting severe effect. 160 are *TNSALP* missense mutations, of which an estimated 75% affect a functional protein domain. The severity of many of these missense mutations has been evaluated by clinical phenotype in carrying patients, 3D protein modeling, and in select cases site-directed mutagenesis. This has allowed for characterization of mutations as “wildtype”, “moderate”, and “severe” alleles reflecting normal, reduced, and absent serum alkaline phosphatase activity. Six clinical forms of hypophosphatasia are currently recognized. Patients with odontohypophosphatasia reflect specific genotype/phenotype correlations. A prenatal benign form probably reflects a specific maternally inherited missense mutation. Perinatal lethal, infantile, childhood, and adult hypophosphatasia are recognized as a clinical spectrum by age at diagnosis, the first two transmitted as autosomal recessive disorders and the latter two milder forms as autosomal recessive or dominant. Thus, genetic counseling with several possible phenotypes within a family remains complex, predicting the population carrier frequency for marriage into a segregating kindred a challenge. The birth prevalence of severe hypophosphatasia (perinatal lethal and infantile forms) in Ontario 50 years ago was estimated to be 1/100,000, which has been used to suggest a carrier frequency of 1/150 by the Hardy-Weinberg principle. We expanded this analysis assuming three alleles: p (wildtype), q (moderate), and r (severe). Assuming a continuous phenotypic spectrum attributable to allele affect on serum alkaline phosphatase activity, adult cases are represented by $(2pr + q^2)$, childhood $(q^2 + 2pq)$, infantile (2qr), and perinatal lethal $(2qr + r^2)$. The original estimate of birth prevalence (1/100,000) thus becomes $2qr + r^2$. Assuming 80% of alleles are severe, r = 4q. The model predicts r = 1/387, q = 1/1548, childhood hypophosphatasia “common” at 1/750, adult cases more common at 1/200. Clinical and laboratory ascertainment thresholds combine with allelic heterogeneity to explain why dominant forms of hypophosphatasia are less commonly recognized.

## 1893/T/Poster Board #442

**Heritability of mammographic density in Korean twin and family: the Healthy Twin study.** *YM. Song[1], J. sung[2], KY. Lee[3], S. Kim[1].* 1) Department of Family Medicine, Samsung Medical Center and Center for Clinical Research, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, 50 Irwondong, Gangnamgu, Seoul, Korea; 2) Department of Epidemiology, the Graduate School of Public Health and Institute of Health and Environment, Seoul National University, 27 Yeongeon-dong, Chongro-gu, Seoul, Korea; 3) Department of Family Medicine, Busan Paik Hospital, Inje University, College of Medicine, 633-165, Kaegum-dong, Busan Jin-Gu, Busan, Korea.

Background: Mammographic density, a known risk factor of breast cancer, has been found to have a high heritability in White women. However, little was known about the mammographic density in Asian women. This study was conducted to determine the heritability of the components of mammographic density and to examine the genetic correlation between dense and nondense areas in Korean women. Methods: Study subjects were 730 women composed of twins and their first degree relatives, who came from the Healthy Twin study. Mammographic density was measured using a computer-assisted method. Interclass correlations of residual variance of each component of mammographic density were calculated between twin pairs, mother-daughter pairs, and sibling pairs. Quantitative genetic analysis was done using computer program the Sequential Oligogenic Linkage Analysis Routines (SOLAR) ver. 2.0 Results: Interclass correlation of age-adjusted residual variance of dense area, nondense area, and percent dense area (PDA) was highest between monozygotic pairs (0.78, 0.78, 0.73) and it was the second highest between dizygotic pairs (0.55, 0.47, 0.64), the third highest sibling pairs (0.31, 0.44, 0.34), and the lowest between mother-daughter pairs (0.02, 0.28, 0.15). When we further adjusted for age and other covariates, interclass correlation of residual variance attenuated only slightly. Age-adjusted heritability coefficients for dense area, nondense area, and PDA were 0.77, 0.74, and 0.71, and were slightly reduced to 0.75, 0.68, and 0.67 when age, age2, and other covariates were adjusted. There were significant inverse genetic correlations between dense area and nondense area and it reduced only slightly when covariates were fully adjusted. Conclusion: Given that mammographic density is associated with an increased risk of breast cancer, genes that have opposite effects on dense and nondense mammographic area and are responsible for familial genetic correlation in PMD need to be elucidated.

## 1894/T/Poster Board #443

**Genetic epidemiology of Montenegro skin test in a Western Amazonian Population.** *A.P. Fioretti[1,2], R.G.M Ferreira[1,2], C.E.M Kawamata[1,2], L.M. Garrido[1,2], A. La Luna[1,2], F.A.B. Santos[1,2], L.M.A. Camargo[1,2], H. Krieger[1,2].* 1) Parasitology, Universidade de São Paulo, São Paulo, São Paulo, Brazil; 2) INAGEMP - Instituto Nacional de Genética Médica Populacional.

Familial distribution of the response to an antigenic extract of Leishmania (Montenegro test) was analyzed by genetic epidemiologic methods. Montenegro skin tests were used to measure the human response to a Leishmania antigen. The present study tested a sample of 313 individuals from Monte Negro (10° 15’ S, 63° 18’ W), a Brazilian small rural county in Rondônia state located at the Amazonian region. The objective was to ascertain the relative importance of genetic components to establish the expression of the phenotype. Statistical analyses were performed using SPSS program and complex segregation analysis was made using POINTER program (Lalouel, J. M. et al., 1983, Am. J. Hum. Genet., 35: 816-826). Stepwise multiple regression suggests that age and gender affects significantly the Montenegro phenotype. Correlations between pairs within families suggested the existence of a significant familial aggregation with a strong genetic component since no significant correlation was found between parents. This familial aggregation was confirmed by complex segregation analysis, which showed that the best model to explain the phenotype transmission in the Monte Negro population was the recessive one, since its likelihood ratio is significantly lower than the sporadic model, while it was not different from the general mixed one (CAPES, CNPq).

## 1895/T/Poster Board #444

**A major gene controls leprosy susceptibility in a hyper-endemic isolated population from the Brazilian Amazon.** *M. Mira[1], F.P. Lazaro[1], R.I. Werneck[1], C.C.O. Mackert[1], F.C. Prevedello[1], R.P. Pimentel[1], G.M.M. Macedo[2], M.A. Eleutério[1], G. Vilar[1], L. Abel[3,4], M.B. Xavier[2], A. Alcais[3,4].* 1) Graduate Program in Health Sciences, Pontifical Catholic University of Paraná, Curitiba, Paraná, Brazil; 2) Tropical Medicine Core, Federal University of Pará, Belém, Brazil; 3) Laboratoire de Génétique Humaine des Maladies Infectieuses, Institut National de la Santé et de la Recherche Médicale, INSERM U550, Paris, France; 4) Université Paris René Descartes, Faculté Médecine Necker, Paris, France.

Leprosy is a chronic infectious disease caused by Mycobacterium leprae that affects 250.000 new individuals worldwide every year. Genetic analysis has been successfully applied to the identification of host genetic factors impacting on susceptibility to leprosy; however, a consensus regarding its mode of inheritance is yet to be achieved. We conducted a Complex Segregation Analysis (CSA) on leprosy using data from the Prata Colony, an isolated, highly endemic former leprosy located at the outskirts of Brazilian Amazon presenting large multiplex, multigenerational leprosy pedigrees. Our enrollment strategy was complete ascertainment leading to the inclusion of the whole colony, totalizing 2005 individuals (225 affected) distributed in 112 pedigrees. CSA was performed using REGRESS, which specified a regression relationship between the probability of being affected and a set of explanatory variables. CSA identified a best fit co-dominant model, with a major gene accounting for the entire familial effect observed. The frequency of predisposing allele was estimated at 0.22. Penetrance for homozygous individuals for the predisposing allele older than 30 years old ranged from 55% to 90%, depending of gender and race, with higher values for black males. Results suggest that the Prata population may be particularly suitable for leprosy gene identification studies.

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## 1896/T/Poster Board #445

**Evaluation of the IBS method in relationship analysis from short tandem repeat data.** *M. Osawa[1], R. Kimura[2], T. Tamura[3], M. Soda[1], F. Satoh[1], I. Hasegawa[1], I. Sato[3].* 1) Dept Forensic Medicine, Tokai Univ Sch Medicine, Kanagawa, Japan; 2) Trasdisciplinary Research Organization, Subtropical and Island Studies, University of the Ryukyus, Okinawa, Japan; 3) Forensic Biology Unit, Scientific Crime Laboratory, Kanagawa Prefectural Police, Kanagawa, Japan.

For paternity test and personal identification, multiplex analysis of short tandem repeat (STR) has been widely employed. Identifiler® system consists of 15 CODIS STR loci that exhibit extensive polymorphism with the heterozygosity of more than 0.7. In this study, relationships between pairs of Japanese individuals have been estimated by the method based on identical by state (IBS) upon the data of DNA profiling in our department. Probabilities of sharing 0, 1, and 2 IBS alleles (zi) were calculated for each locus on basis of allele frequencies reported for the Japanese; 0.11, 0.52 and 0.37 of z0, z1 and z2, respectively, in full sib pairs, for instance. Then, distribution of the number of loci having 0, 1, and 2 IBS alleles (li) in the set of 15 loci was accomplished by listing all possible combinations of IBS probabilities, zi, in the loci. The expected values of l1 and l2 (E(l1) and E(l2)) for parent-child pair were calculated to be 11.7 and 3.3, respectively. E(l0), E(l1), and E(l2) were 1.6, 7.7 and 5.7 for full sib pair, 3.2, 9.6 and 2.2 for 2nd degree relatives, 6.1, 7.5 and 1.3 for non-related pair, respectively. In DNA profiles of real identification cases, the distribution of li was consistent with the theoretical one. Since the distributions between different categories of relationship considerably overlapped, the total number of 15 loci seemed to be too small to judge biological relationship between arbitrary pairs. Even in testing sib relationship, the total number of 15 loci is clearly small because no significant relationship can be obtained in approximately 25%. The exact method generally displays higher likelihood ratio than the IBS method. However, the merits of the IBS method are its conciseness in calculation and the ability to exclude incidental effects of rare alleles, of which involvement is inevitable in examination to more number of STR loci. Therefore, the IBS method, being alternative to the exact method, would be useful in applied genetics and forensic investigation.

## 1897/T/Poster Board #446

**Statistical methods to detect viral integration site hotspots.** *A.P. Presson[1,2], S. Kim[3], Y. Xiaofei[4], I.S.Y. Chen[3].* 1) Dept Biostatistics, Univ California, Los Angeles, Los Angeles, CA; 2) Dept Pediatrics, Univ California, Los Angeles, Los Angeles, CA; 3) Dept Microbiology, Immunology and Molecular Genetics, Univ California, Los Angeles, Los Angeles, CA; 4) Dept Statistics, Univ California, Los Angeles, Los Angeles, CA.

Modern gene therapy methods suffer from insufficient control over where a therapeutic viral vector inserts into the host genome. Viral integration patterns vary by vector type and often prefer regions containing genes, so that the probability of an integration event varies by location. Since integration can activate local gene expression, it is important to characterize insertion patterns of potential gene therapy vectors. Currently, viral integration hotspots are defined by a minimum density of events (2-4 within a 36-104kb region). While this guideline may be useful for some data sets, it relies on the number of observed integration sites. This is problematic for comparing hotspots among different vectors or collections of experiments where the number of observed integration sites can vary substantially. Furthermore, this definition targets small genomic regions. As the definition of viral integration hotspots is essential to understanding their mechanism and safety, an accurate and more general definition is warranted. We propose a Bayesian change point (bcp) framework to estimate integration hotspots from consecutive changes in integration rate along a chromosome. We identify change points using both a) a traditional bcp model and b) a modified version that samples the number of change points as a proposal in the Markov chain. We test these models on 1) our data from rhesus macaque animals transplanted with hematopoietic stem cells containing lentivirus vectors (LV), 2) published LV data, 3) published retrovirus data and 4) control data from pre-transplant stem cells containing LV. Preliminary results identified 35 hotspots in our LV transplant data (1). While the LV control data (4) contained four times the number of integration sites observed in (1), the bcp method identified only two additional hotspots. In comparison, a definition of ≥2 insertions within a 100kb region identified 43 hotspots in (1) and 150 in (4). The published transplant data sets (2-3) also suggest preferential integration. The average hotspot size in our LV data was about 850kb. Bayesian change point models effectively distinguish true hotspots from random integration events. Statistical models that can reliably define hotspots will allow us to compare integration preferences among different vector types and assess their safety and efficacy for gene therapy trials.

## 1898/T/Poster Board #447

**Sensitive and accurate detection of copy number variants using depth of coverage.** *S. Yoon[1], Z. Xuan[1], K. Ye[2], J. Sebat[1].* 1) Cold Sprg Harbor Lab, Cold Sprg Harbor, NY; 2) Albert Einstein College of Medicine, Bronx, NY.

Methods for the direct detection of copy number variation (CNV) genome-wide have become effective instruments for identifying genetic risk factors for disease. The application of next generation sequencing platforms to studies of disease promises to improve sensitivity to detect CNVs as well as inversions, indels and SNPs. New computational approaches are needed to detect these variants. Existing approaches for CNV detection are primarily based on paired-end read mapping (PEM). Due to limitations of this approach, some classes of CNVs are difficult to ascertain, including large insertions and variants located within complex genomic regions. To overcome these limitations, we developed a method based on analyzing depth of coverage. Event-wise testing (EWT) is a method based on significance testing. In contrast to standard segmentation algorithms which typically operate by performing likelihood estimation for every point in the genome, EWT works on intervals of probes, rapidly searching for specific classes of events. Overall false-positive rate is controlled by testing the significance of each possible event and adjusting for multiple testing. Deletions and duplications detected in an individual genome by EWT are examined across multiple genomes to identify polymorphism between individuals. We estimated error rates using simulations based on real data, and we applied EWT to the analysis of chromosome 1 from paired-end shotgun sequence data (30X) on three individuals obtained by participation in the 1000 genomes project (www.1000genomes.org). Our results suggest that analysis of read depth is an effective approach for the detection of CNVs, and it captures structural variants that are refractory to established PEM-based methods.

## 1899/T/Poster Board #448

**Automated High Resolution HLA Typing of Class I & II Alleles Using the SBT Method.** *W. Dong[1], C. Carozza[1], E. Pimpinella[1], J. Barone[1], S. Hsu[1], N. Flaherty[2], L. Tack[2].* 1) Histocompat/Molecular Genetics, American Red Cross Penn-Jersey Region, Philadelphia , PA; 2) PerkinElmer, Inc, 2200 Warrenville Rd, Downers Grove, IL.

Human Leukocyte Antigen (HLA) matching is a critical element for solid organ or bone marrow transplantation success. The HLA Lab of the PJ Region of the American Red Cross provides allelic level HLA typing supporting bone marrow transplantation to hospitals and also carries out DNA-based typing of unrelated donors and cord blood units as a contract lab for the National Marrow Donor Program. We screen for HLA polymorphisms using molecular methods such as Sequence Specific Oligonucleotide Probing (SSOP) and Sequencing-based Typing (SBT) methods. Our strategy is to use the SSOP method as an intermediate resolution testing process along with SBT for higher resolution typing. High resolution typing using the more informative “Gold Standard” SBT method is common for successful bone marrow and stem cell transplantation. SBT also allows the detection and identification of new alleles, not possible with most other techniques. The AlleleSEQR SBT kits from Celera are used for testing both Class I (HLA-A, B, C) and Class II (HLA-DRB1, DQB1 and DPB1) loci and requires setting up hundreds of PCR reactions, making this procedure an ideal candidate for automation. Our lab processes more than 1000 samples/week for HLA typing. To meet the turn around time of 3 to 9 days for clinical purposes, we used a JANUS Liquid Handling Workstation from PerkinElmer to automate the SBT typing process. The JANUS workstation sets up (1) primary PCR amplification plates, and (2) SBT cycle sequencing reactions using forward and reverse sequencing primers to detect polymorphic sequences in both Class I and Class II regions. Amplified dye-terminator labeled reaction products are analyzed using an ABI Prism 3730XL DNA Analyzer. Sequence files are imported into the Assign-SBT data analysis software and electropherograms aligned. Data for each sample template analyzed is compared to known alleles in the library for identification. Automation, by increasing SBT throughput, shortens turnaround time, reduces the potential for manual procedural errors, and maintains consistency between runs.

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## 1900/T/Poster Board #449

**Systematic identification of loci controlling gene co-regulation.** *K.S. Kompass, J.S. Witte.* Department of Epidemiology and Biostatistics, University of California-San Francisco, San Francisco, CA, USA.

In normal tissues, genes in *trans* that participate in similar biological processes, such as encoding components of the same pathway or a larger protein complex, are often co-regulated in clusters. When gene clusters are transcriptionally regulated, much of this organization is determined by *cis* acting sequences, as genes with common regulatory elements are more likely to be bound by the same transcription factors. Experimental work has shown that polymorphic sequence changes in the *cis*-regulatory elements of individual genes can greatly alter the affinity of coordinate transcription factor binding and lead to aberrant transcription, and regulatory changes in upstream transcription factors that bind regulatory elements of many target genes are key components in the development of certain cancers (Pittman et al., Genome Research 2009). We performed a preliminary study to systematically identify loci that interact with multiple promoters in *trans* using publicly available data (NCBI GEO GSE14860; Salvesen et al., PNAS 2009). To this end, we modified an existing rank-based gene biclustering algorithm (Owen et al., Genome Research 2003) to generate high-quality, stable clusters of genes by recursively minimizing the algorithm's cross-validation error. Using this method, we generated 875 unique clusters of genes from 33 human samples from GSE14860 and tested their association with genotype using Affymetrix 100k SNP arrays. Approximately 25 *trans* loci were identified that significantly interact ($p<10^{-7}$) with clusters of co-regulated genes. The most significant interaction identified was between a region at 8q.22.1 containing PGCP and a cluster of genes containing CFL1, MYH9, SRM, and GNB2. Another cluster containing HLA-DRB1, CUX1, HLA-DPB1, HLA-DRB5, MTMR14, and HLA-DQB1 interacted significantly with a region at 9q33.1 containing PAPPA. Further studies will incorporate other publicly available data to systematically identify *trans* loci that interact with distant regulatory elements to exert broad regulatory control over the genome. These studies should allow for improved genetic identification of pathways, improved functional predictions for unstudied transcripts, and improved detection of regulatory aberrations in disease.

## 1901/T/Poster Board #450

**A comprehensive resequence analysis of the KLK3 gene region on chromosome 19q13.33.** *H. Parikh[1], Z. Deng[1,2], J. Boland[1,2], C. Mathews[1,2], I. Collins[1], J. Jia[1], A. White[1], L.A. Burdett[1,2], A. Hutchinson[1,2], M. Yeager[1,2], J. Ahn[1], S. Berndt[1], L. Qi[1,2], R. Hoover[1], G. Thomas[1], D. Hunter[3], R. Hayes[1,4], S. Chanock[1,2], L. Amundadottir[1].* 1) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; 2) Core Genotyping Facility, Advanced Technology Program, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD 21702; 3) Program in Molecular and Genetic Epidemiology, Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115, USA; 4) Division of Epidemiology, Department of Environmental Medicine, New York University School of Medicine, New York, NY 10016.

Prostate cancer is the most commonly diagnosed non cutaneous cancer in men in the U.S. The PSA test, a widely used test for prostate cancer screening, is based on measuring prostate specific antigen (PSA) levels in serum as they are often increased in men with prostate cancer. However, elevated levels of PSA can also be caused by nonmalignant conditions such as BPH and prostatitis. Although the PSA test has led to the diagnosis of earlier stage prostate cancers it has serious limitations because of low sensitivity and specificity. A recent genome wide association study (GWAS) of prostate cancer described single nucleotide polymorphisms (SNPs) in the vicinity of the KLK3 gene that associate with an increased risk of prostate cancer. The SNP most significantly associated with prostate cancer risk is rs2735839 (P value $1.5x10^{-18}$). In contrast, our analysis of tag SNPs from the KLK3 gene region in subjects from the Prostate, Lung, Colon and Ovarian (PLCO) Cancer Screening Trial indicated that the variants associate with altered PSA levels but not with prostate cancer risk unless control subjects are restricted to those with very low PSA levels. As a first step in resolving the question whether genetic variation in the KLK locus associates with prostate cancer, PSA levels or both, we set out to thoroughly characterize common genetic variation in the region. We conducted a next-generation sequence analysis in 78 individuals of European background to characterize common (minor allele frequency over 1%) genetic variation in a 56 kb region on Chr19q13.33 (Chr19:56,019,829-56,076,043 bp). This region is centered on the KLK3 gene but also includes the KLK2 and KLK15 genes. Sequence depth was on average about 70 fold. Four HapMap families (CEU and YRI) were included for quality assessment of genotypes (99.8%; concordance). We identified 555 polymorphic loci including 298 novel SNP and indel polymorphisms not in db-SNP. Twenty variants are predicted to alter exons or 5'/3'UTR sequences of the KLK3 gene including five non-synonymous amino acid changes. In the neighboring genes KLK15 and KLK2, there were 7 and 15 coding SNPs, respectively. We have also begun to correlate SNPs in region with expression levels of KLK2, KLK3 and KLK15 in the NCI60 cell line dataset. The detailed map of genetic variation in the region will aid in selecting tag SNPs for fine mapping the association signal and determine its relevance to prostate cancer risk and regulation of PSA expression.

## 1902/T/Poster Board #451

**A method to analyze extreme quantitative trait data attributable to rare variants: application to the analysis of next generation sequence data.** *D.W. Covarrubias[1,2], B. Li[1], S.M. Leal[1,2].* 1) Dept. Molecular and Human Genetics, Baylor College Medicine, Houston, TX; 2) Dept. Statistics, Rice University, Houston, TX.

It has been demonstrated that rare variants are involved in etiology of quantitative traits. It is now possible to identify rare variants which influence quantitative trait loci (QTL) using next generation sequencing. For the analysis of QTL, one strategy which is often used is to only analyze individuals with extreme quantitative trait values (QTVs). Although methods which are used to detect associations with common variants that influence QTL can be used to analyze rare variants, they are underpowered. We extended the combined multivariate collapsing (CMC) method to analyze quantitative traits; the CMC method was originally developed to test for associations with rare variants in case-control data. The quantitative CMC (QCMC) method can be used to analyze data for which either all individuals regardless of their QTVs are sequenced or only individuals with extreme QTVs are sequenced. In order to evaluate the power and robustness of the QCMC method, sequence data was simulated via coalescence theory using parameters estimated from population genetic data and the QTV distributions were based-upon clinically relevant quantitative trait data. It was shown in most situations that there was only a slight gain in power when the complete sample was sequenced and analyzed, compared to when only those individuals with extreme QTVs in the upper and lower 25% were analyzed. When only individuals with extreme QTVs are sequenced and analyzed it is advantageous to analyze their QTVs instead of dichotomizing and implementing the CMC method, since power loss can be substantial. The QCMC is robust to misclassification (e.g. inclusion of non-causal variants and exclusion of causal variants). In conclusion, implementing extreme QTV sampling is a beneficial strategy to reduce sequencing cost and the power of the study design can be greatly increased by analyzing full QTVs. The QCMC method can easily be implemented to analyze rare variant quantitative trait data obtained from candidate genes or whole exome sequencing.

## 1903/T/Poster Board #452

**A novel adaptive method for the analysis of next generation sequencing data to detect complex trait associations with rare variants due to gene main effects and interactions.** *S.M. Leal[1,2], D.J. Liu[1,2].* 1) Dept. Molecular and Human Genetic, Baylor Col Med, Houston, TX; 2) Dept Statistics, Rice University, Houston, TX.

Increasing evidence suggests complex traits may not only be due to common variants but also be due to rare variants which have stronger phenotypic effects than variants with higher frequencies. Next generation sequencing technologies make it possible to carry out direct association studies of rare variants for complex traits. Although statistical methods developed for the analysis of rare variant sequence data have been shown to be more powerful than applying methods commonly used to analyze common variants; analysis of rare variants is still problematic, especially in the presence of variant misclassification (i.e. inclusion of non-causal variants and exclusion of causal variants) and unknown patterns of gene interactions. Working in a novel framework to address the problems of rare variant analysis, the Kernel Based Adaptive Clustering (KBAC) method was developed. The KBAC combines variant classification and association testing in a coherent framework. This method not only exhibits robustness against variant misclassification errors, but also allows for flexible disease models. In order to evaluate the power and robustness of the KBAC method, sequence data was simulated via coalescence theory using parameters estimated from population genetic data and realistic disease models with and without gene interactions were generated based-upon Hirschsprung's Disease and Breast Cancer. It was demonstrated especially in the presence of variant misclassifications and gene interactions that the KBAC method has superior power and robustness over other methods, such as combined multivariate and collapsing, Hotelling T^2 and minimum p-value.

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## 1904/T/Poster Board #453

**Fine mapping of common and rare variants associated with low-density lipoprotein cholesterol (LDL-C) via sequencing candidate loci following genome-wide scans.** *B. Li[1], Y. Li[1], S. Sanna[2], D. Schlessinger[2], S. Najjar[2], A. Scuteri[2], E. Lakkata[2], M. Boehnke[1], G. Abecasis[1], M. Uda for Sardinian Project[2].* 1) Center for Statistical Genetics, Department of Biostatistics, University of MIchigan, Ann Arbor, MI; 2) Istituto di Neurogenetica e Neurofarmacologia (INN), Consiglio Nazionale delle Ricerche, c/o Cittadella Universitaria di Monserrato, Monserrato, Cagliari 09042, Italy.

Coronary artery disease is one of leading causes of morbidity and mortality in developed countries and strong associations have been established between lipoprotein levels and coronary heart disease. High levels of low-density lipoprotein cholesterol (LDL-C) and genotypes increased with high LDL-C levels are associated with increased risk of coronary heart disease. Our previous studies of >8,000 individuals through genome-wide association scans identified a number of loci associated with LDL-C levels, including previously reported and also newly implicated loci. To further understand the genetic contributions of both common and rare variants to the LDL-C level, we sequenced exons of 9 genes in associated loci in 256 unrelated Sardinian individuals with either extremely low or high LDL-C levels, along with 120 HapMap samples. Among all variants identified, 71% (81/121) nonsynonymous and 56.3% (40/71) synonymous mutations have frequency below 1%. In addition, two frame shift (in *APOB*) and two truncation mutations (in *PCSK9*) were identified. Comparisons between high LDL-C and low LDL-C groups showed that rare coding variants are enriched in one of the two groups for a set of genes (*APOB*, *LDLR*, *PCSK9*, *SORT1*). To increase power of detecting associations of variants in coding regions with LDL-C levels, we are using imputation to extend our findings to additional genotyped individuals in our 6148 sample Sardinian cohort. Equipped with this larger amount of data after imputation, fine mapping and evaluation of potential functional variants should be achieved with greater power.

## 1905/T/Poster Board #454

**Difference in Estimators of Population Parameter between Data I and Data II in 1000 Genomes Project Pilot 1.** *Z. Hou[1,2], H. Siu[1,2], P. Hu[1,2], F. Yu[4], Y. Fu[3], L. Jin[1,2], M. Xiong[1,3].* 1) Laboratory of Theoretical Systems Biology, School of Life Science, Fudan University, Shanghai, 200433, China; 2) State Key Laboratory of Genetic Engineering and MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai, 200433, China; 3) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77225; 4) Human Genome Sequencing Center, Molecular and Human Genetics Department, Baylor College of Medicine, Houston, TX 77030.

The 1000 genomes project provides the high density sequencing data in pilot1, and the data release in two versions (Data I with 210 chromosomes in Feb. and Data II with 344 chromosomes in Apr.). Number of segregation sites in Data II is increased over twice than that in Data I. We contrast the frequencies of derived alleles with the theoretic model, and obtain the mean errors between the observed allele frequencies and theoretic allele frequencies in three populations (Data I: ASI.I{23.2%}, CEU.I{16.9%} and YRI.I{13.4%}; Data II: ASI.II{11.1%}, CEU.II{14.3%} and YRI.II{9.7%}). It is clear that the estimated sequencing error in data I is large than that in Data II. We calculate several common statistics of $\theta$-estimators in a sliding window set (100kb), and obtain the average population parameter as shown in Table 1. We can see the $\theta$-estimators in Data II are greater than those in Data I. We also observe the differences between Tajima's estimator and Watterson's estimator are relatively small. This demonstrates that sequence error rates are not large. Using Fu and Li's method and ignoring the mostly sequencing errors which are from singletons, we can reduce the differences in both mean values and variances among the estimators of $\theta(\pi\text{-}\xi)$, $\theta(S\text{-}\xi)$, $\theta(\pi\text{-}\eta)$ and $\theta(S\text{-}\eta)$. Furthermore, we observe that $\theta(\pi\text{-}\xi)$ is same as $\theta(\pi\text{-}\eta)$ and $\theta(S\text{-}\xi)$ is same as $\theta(S\text{-}\eta)$ in both datasets. This shows that impact of sequencing errors in high-frequency mutation on $\theta$-estimators is small. If the errors of the estimates of population parameter are mostly from singleton, Fu and Li's correction is an efficient method to estimate the population parameter $\theta$. Changing the width of the sliding window from 100 kb to 10kb we obtain the similar results.

## 1906/T/Poster Board #455

**Functional Principal Component Analysis for Testing Association of Full Spectrum of DNA Variation.** *L. Luo, E. Boerwinkle, M. Xiong.* Human Gen Ctr, Univ Texas, Houston, Houston, TX.

Although great progress in GWAS has been made, the identified significant SNP associations account for only a few percent of the genetic variation. More disease risk variants remain to be identified. Next-generation DNA sequencing technologies will detect millions of rare variants. Most traditional statistical methods are difficult to be applied to rare variants due to the low frequency and high genotyping error. Functional Data Analysis (FDA) techniques are ideally suited for association analysis of next-generation sequencing data. A genotype profile of each individual can be viewed as a realization of the Poisson process and hence as a function of genomic location. They use data reduction techniques to compress the signal into a few components by Functional Principal component analysis (FPCA), which in turn can reduce both false positive and negatives. FPCA can also decorrelate dependent functional data, where many traditional statistics for independent variables can be easily applied. We develop a novel FPCA-based statistic for testing association of full spectrum of genetic variants within a gene ranging from rare variants to common SNPs. Its type 1 error rates were not appreciably different from the nominal levels by simulation studies. The proposed statistic was applied to association studies of Psoriasis. The best SNP P-values, adjusted for multiple testing, of two MHC class genes MICA and HLA-DMA are 3.2E-6 and 1.6E-4, respectively. Their P-values by gene-based linear combination test are 3.4E-4 and 1.9E-4, respectively. While their P-values by new statistic are respectively,1.4E-7 and 2E-7. We also applied the FPCA approach to the next-generation sequencing data of gene ANGPTL4 in the Dallas Heart Study where we compared the number of sequence variants in the top and bottom quartiles of the distribution to examine the genetic effects of sequence variants. After excluding variants with MAF greater than 0.03, FPCA approach declared the significant association of gene ANGPTL4 with three phenotypes: BMI (P-value: 6.03E-05), Triglycerides (P-value: 0.0035) and VLDL (P-value: 0.0148). However, Fisher exact test declared no significant association of gene ANGPTL4 (BMI, 0.21, Triglycerides,0.87, and VLDL,0.94). These examples demonstrate that the FDA-based statistic is powerful for testing association of entire spectrum of genetic variants. FDA is expected to emerge as a major analytic tool for association studies of next-generation sequencing data.

## 1907/T/Poster Board #456

**Assessing the utility of imputation for fine-mapping in association studies via simulations.** *S.R. McGee[1], D.A. Nickerson[1], C.S. Carlson[2,3], T. Bhangale[1,4].* 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Department of Epidemiology, University of Washington, Seattle, WA; 3) Division of Public Health Prevention, Fred Hutchinson Cancer Research Center, Seattle, WA; 4) Department of Biostatistics, University of Washington, Seattle, WA.

Hundreds of associations for common human diseases/traits have been established via genome-wide association studies (GWAS). This is rapidly moving variation analysis towards the discovery of the underlying causal variant(s) to provide a greater understanding of human phenotypic variation. Imputation has been widely applied in association analysis but could be of great utility in fine mapping causal variation as well. The advent of the Thousand Genomes Project (TGP) is expected to provide considerable new information for alleles with lower minor allele frequencies (MAF < 10%) and greater insights into the patterns of linkage disequilibrium (LD) within and among human populations. In this study, we used simulations to characterize the extent to which imputation from the HapMap and TGP data will aid in uncovering the causal variation in human association studies. To explore causal variant mapping we simulated sequence variation among samples using a neutral model for two major founder populations (African-descent and European-descent). We then trimmed the simulated variation data to match the densities of the Phase II HapMap and TGP as well as commercially available genotyping arrays. Causal variants were picked to cover a wide range of MAFs and effect sizes. The locations of significantly associated variants were then detected using 1) genome-wide arrays, 2) after imputation with simulated HapMap, and 3) TGP data. We then assessed the probability of discovering the true causal variant within a distance-range defined by a set of the most significantly imputed variants. For example, TGP-based imputation can provide a three-fold increased probability of locating the causal variant (with MAF < 5%) within a 20 kb window defined by the most significantly associated variants, compared to HapMap-based imputation. Furthermore, when simulating an African population, imputation with TGP-based data identified the causal variant as the most significantly associated variant approximately 20% of the time. Our approach can be used to set confidence limits on the search windows for follow-up resequencing to identify causal variations in human association studies.

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## 1908/T/Poster Board #457

**Clustering binary sequences and inferring haplotype sequences from genotypes using modal expectation-maximization (MEM).** *X. Mao[1], B. Lindsay[1], S. Chen[2].* 1) Dept Statistics, Penn State Univ, State College, PA; 2) School of Math. and Stat. Sci., Arizona State University, Tempe, AZ.

DNA sequences are widely used in biomedical research and other fields. Many methods are developed to study the variation within sequences, to infer haplotype from genotypes, to quantify the differences between sequences, to cluster sequences and further to infer the cause of the physiological changes. The proposed method is a modal expectation-maximization (MEM) method for clustering DNA haplotype sequences. First we define a kernel density estimator for the sequences and use it to define a weight function for each sequence. Then we start from each data sequence and examine all other sequences along with the weight function to find the nearest mode of the density. We may cluster the sequences that share the same mode. We can create a hierarchical clustering of all the sequences by adjusting the tuning parameters in the kernel density function. We generalize this method to create a new tool for the haplotype inference problem. We define a density estimator for the genotype sequences and find the objective function that needs to be maximized. By adjusting the tuning parameters, one may also obtain modes for the sequences as the ancestor (as in haplotype) for the genotypes or obtain the haplotype configuration for the genotype sequences. We only present the method for inferring the haplotypes. Both methods are tested on large datasets with the comparison to the available methods such as Phase, fastPhase and others . It shows that the methods yield similar results as they require less or equal amount of computational time and they provide some advantages of statistical inference.

## 1909/T/Poster Board #458

**DNA Sequence-based Analysis Via Regression Modeling that Leverages Genomic Annotations.** *O. Libiger, A. Torkamani, N.J. Schork.* The Scripps Translational Science Inst, Scripps Health and The Scripps Research Inst, La Jolla, CA.

Recent whole genome association studies (GWAS) yielded unequivocal statistical associations between a number of common single nucleotide polymorphisms (SNPs) and a variety of diseases. However, each of these SNPs only accounts for a very small proportion of the incidence of the associated disease. Possible explanations are that structural variants or rare single nucleotide variants not captured by the variation assayed in the GWAS are important genetic determinants of complex diseases. It could also be the case that many loci contribute to the manifestation of a disease either in isolation or through epistatic interactions. We devised a novel approach to assessing associations between groups of variants and disease based on high-dimensional regularized regression models. Our approach can accommodate the analysis of the full range of genomic variations identified by DNA sequencing assays, and addresses locus heterogeneity and interaction effects by leveraging genomic annotations for collections of variations. We have assessed the utility of the method through analytical and simulation studies and showcase its application in a number of empirical studies including studies of complete mtDNA sequences obtained on individuals with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) as well as two genes sequenced in their entirety for a study of obesity.

## 1910/T/Poster Board #459

**Bayesian network model for SNP calling in shallow sequencing with haplotype based prior.** *D. Zhi, N. Liu.* Section on Statistical Genetics, Dept Biostatistics, Univ Alabama, Birmingham, Birmingham, AL.

Traditionally, deep sequencing (e.g., >30X for solexa short-reads) of individuals' genome is conducted to ensure high quality ascertainment of genetic variations. Alternatively, shallow (low-coverage) sequencing approach allows for affordable survey of genetic variation with large samples, even the genotype of each individual may not be accurately ascertained. This approach is adopted by the 1000 Genomes Project (1KG, http://www.1000genomes.org/page.php) to sequence 1200 individuals at 4X coverage which promises to deliver the most complete catalogue of single-nucleotide polymorphisms (SNPs) with minor allele frequency >1%. While the large sample size in 1KG warrants accuracy of SNP calling, leveraging haplotype information would improve SNP calls further. Moreover, phased sequences for 1KG individuals would be invaluable for genetic research, such as for untyped marker imputation. Recently, hidden Markov models (HMMs) are used to efficiently describe individuals as mosaic of reference or ancestral haplotypes. However, current HMM-based approaches do not consider information about mate pairs, which carry essential clues for haplotyping. In this study we set forth to develop a Bayesian network model which generalizes existing HMM models to include mate pair information and sequencing error models. Our preliminary results indicate that our model significantly improves upon current HMM-based approaches, especially when sequencing coverage is relatively low.

## 1911/T/Poster Board #460

**SERPING1 Mutation rs2511989 is Variably Associated with Macular Degeneration and Not Associated with Severity of Disease.** *C. Ramsden[1,4], M. Cooper[2], A. Tan[2,3,4], L. McDougall[4], B. Longville[2,3,4], J. Xiao[2,3,4], D. Mackey[4], I. Constable[4], L. Palmer[2].* 1) Department of Ophthalmology, Sir Charles Gairdner Hospital, Nedlands, WA 6009, Australia; 2) Centre for Genetic Epidemiology and Biostatistics, University of Western Australia, Nedlands, WA 6009, Australia; 3) Western Australian Institute for Medical Research, Centre for Medical Research, University of Western Australia, Nedlands, WA 6009, Australia; 4) Lions Eye Institute, Centre for Ophthalmology and Visual Science, University of Western Australia, Nedlands, WA 6009, Australia.

Background: Age Related Macular Degeneration (AMD) is the most common cause of blindness in developed countries occurring in over 10% of individuals aged over 60. There is a spectrum of disease, from early to late, late being further divided into geographic atrophy and choroidal neovascularisation. A plethora of robust research has implicated the alternative and classical complement pathways of innate immunity in causing AMD. C1 esterase inhibitor, coded by the SERPING1 gene, an inhibitor of the classical pathway has been variably reported to be associated with AMD. We intended to test this association and that of SERPING1 with the severity of disease. Methods: A single nucleotide polymorphism rs2511989 was genotyped in 1013 individuals from a cohort of patients with recorded AMD from the Western Australian Macular Degeneration Study (WAMDS) and analysed for disease association against 4 control populations previously described in the UK and US using R. Further analysis was conducted comparing early disease to late. Multivariate analysis considered age, sex, smoking, hypertension, dyslipidaemia and the use of alcohol, non-steroidal anti inflammatory drugs and antioxidants. Results: Of the 1013 patients 74% had late AMD. SERPING1 minor allele genotype of rs2511989 AA occurred at a frequency of 18.2%, GA at 45.6%, GG at 36.2%. Genotyping was successful in 97% of samples. In comparison to four established control groups, taken from previously published work by other groups, rs2511989 was variably associated with AMD. A GG genotype association with AMD was only shown with one UK control population ($p = 0.003$) and not in three US control population ($p = 0.36$, 0.30 and 0.66). This relationship did not alter when late AMD was compared to the control populations. Within the WAMDS population, rs2511989 genotype variation was not associated with late disease in comparison to early ($p=0.61$). There was no association with any genotype and visual acuity, retinal detachment, haemorrhage, lesion size or lesion characteristic in the neovascular subgroup. Conclusion: Our results suggest that SERPING1 mutation rs2511989 is not associated with severity in macular degeneration, but is variably associated with the occurrence of disease when compared to previously described control populations.

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## 1912/T/Poster Board #461

**A Scalable Information Content-Based Approach for Genomewide-Association Studies.** *S. Bolte[1,3], E. Luczkowski[2], P. Jailwala[2], M. Serafin[2], K. Stamm[3], T. Wang[4], S. Twigger[5], S. Ghosh[2], C. Struble[3].* 1) GE Healthcare, Wauwatosa, WI; 2) The Max McGee National Research Center for Juvenile Diabetes and The Human and Molecular Genetics Center, Department of Pediatrics at the Medical College of Wisconsin and the Children's Research Institute of the Children's Hospital of Wisconsin, Milwaukee; 3) Department of Mathematics, Statistics & Computer Science, Marquette University, Milwaukee, WI; 4) Division of Biostatistics, Department of Population Health, Medical College of Wisconsin, Milwaukee, WI; 5) Department of Physiology, Medical College of Wisconsin, Milwaukee, WI.

Complex, common diseases such as Type 1 Diabetes, inflammatory bowel disease, coronary artery disease, depression and allergic disorders have strong genetic components. Understanding the etiology of complex diseases will help develop new strategies for prevention and cure. Recent genome-wide association (GWA) studies for complex diseases using single nucleotide polymorphisms(SNPs) have yielded important pathogenesis regions. To date, these studies have mostly employed single-marker analytical tools that do not fully exploit the information available from multiple markers. An approach to multiple marker analysis is information content (IC), a measure of variability, of SNP typing data. Instead of several haplotype frequencies to compare between cases and control subjects, we gain power by incorporating the underlying haplotype information into one IC score, allowing for a single statistical test to be performed. Chromosomal intervals differing in IC between cases and control subjects potentially harbor susceptibility genes. Nevertheless, multiple marker analysis increases the computational complexity beyond the capabilities of single desktop workstations. In this study, we present our IC-based framework as a means for identifying disease genes. The flexible framework allows for a variety of GWA studies. Adjustable parameters such as window size & structure of genomic regions for IC calculations, bootstrap sample size, and number of cases & controls balance statistical power and computational time. No assumptions are made about the location of potential associated variations, analyzing available SNP data for non-coding and coding regions. Furthermore, our framework does not depend on identifying haplotype blocks or tag SNPs as a preprocessing step. Our framework is implemented using the previously developed tagsnps program of Nicolas et al and Condor, middleware for high-throughput computation, from the University of Wisconsin--Madison. Early results analyzing the human MHC indicate the framework is scalable on our modest sized computational resources, a 42-core Macintosh grid. An analysis of 971 cases and 937 controls completed in under 10 minutes representing an over 10-fold speedup in computational time compared to a single machine. To understand the impact of framework parameters, power studies are presented for two simulated data sets. We conclude by applying the framework to two real-world data sets.

## 1913/T/Poster Board #462

**Extreme Point Methods in Calculating Power Function of Any Test of HWE in Multi-Allelic Markers.** *R. Chakraborty, M. Rao, S. Venkatesan.* Ctr Genome Infor/Env Hlth, 108, Univ Cincinnati, Cincinnati, OH.

In the 58th ASHG Annual Meeting, a poster was presented outlining how the power function of any test of the Hardy-Weinberg Equilibrium (HWE) can be calculated explicitly in the case of bi-allelic markers using extreme point methods (Abstract 2351, 2008). The goal of this presentation is to extend the methodology to multi-allelic markers. For simplicity, let us assume that we have a tri-allelic marker with alleles $A_1$, $A_2$, and $A_3$ with population frequencies $p_1$, $p_2$, and $p_3$, respectively. Let the distribution of the genotypes $A_1A_1$, $A_1A_2$, $A_1A_3$, $A_2A_2$, $A_2A_3$, and $A_3A_3$ be cast in the form of a Wright's model (1977) with inbreeding coefficient '$\theta$.' Testing HWE is equivalent to testing '$\theta$' equal to zero. Let T be any test of HWE built on data collected on the genotypes. Using extreme point methods, we give an explicit formula for the power function of T. The underlying convex set is the set of all genotype distributions with '$\theta$' ranging over the permissible values of '$\theta$.' This convex set has only two extreme points. The power function formula is built on these extreme points. Some illustrations of the computations are provided.

## 1914/T/Poster Board #463

**Hypothesis Mining: A web based query tool utilizing relational databases to mine GWAS results.** *Q.D. Gibson, P.F. McArdle.* Univ Maryland Sch Medicine, Baltimore, MD.

Since ambiguous temporality is not an issue in genetic epidemiology, cross sectional study designs are a cost effective approach. Once a GWAS design has been selected, genotype costs remain fixed and thus overall efficiency increase as the number of phenotypes in the study increases. Assuming association analyses have been conducted for a genome wide set of markers and multiple phenotypes, we propose a data mining application to help distinguish true positive associations from false positive ones. Our application relies on storage of association results in a relational database. The application allows the researcher to input a hypothesis through a user interface that then queries the database to identify data that is consistent with the hypotheses. This approach allows researchers to identify markers consistent with a biological pathway, even though the individual association results were not among the top signals or reach "genome wide significance". The pathway mining application allows for greater utility of the data thereby provide added value to the cost of each study.

## 1915/T/Poster Board #464

**An immersive 3-D visualization environment for exploring high-dimensional genetic analysis results.** *D. Hill, R. Cowper, J.H. Moore.* Department of Genetics, Dartmouth College, Lebanon, NH.

This is an exciting time in biomedical research due to the availability of technology that allows us to measure tremendous amounts of information about genes, proteins and other biomolecules that play an important role in the molecular pathology of disease. However, it is also a challenging time due to the bioinformatics needs associated with storing, managing, analyzing and interpreting 'omics' data. While we have made great progress in developing the databases and data analysis tools for measuring statistical relationships in high-dimensional datasets, the bioinformatics methods for knowledge discovery in the large volumes of statistical results generated from 'omics' analyses are in their infancy. To address this interpretation challenge, we have developed an innovative three-dimensional (3-D) visualization approach to the exploration of statistical analysis results from genome-wide association studies (GWAS) that capitalizes on the power of human visual perception and our evolved ability to recognize complex patterns. The overall goal of this study is to replace the traditional approach of sifting through thousands of rows of p-values in an Excel spreadsheet with an innovative and unconventional visual approach that presents the results in an interactive 3-D graphical format that makes important local and global patterns much easier to identify by the user. To implement this we have harnessed the power of cutting-edge computer graphics technology in the form of fast Graphical Processing Unit (GPU) video card hardware and sophisticated 3-D video game engine software (Unity3D) that is designed for real time rendering of 3-D visual environments and animation. Here, we map multiple different analytical results for each gene onto a 3-D structure such a tree or a tropical fish, for example. This allows the user to visually explore forested landscapes or schools of tropical fish for visually interesting patterns that reflect important multivariate information that would otherwise not be revealed in an Excel spreadsheet. This immersive and interactive 3-D virtual environment puts the user in a visually appealing environment that enhances scientific discovery.

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## 1916/T/Poster Board #465

**PHENOMIM: an OMIM-based secondary database with a controlled vocabulary.** *X.L. Ji[1,2], C.X. Liu[3], Y. Fang[1], S.Q. Liu[2], Y.Y. Zhao[4], D.Y. Zhao[1], J. Li-Ling[1,3,4].* 1) Sino-Dutch Biomedical and Information Engineering School, Northeastern University, Shenyang 110003, China; 2) Laboratory for Conservation and Utilization of Bio-Resources & Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming 650091, China; 3) Department of Gynecology and Obstetrics, Liaoning Center for Prenatal Diagnosis, Shengjing Hospital of China Medical University, Shenyang 110003, China; 4) Department of Medical Genetics, China Medical University, Shenyang 110001, China.

It has been recognized that phenotype analysis may provide crucial information for gaining insights into molecular interactions underlying various diseases. However, so far few attempts have been made to systematically carry out analyses at the phenotype level, mainly owing to lack of a unified system of phenotype descriptors. Here we present a secondary database, PHENOMIM, for presenting the clinical synopses (CS) from the Online Mendelian Inheritance in Man (OMIM) database with a structured and controlled vocabulary. The OMIM-based phenotype vocabulary has combined medical knowledge and mechanisms of embryonic development. A web interface has also been developed for visualization of the hierarchical structure of the vocabulary and related information from the OMIM database. Our downloaded OMIM databank contained over 18,000 detailed entries about human genes and genetic disorders, of which 4,741 entries have a CS field that lists the clinical features of a given disease. Through text parsing, a total of 58,075 clinical features were retrieved. The data were then sorted according to a hierarchical ontological system designed based on human embryology and clinical knowledge. To assess the value of PHENOMIM, a simple statistical test has been performed to evaluate the vocabulary. OMIM entries were compared to each other based on their phenotypic maps. The OMIM entry pairs with most similar phenotypic mapping were identified. Interestingly, besides variants of a same syndrome, e.g., Cockayne syndromes type A (OMIM 216400) and type B (OMIM 133540), many entries with drastically different labels (e.g., OMIM 170995 and 214110; OMIM 194190 and 601803, etc.) were found to have very similar maps. Since such pairs maybe either clinically or genetically related, our phenotypic analysis may provide crucial information for underlying genetic interactions. Availability and Implementation: Freely available on the web at http://yunda.org:8080/test/phenomim/index.php. Website implemented in PHP, MySQL and Apache, with all major browsers supported. Contact: li-ling@mail.cmu.edu.cn Supplementary Information: Detailed documentation is available at http://yunda.org:8080/test/phenomim/index.php.

## 1917/T/Poster Board #466

**Classifying Clinical Outcomes in Rare Renal Diseases Using Molecular Markers: Optimal Feature Selection When Features Are Rare, Weak and Correlated.** *J. Jin[1], P. Song[2].* 1) Statistics, Carnegie Mellon University, Pittsburgh, PA; 2) Statistics, University of Michigan, Ann Arbor, MI.

We consider two types of rare renal diseases: Membranous Nephropathy (MN) and Focal and Segmental Glomerulosclerosis and Minimal Change Disease (FSGS/MCD). Data are collected from 450 patients, roughly half in each of the two types. For each patient, over 20,000 genetic features (i.e. gene expression levels) are measured through microarray experiments on renal biopsy tissues, accompanied with dozens of auxiliary features such as histopathology, laboratory, clinical, treatment variables. The main interest is, based on both molecular and auxiliary features, to predict patient's primary clinical outcome as to be either {em complete/partial remission} or {em no remission} of urinary protein excretion. We model the feature vector $X = (Y, W)'$ as samples drawn from a high dimensional Gaussian distribution, where $Y$ and $W$ correspond to genetic features and auxiliary features, respectively. We model the covariance matrix as $Lambda + H$, where $Lambda$ is a diagonal matrix and $H$ is a low-rank matrix characterizing the correlation between $Y$ and $W$. In addition, the $Y$ component is highly sparse, and the $W$ component is relatively dense. We propose a new approach for feature selection in the $W$, and apply the recent innovation of Higher Criticism Thresholding (Donoho and Jin (2008), Proc. Natl. Acad. Sci.) to select features in the $Y$. We show that the feature selection approach for the $W$ is optimal when useful features are weak but relatively dense, and that the feature selection approach for the $Y$ is optimal when useful features are both weak and rare. These two approaches complement with each other in different sparsity regimes. In addition, we find that the correlation matrix $H$ plays an important role in the power determination for a classifier. We derive an explicit formula on how matrix $H$ affects feature weights in a classifier. Results on both simulations and applications to rare renal diseases are reported. This is joint work with Peter Song, Department of Biostatistics, University of Michigan.

## 1918/T/Poster Board #467

**Software for designing and forming predictive genetic tests.** *Q. Lu[1], C. Ye[1,2], J. Zhu[2], R.C. Elston[3].* 1) Department of Epidemiology, Michigan State University, East Lansing, MI; 2) Institute of Bioinformatics, Zhejiang University, Zhejiang, P. R. China; 3) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH.

Early disease prediction capitalizing on genetic loci and clinical risk factors holds great promise for improving health care and is anticipated to change health care from treatment to prevention. However, linking the genetic and clinical risk factors, including those from genome-wide association studies, into individual genotype/clinical profiles to predict an individual's risk of disease (i.e., forming a predictive genetic test), remains a challenge. To facilitate this kind of translational research, we develop a graphical user interface package, PredTest, in R. This package is built for both designing and analyzing predictive genetic tests. In the design stage, it provides an estimated classification accuracy of the test using essential information gained from public resources and/or previous studies, and determines the sample size required to verify this accuracy. In the analysis stage, it adopts a robust and powerful method for forming predictive genetic tests. The package is developed based on the optimality theory of the likelihood ratio and, therefore, theoretically could form a test with the highest performance. It can be used to incorporate a relatively large number of genetic and clinical predictors, with consideration of their possible interactions, and so is particularly useful for forming predictive genetic tests under the common complex diseases scenario.

## 1919/T/Poster Board #468

**GWAMA: software tool for meta-analysis and visualization of whole genome association data.** *R. Magi[1,2], A.P. Morris[2].* 1) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford OX3 7LJ, UK; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, UK.

Genome-wide association (GWA) studies have proved to be extremely successful in identifying moderate genetic effects contributing to complex human phenotypes. However, to gain insights into increasingly more modest signals of association, samples of many thousands of individuals are required. One approach to overcome this problem is to combine the results of GWA studies from closely related populations via meta-analysis, without direct exchange of genotype and phenotype data. We have developed the GWAMA (Genome-Wide Association Meta-Analysis) software to perform meta-analysis of the results of GWA studies of binary or quantitative phenotypes. Fixed-effects meta-analyzes are performed for both directly genotyped and imputed SNPs using estimates of the allelic odds ratio and 95% confidence interval for binary traits, and estimates of the allelic effect size and standard error for quantitative phenotypes. The software incorporates error trapping facilities to identify strand alignment errors and allele flipping, and performs tests of heterogeneity of effects between studies. Comprehensive log files of all errors and warnings are composed with unique error codes for each exception. This allows researchers to quickly and easily discover all problems in their data set. The software package also includes R scripts for creating publication quality Manhattan- and QQ-plots from the output data to summarize results. Both Manhattan- and QQ-plots can be drawn for illustrating the results. GWAMA is an open source software package and can be downloaded from http://www.well.ox.ac.uk/gwama/. A userguide and example files are provided with the software.

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## 1920/T/Poster Board #469

**Sparsity Pruning with Random Forests.** *A.E. Hubbard[1], B.A. Goldstein[1], A. Cutler[2], L.F. Barcellos[3].* 1) Division of Biostaitsics, University California Berkeley, Berkeley, CA; 2) Department of Mathematics & Statistics, Utah State University, Logan UT; 3) Division of Epidemiology, Univ of California, Berkeley, CA;.

One of the significant challenges in genome wide association (GWA) studies is to separate the few true effects (signals) from the rest of the data (noise). Many machine learning techniques are available for dealing with such sparse data. Random Forests (RF) is a flexible algorithm that has seen growing application in genetic studies. The algorithm produces a series of categorization trees using a large set of predictor variables and a specified outcome. The collection of trees can be used to predict an outcome, cluster observations, and calculate variable importance measures. In a recent application to GWA data, we found that by recursively removing sparse data, SNPs associated with disease can more effectively be discovered. However, little work has been done to characterize the effectiveness of sparsity pruning using RF. Using simulated GWA data, the effectiveness of sparsity pruning and filtering techniques is examined. We define the sparsity of the final forest through the two main RF tuning parameters: mtry (the number of variables to search over at a node) and ntree (the number of trees in the forest). Different genetic causal models are explored with a focus on epistatic and heterogeneous effects, as well as dominant and recessive effects. The power of such sparsity pruning to retain a true association is examined, revealing that most true associations are retained while the dimension size is decreased. Power is strongest to detect main effects, though still more than capable of isolating interactions as well as heterogeneous effects. This dimension reduction subsequently allows one to effectively analyze GWA data for causal effects with more targeted parameters.

## 1921/T/Poster Board #470

**Some Strategies of Testing Hardy-Weinberg Equilibrium in Tri-Allelic Markers.** *M. Rao[1], R. Chakraborty[1], S. Venkatesan[1], R. He[2].* 1) Environmental Health, University of Cincinnati, Cincinnati, OH; 2) Novartis Consumer Health, Inc., 200 Kimball Drive, Parsippany, NJ.

The focus of the presentation is testing the Hardy-Weinberg Equilibrium (HWE) in tri-allelic markers. Let $A_1$, $A_2$, and $A_3$ be the alleles with population frequencies $p_1$, $p_2$, and $p_3$, respectively. Let the resultant genotype distribution be cast in the form of a Wright's model (1977) with inbreeding coefficient $\theta$. The Wright's model has four parameters: the allele frequencies and the inbreeding coefficient. Testing HWE is equivalent to testing $\theta = 0$. One could use a likelihood ratio test based on the sample genotype frequenices to test HWE. One could use Mathematica for the optimization work involved in the likelihood ratio test. In spite of spending hours and hours on Mathematica, no solution is forthcoming on certain data sets. There is a way out. Let us reduce the genotype distribution into three 2x2 genotype distributions stemming from $A_1$ versus not $A_1$, $A_2$ versus not $A_2$, and $A_3$ versus not $A_3$ with respective inbreeding coefficients $\theta_1$, $\theta_2$, and $\theta_3$, respectively. It can be shown that $\theta_1 = \theta_2 = \theta_3 = \theta$. One can then easily test HWE in each of the 2x2 set-ups using any one of the tests available in the literature. One can then string all these three tests together to test HWE in the 3x3 case. We will also show how to compute the power function of such a test. Computations will be illustrated using some examples.

## 1922/T/Poster Board #471

**A software tool for scoring CNVs and runs of homozygosity using linkage results.** *R. Karlsson, L. Graae, M. Lekman, D. Galter, A. Carmine Belin, S. Paddock.* Neuroscience, Karolinska institutet, Stockholm, Sweden.

**Background:** High-density SNP platforms can reveal copy number variants in the human genome that have previously been difficult to discover. Linkage studies have been performed for many diseases, however resolution is low, and follow-up was prohibitively expensive. Thus interesting but broad linkage peaks may have been left uninvestigated. Family-based DNA samples that were collected for linkage studies can now be re-genotyped relatively cheaply on genome-wide SNP platforms, where software (e.g. PennCNV) can be used to call CNVs with high confidence. The high resolution of modern SNP genotyping together with the inheritance information available in family-based samples can lead to new insights. We therefore suggest scoring CNVs using linkage data to find candidate CNVs for complex diseases. **Methods:** We present a simple approach to linkage-based CNV scoring. The genome is segmented so that every start or end of a CNV in any individual becomes a segment breakpoint. CNVs are counted and summarized for each individual and each pedigree. Filters for inclusion can be applied, for example on copy number or minimum CNV count per family and region (to exclude singleton de novo events). CNVs that pass the filters are scored by the sum of average family-wise linkage scores over the segment for all families that have a CNV in that specific chromosomal segment. Input files can be derived from parametric or non-parametric linkage analyses. We have implemented this analysis in a freely available software package. Our implementation supports output from Merlin for linkage, and PennCNV for CNV analysis, but can easily be extended to accept other popular file formats. It is also possible to score other individual-based chromosomal segments by this method. One alternative, implemented in our tool, is to replace CNVs by runs of homozygosity output from PLINK and perform an analogous scoring. **Results:** In a preliminary study we applied our method to a 47 pedigree, 277 individual subsample of the NIMH genetics initiative repository of DNA from families affected by bipolar disorder. The sample was re-genotyped on the Illumina HumanHap610 platform. Using nonparametric linkage data, regions 2p25, 15q21, and 12p12 got the highest scores. Using parametric linkage scores identified a candidate region on 3p14. These CNVs will be validated using qPCR and further investigated by re-sequencing and functional assays to assess their significance in the etiology of bipolar disorder.

## 1923/T/Poster Board #472

**Modelling Single SNP and Haplotype Mendelian Randomisation in R.** *G. Cadby, PA. McCaskie, LJ. Palmer.* Centre for Genetic Epidemiology and Biostatistics, University of Western Australia, Perth, Western Australia, Australia.

One aim of epidemiology is to discover modifiable disease exposures and translate this knowledge into public health for the prevention of disease. Researchers often rely on observational studies to find associations between exposure and disease as randomised controlled trials (RCTs) are not always feasible, however many observational epidemiology results have not been replicated in RCTs. This may be as the reported associations are due to artefacts of the study design, including confounding, selection bias and reverse causation.

If it is possible to identify a genetic variant (or instrument) which is associated with the exposure, then due to the random assortment of alleles from parents to offspring, this variant should not be associated with confounding factors or subject to selection bias or reverse causation. If an association is detected between the variant and disease, we can assume the exposure is causally related to disease. This is referred to as Mendelian Randomisation (MR) and is a form of Instrumental Variables (IV) analysis.

Several software packages implement IV methods, however none of these are designed specifically for a genetic association analytic framework. As such, they can accommodate the inclusion of simple single nucleotide polymorphism (SNPs) as instruments, however they do not allow the inclusion of imputed haplotypes. We have designed a MR software application in R which models epidemiological relationships using either SNPs or haplotypes as instruments. Both SNP and haplotype MR analyses use Generalised Method of Moments and Two Stage Least Squares. In addition, a multiple-imputation approach to haplotypic analysis, identical to that utilized in the program SimHap, is used to estimate the phase of the haplotype instrument.

The use of MR modeling in the genetic epidemiological framework will assist in the detection of casual relationships between exposures and disease, which will in turn lead to improved health interventions acting on modifiable environmental exposures, resulting in better health outcomes.

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## 1924/T/Poster Board #473

**PATH2: Analysis of Genetic Pathways Incorporating Prior Information From Electronic Databases.** *D. Daley, B. Tripp, D. Zamar.* i-CAPTURE Ctr, Univ British Columbia, Vancouver, BC, Canada.

Statistical models are becoming more complex as we seek to better understand biologic disease pathways. For complex diseases, susceptibility may be the result of interactions (gene-gene or gene-environment) and statistical models need to be able to incorporate these interactions. Hierarchical models provide a framework to tackle many of the problems encountered in the genetic analysis of common complex diseases, as they allow for the incorporation of prior information into the statistical model. In the "information age" there is a vast quantity of knowledge that can be mined from electronic databases, but the challenge is how to automate data retrieval and appropriately incorporate this information into the statistical model. We have developed Path (Path: a tool to facilitate pathway-based genetic association analysis), to aid researchers in the incorporation of prior information in statistical analyses, the software can be freely obtained from http://genapha.icapture.ubc.ca/index.php/research/software/. Path is designed to help researchers interface their data with biological information from several bioinformatics resources. Path brings together currently available information from nine online bioinformatics resources including the National Center for Biotechnology Information (NCBI), Online Mendelian Inheritance in Man (OMIM), Kyoto Encyclopedia of Genes and Genomes (KEGG), UCSC Genome Browser, Genetic Association Database, the Single Nucleotide Polymorphism database (dbSNP). Path allows users to view KEGG pathway diagrams and to search for genetic pathways by gene name, or by rs number. Path displays KEGG pathway diagrams and indicates the genes that have been genotyped in the pathway, allowing users to build custom association graphs, plots of interest, and to test for interactions. Path has been incorporated in the Genapha website (http://genapha.icapture.ubc.ca/). We are currently expanding Path to include additional data sources and hierarchical model structure, to guide users in the incorporation of prior information gathered from external sources. These models will be applied to an asthma dataset with 98 candidate genes and 5,565 individuals from Canada and Australia, in order to better understand the contribution of gender, genes, and environment and their complex interactions in the development of asthma. We have published our primary results (Daley, D et al (2009) Hum Genet 125, 445-59). Hierarchical model results will be presented.

## 1925/T/Poster Board #474

**An Application of Random Forests to a MS GWA dataset: Methodological Considerations & New Findings.** *B.A. Goldstein[1], A.E. Hubbard[1], F.B.S. Briggs[2], A. Cutler[3], L.F. Barcellos[2], IMSGC.* 1) Division of Biostatistics, Univ California, Berkeley, Berkeley, CA; 2) Division of Epidemiology, Univ of California, Berkeley, CA;; 3) Department of Mathematics & Statistics, Utah State University, Logan UT.

As computational power improves, the application of more advanced machine learning techniques to the analysis of large GWA datasets becomes possible. While most traditional statistical methods of analysis can only elucidate main effects of genetic variants on risk for disease, machine learning approaches are particularly suited to discover higher order and non-linear effects. One such approach is the Random Forests (RF) algorithm. In brief, the algorithm produces a series of categorization trees using a large set of predictor variables. Each tree serves as a weak predictor for a chosen outcome. After growing a series of trees, the results are aggregated to create a robust predictor. The algorithm computes an importance score for each predictor variable considered. While recent years have seen a growth in the use of RF for SNP discovery in complex diseases, most work has focused on small datasets or simulation studies which are limited. Using a multiple sclerosis (MS) case-control dataset comprised of 500K SNP genotypes characterized across the genome, we outline an approach and several considerations for optimally tuning RF based on the empirical dataset. For large GWA data, it is found that the default values typically used for RF analyses are not appropriate, particularly the mtry (the number of variables to search over at a node) value. Furthermore, prediction and inference notably improve after recursively sub-sampling the data, and after removing very strong effects (predictors) from the analysis. The RF results obtained in this study are compared to previously published and replicated GWAS results using the same dataset. RF is able to replicate the positive findings for SNPs within EVI5 (Chr 1), KANK1 (Chr 9), and IL2RA (Chr 10). Moreover, SNPs within four new loci are identified as important for distinguishing between MS cases and controls: PHACTR2 (Chr 6), IL7 (Chr 8), CTNNA3 (Chr 10), and MPHOSPH9 (Chr 12). This study represents one of the first applications of a machine learning algorithm to a GWA dataset and demonstrates the utility of RF to reveal novel genetic associations.

## 1926/T/Poster Board #475

**A Bonferroni procedure using weights accounting for Hardy-Weinberg disequilibrium information in genome-wide association studies.** *G. Kang, G. Gao.* University of Alabama at Birmingham, Birmingham, AL.

In genetic case-control studies, traditional tests for detecting association between a single marker and a disease include Pearson's Chi-square allelic test and genotypic test, and Cochran-Armitage trend test (for additive model). These tests can be powerful for additive (ADD) and multiplicative (MUL) disease models, but are less efficient for dominant (DOM) and recessive (REC) disease models. In addition, testing for Hardy-Weinberg disequilibrium (HWD) has been used for detecting association between a single marker and a disease. The HWD methods can be powerful for DOM and REC models, but have very low or no power for ADD and MUL models. Recently, Song and Elston proposed a method combining the Cochran-Armitage trend test and a measure of HWD to create an approach powerful for the four models discussed above. When the methods described above for testing association for a single marker are applied to genome-wide association studies (GWAS) that test hundreds of thousands of single nucleotide polymorphisms (SNPs), the Bonferroni procedure is often used to control family-wise error rate (FWER), the power of GWAS can be low. In this study, we adapt a generalized sequential Bonferroni (GSB) procedure of Holm to GWAS. The GSB assigns a weight to each test (or marker) according to prior information instead of treating each marker equally and assigning the same significance threshold to each marker as in the Bonferroni procedure. We propose to calculate a weight for each marker by using information of HWD among cases, and then to incorporate the weights for all markers into the allelic association tests used for GWAS. We refer to this GSB procedure with HWD weight as GSB-HWD procedure. Simulation results show that our GSB-HWD method: 1) controls FWER quite well, 2) always has much higher power in GWAS than the traditional association tests with Bonferroni correction under DOM and REC models, 3) can have power very comparable to the traditional association tests with Bonferroni correction in GWAS under ADD and MUL models, and 4) generally outperforms Song and Elston's method with Bonferroni correction in multiple testing under the four models described above. We applied our GSB-HWD procedure to GWAS of a coronary artery disease dataset with about 340K SNPs and to GWAS of a hypertension dataset with about 350K SNPs. Our GSB-HWD procedure identified more significant SNPs than the traditional association tests with Bonferroni correction.

## 1927/T/Poster Board #476

**Analyses and Comparison of Imputation-based Association Methods.** *Y. Pei[1,2], J. Li[2], L. Zhang[1,2], H. Deng[1,2,3].* 1) Key Laboratory of Biomedical Information Engineering, Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, P. R. China; 2) School of Medicine University of Missouri-Kansas City, 2411 Holmes Street Kansas City, MO 64108, USA; 3) College of Life Sciences and Engineering, Beijing Jiaotong University, Beijing, China.

Genotype imputation methods have become increasingly popular for recovering untyped genotype data. An important application with imputed genotypes is to test genetic association with complex traits. Association testing with imputed genotypes can provide additional insight beyond what is provided by testing on only typed tagging SNPs in genome-wide association studies. Many sophisticated imputation-based association methods have been proposed and proven to be effective. However, their performances are affected by a variety of genetic factors, which have not been well studied. In this study, using both simulated and real data sets, we investigated the effects of LD, MAF of untyped causal SNP and imputation accuracy rate on the performances of five popular imputation-based association methods, MACH, IMPUTE, SNPTEST, BIMBAM and SNPMStat, and compared their relative performances. Results show that: 1). all the methods investigated could boost signals and improve power under medium and high LD levels, and the power increased with increasing level of LD; 2) the power increased with higher MAF of untyped causal SNPs; 3). high imputation accuracy rate could not guarantee an improvement in power; 4). among the methods, SNPTEST performed best under most scenarios for both quantitative and qualitative traits. Our results are helpful in guiding the applications of imputation-based association methods in empirical data.

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## 1928/T/Poster Board #477

**Robust Score Tests for Multivariate Linkage Analysis.** *N. Morris, Y. Song, R. Elston, C. Stein.* Epidem/Biostatistics, Case Western Reserve Univ, Cleveland, OH.

There have been numerous papers discussing robust score tests for univariate quantitative trait linkage. In this work we suggest several robust tests for multivariate linkage analysis. While previous investigations have been hampered by inaccurate knowledge of the distribution of the multivariate linkage test statistic, we show how recent advances can solve this problem. Our tests include a transformation method and several empirical variance estimators. We perform a simulation study to determine the power and validity of these tests in terms of type I error. We also derive theoretical approximations to the power curve for one of these score tests. This theoretical approximation is used to further investigate how the power of these test is influenced by the model parameters, and when multivariate linkage is likely to be beneficial. Our method for linkage analysis has been implemented in the program RELPAL in the S.A.G.E. software suite.

## 1929/T/Poster Board #478

**Mitochondrial Sequence Data Analysis Tool.** *M. Schmidt, J. McCauley.* Miami Inst Human Genomics, Miller School of Medicine, Univ Miami, Miami, FL.

With the increasing ability to sequence mitochondrial DNA (mtDNA), the need for a flexible multi-purpose analysis tool arises. We have developed software to aid in examining mitochondrial variation for the purposes of genetic studies. Using this tool, one can complete various tasks which include quality control, detection of insertions/deletions, detection of single-nucleotide polymorphisms (SNPs), classification of haplogroups, and other data analyses. Analysis starts with a reference sequence and descriptor files for things such as gene location, amino acid coding, haplogroup classifications, and other user defined labels that can describe regions or sequence of interest. Using the quality control feature, one is able to compare sample data to a user provided reference sequence (e.g. the Revised Cambridge Reference Sequence (rCRS)), providing a summary about missing data, variant calls, or understanding sequence irregularities that may result as an artifact of the sequencing process. This tool is not limited to human mtDNA but could be adapted for analysis of any species' mitochondrial DNA sequence provided a user defined reference sequence and descriptor files. The overall focus of this application is to detect and summarize SNP variation along with insertions and deletions in sample data. All deviations from the reference are reported. For all positions in the mtDNA, base and affection status are tallied and provided in the summary for further consideration. Base changes leading to amino acid (i.e. non-synonymous) changes may be of particular interest when comparing mitochondrial sequences between cases and controls in a disease study. These and other variations may be subjected to a chi-square test with the summary file reporting significance values. In addition to examination of single SNPs this software also considers the collective variation across the mtDNA genome or within each gene (or other user defined regions). It tallies this variation, again by affection status, with the ability to perform additional tests for association. Haplogroups are also reported and analyzed for association through use of a defined descriptor file. This software is written in C++ and runs on Windows and Linux. Included with the distribution are the C++ source files for the program and a set of descriptor files for human mtDNA, including a haplogroup defined descriptor file utilizing the simplified mtDNA lineages found at mitomap.org.

## 1930/T/Poster Board #479

**Performance of population stratification correction: a simulation study using observed genotypes from a large European Reference Panel.** *E. Genin[1,2], M-C. Babron[1,2,3,4], R. Kazma[4,1], G.M. Lathrop[5,6], S. Heath[5,6].* 1) INSERM UMRS-946, PARIS, France; 2) Univ Paris-Diderot, Paris, France; 3) Inserm UMRS-535, Villejuif, France; 4) Univ Paris-Sud, Villejuif, France; 5) Centre National de Génotypage, CEA, Evry, France; 6) Fondation Jean Dausset/CEPH, Paris, France.

With the development of high density SNP-arrays spanning the whole human genome, genome-wide association studies have become a reality. In these studies, large samples of several hundreds of cases and controls are required to keep reasonable power after accounting for multiple testing. This is usually only feasible through multi-centric collaborative studies and stratification issues are likely to arise. Indeed, even within Europe, SNP allele frequency and disease prevalence variations exist that can lead to false positive results in association studies. Different methods have been proposed to account for stratification in association tests. One of the most widely used is the principal-component (PC) corrected chi-square implemented in Eigenstrat (Price et al., 2006) which computes association statistics using ancestry-adjusted genotypes and phenotypes. Simulations have shown that the method performs very well in keeping type-one error rates at the expected sizes while maintaining good power to detect association. In the different simulation studies however, power rates are usually computed using simulated genotype data and not observed ones and global type-one error rates are reported; i.e. without any account of markers that give false-positive results. In this study, we explored the performance of the Eigenstrat method using a large panel of 5,811 individuals from 13 European countries genotyped on Illumina 317K or 370K chips (Heath et al., 2008). We simulated case-control statuses (with 2,000 cases and 3,811 controls) of the 5,811 individuals from the sample assuming different disease gradients within Europe and different genetic models for the disease predisposition with different SNPs involved (3 among the most stratified ones and 3 among the less stratified ones). We compared the results obtained with Eigenstrat using corrections based on different numbers of PCs and PCs computed with different densities of markers to those obtained with the Cochran-Mantel-Haenszel (CMH) test after stratification on the individual reported country of origin. Although the different SNPs chosen as disease susceptibility loci had similar average allele frequencies over Europe, the impact of the PCs used to correct for stratification is not the same both in terms of power and type-one error rates. The CMH tests is shown to perform relatively well with a correct size and a limited lack of power compared to Eigenstrat.

## 1931/T/Poster Board #480

**Comparison of methods for the analysis of genetic association studies with very rare variants.** *S. Bacanu, M.R. Nelson, J.C. Whittaker.* GlaxoSmithKline, Res Triangle Park, NC.

Recent genome wide association studies of complex traits have been very successful and are predicted to detect the majority of common genetic variants of moderate to large effect. Despite this, for most traits only a small proportion of the genetic variance believed to exist is explained by the variants found. It is possible that much of the currently unexplained variance is due to rare variants having larger effects than those of common variants. Until recently it was cost-prohibitive to investigate such variants on anything but a small and targeted scale. However, current sequencing technology allows for investigation of rare variants on a larger scale, for instance resequencing the entire exon-coding regions of the genome (exome) in hundreds or thousands of individuals. As is often the case, the development of methods for data analysis lagges behind the technology for data generation. The most commonly used analysis method computes an overall gene statistic by contrasting the aggregate number of rare, putatively functional variants in subjects with phenotypes in the extreme tails of the distribution, assuming that all these putatively functional variants with frequency below an arbitrary threshold have the same effect on the trait of interest. Here we explore a number of alternative analysis methods, based on (1) aggregating the rare variants by exon/gene part, (2) smoothing the aggregated number of rare variants by genetic position (and possibly allele frequency) and (3) weighted aggregation of rare variants based on predicted functionality, calculated using structural or conservation based metrics. We consider both binary and quantitative traits, investigating the performance of our methods via extensive simulation, varying sample size, models for etiology and assumptions about the functionality of rare variants. We provide guidance for choosing the quasi-optimal method based on these parameters. Finally, we demonstrate our methods on data from a number of recent studies involving large scale resequencing of candidate genes.

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## 1932/T/Poster Board #481

**Comparison of Genotype Imputation Methods for SNP Array Data.** *C.T. Chang[1], Y.J. Lin[1], C.Y. Tang[1], W.P. Hsieh[2].* 1) Computer Science, National Tsing Hua University, HsinChu, Taiwan, Taiwan; 2) Institute of Statistics, National Tsing Hua University, HsinChu, Taiwan.

The high throughput technology for genotyping has made genome-wide association possible. The SNP data derived from the array-based technology cannot avoid missing values since they are transformed from intensity data and are subject to the probe quality. Hence, there are many algorithms developed to impute the missing genotypes by utilizing the LD structure or the haplotypes from HapMap samples. Imputation can increase the power of the association study and does not cost extra money to type the missing SNPs. More and more genotype imputation software have been developed for this purpose and they have not been evaluated in a genome-wide association study. We compare the following software: BIMBAM v0.99, BEAGLE v3.0.3, fastPHASE v1.4.0, IMPUTE v0.5.0, MACH v1.0.16, PLINK, SNPMSTAT v3.1, and test them on linux kernel version 2.6 amd64 platform. Our purpose of this study is to integrate all the methods into a unifying format and facilitate genome-wide association study. The dataset used for evaluation is the HapMap data. Randomly selected SNPs from chromosome 21 of HapMap samples are masked to evaluate the imputation methods. The assessment is made on the accuracy, concordance rate, processing speed, and memory usage. We found that the performances of each method differ from populations. Therefore, an optimal imputation method is constructed by a voting and fusion system. When the results of the included methods are inconsistent, the final result is voted by all the modules. It summarizes the results and gives a better overall imputation.

## 1933/T/Poster Board #482

**LD Mapping of Disease Gene and Haplotype Analysis on Textile Plot.** *N. Kumasaka, Y. Nakamura, N. Kamatani.* Ctr Genomic Med, RIKEN, Tokyo, Japan.

We have been introducing textile plot (Kumasaka et al., 2008) to provide a visualization of LD structure so as to maximize deep insight into genetic variation present in the multiple SNP genotype data. The plot can accentuate LD by specific geometrical shapes. The LD between adjacent SNPs is shown by line crossings between adjacent loci so that low-grade line crossing indicates high dependency of alleles between SNPs, and vertical dispersion of genotypes approximates the structure of pairwise correlation coefficients for any number of SNPs. The haplotype structure can be also directly confirmed through the diplotype configuration as the queues of multiple genotypes on the textile plot. Application of this technique to simulated and real data sets illustrates the potential usefulness of the textile plot as an aid to the LD mapping of disease gene in conjunction with haplotype analysis.

## 1934/T/Poster Board #483

**Bayesian Methods for Investigating Hardy-Weinberg Equilibrium.** *J. Wakefield.* Statistics & Biostatistics, Univ Washington, Seattle, WA.

Testing for Hardy-Weinberg equilibrium is commonplace and is usually carried out via frequentist approaches. Unfortunately, the latter suffer from a number of drawbacks. First, the discreteness of the sample space means that uniformity of p-values under the null cannot be assumed. Second, the interpretation of the subsequent p-values, and choice of significance threshold depends critically on sample size, since equilibrium will always be rejected at conventional levels with large sample sizes. Finally, when applied in genome-wide contexts multiple testing is also a significant issue. We describe a Bayesian approach to the investigation of Hardy-Weinberg equilibrium using both Bayes factors (for hypothesis testing), and the examination of posterior distributions (for estimation). The choice of prior distribution is particularly important for testing, and we describe this choice with respect to a number of case studies including an example with nine alleles, and a genome-wide association study. The parameterization adopted under the alternative will vary depending on the nature of the data, with the use of tests for Hardy-Weinberg as a quality control measure in GWAS clearly differing from the more traditional application in population genetics. For the latter we parameterize in terms of fixation indices. The use of Bayesian methods is appealing for a number of reasons: the discreteness of the sample space is irrelevant since only the observed data are considered in the calculation, the choice of significance threshold in a hypothesis testing situation can be driven by the costs of making the two potential sources of error, and the examination of posterior distributions gives insight into reasons for departure from equilibrium. Freely-available software allows the straightforward application of the methods described.

## 1935/T/Poster Board #484

**Evidence of *CACNA1C, IGF2BP2, JAZF1, TCF7L2,* and *WFS1* Variants for Risk of Type 2 Diabetes in the NHLBI Family Heart Study GWA Data.** *P. An, M. Feitosa, M.A. Province, I.B. Borecki.* Division of Statistical Genomics, Department of Genetics, Washington University School of Medicine, St Louis, MO.

Variants of known type 2 diabetes (T2D) genes and the KEGG T2D (hsa04930) pathway genes were assessed for their association with T2D status in the NHLBI Family Heart Study (FHS). A total of 897 Caucasian family members including 14% T2D cases had phenotype and genotype data with up to 2.5 million typed and imputed SNPs in the FHS. A total of 4,944 SNPs covering 15 known T2D genes and 37 KEGG T2D pathway genes were assessed using logistic regression under an allele dosage model with generalized estimating equation to account for familial dependencies. Covariates in this analysis included age, age2, sex, and field center. $P<0.0055$ from a modified FDR approach was used to flag significance considering correlations of these genes and SNPs in the presence of prior knowledge these selected genes. Significant association was found in 5 genes that included 2 SNPs in *CACNA1C* (rs2108704, rs12426831, $p$= 0.0010), 28 SNPs in *IGF2BP2* (around rs4402960, $p$=0.0034), 1 SNP in *JAZF1* (rs6462063, $p$=0.0040), 2 SNPs in *TCF7L2* (rs4506565, rs7903146, $p$=0.0046, 0.0033, respectively), and 40 SNPs in *WFS1* (around rs5018648 including 2 coding SNPs rs1801206 and rs734312, $p$=0.0011, 0.0005, 0.0033, respectively). For risk of T2D, risk allele frequencies of these variants were 0.21-0.70, effect size estimates were <1.5%, and odds ratio point estimates were <1.6. Among the 28 emerged variants in this analysis, 3 exact SNPs (*IGF2BP2* rs4402960, *TCF7L2* rs4506565, and *WFS1* rs5018648) replicated reports in multiple large GWA studies. Mechanisms of these gene variants in raising risk of T2D are yet currently unknown. Taken together, this analysis reconfirmed effects of variants in *CACNA1C, IGF2BP2, JAZF1, TCF7L2,* and *WFS1* genes for risk of T2D. Survey of SNP*SNP and BMI*SNP interacting effects of these variants for risk of T2D is underway in the FHS cohort.

## 1936/T/Poster Board #485

**Heritability of Longevity in the Amish.** *K.L. Spencer[1], A.C. Davis[1], L. Jiang[1], R. Laux[1], P.J. Gallins[2], N. Schnetz-Boutaud[1], L.L. McFarland[1], D. Fuzzell[1], C. Knebusch[1], M. Creason[2], L. Caywood[2], C.E. Jackson[3], W.K. Scott[2], M.A. Pericak-Vance[2], J.L. McCauley[2], J.L. Haines[1].* 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Miami Institute for Human Genomics, Miller School of Medicine, University of Miami, Miami, FL; 3) Scott & White, Temple, TX.

Purpose: Successful aging is a complex trait, influenced in part by genetic factors. Longevity, or age at death, is an important component of this phenotype. We set out to determine the heritability of age at death in an Amish population whose present-day members reside in Holmes County, Ohio and Elkhart and LaGrange Counties, Indiana. Because their lifestyle has remained consistent over the last few centuries, environmental variance is minimized, making this an ideal population for studying longevity. Methods: Using information from 2,988 individuals in a single Amish pedigree dating back to the 1700s, we estimated the heritability of longevity using the software program SOLAR, with and without considering gender as a covariate. We excluded individuals who died before age 30 to eliminate deaths due to childhood illnesses and accidents. Results: The average at death was 70.1 years (standard deviation=15.1 years, range=30 to 106 years). The unadjusted estimate of narrow sense heritability ($h^2$) was 0.13 and highly significant (p<1.0x10-12). Adjusting for gender did not substantially change the results ($h^2$=0.14, p<1.0x10-12). Conclusion: A significant portion of the variance underlying longevity in the Amish is heritable. Our heritability estimate was somewhat lower than a previous study of the Old Order Amish of Lancaster, Pennsylvania (Mitchell et al. 2001, $h^2$=0.25), though still consistent with a significant genetic effect. Given these data, the Amish represent a useful research population for gene discovery in longevity.

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## 1937/T/Poster Board #486

**A new method for detecting IBD regions in multiple individuals: making disease mapping based on very few individuals possible.** *I. Moltke[1], A. Albrechtsen[2], T. van Overseem Hansen[3], F.C. Nielsen[3], R. Nielsen[4,5].* 1) Dept Biology, Univ Copenhagen, Denmark; 2) Dept Biostatistics, Univ Copenhagen, Denmark; 3) Dept Clinical Biochemistry, Rigshopitalet, Denmark; 4) Dept Integrative Biology, Univ California Berkeley, USA; 5) Dept Statistics, Univ California Berkeley, USA.

All individuals in a population are related if traced back long enough, which means that they will share regions Identical By Descent (IBD). The more distantly related the individuals are, the less they are likely to share. This makes it possible to detect regions containing disease causing genetic variants using seemingly unrelated affected individuals by identifying which region they share IBD. With today's easy access to genome wide SNP data a method for identifying IBD regions based such data is therefore highly desirable.

Here we present an MCMC method for detecting IBD regions among multiple individuals from a population sample. It is based on a probabilistic model applicable to un-phased SNP data which simultaneously models IBD sharing among chromosomes from multiple individuals. The new method can take inbreeding, allele frequencies, genotyping errors and genomic distances into account and it can detect IBD regions without any pedigree information. The simultaneous modeling of multiple chromosomes makes the method more powerful and accurate than existing methods that are all based on pairwise relatedness.

To demonstrate the potential of the method we have applied it to SNP chip data from five seemingly unrelated individuals affected by Breast and/or Ovarian cancer. This allowed us to successfully map the disease causing founder mutation to a region of approximately 3 Mb containing the BRCA1 gene.

## 1938/T/Poster Board #487

**Estimation of the contribution of rare disease-predisposing variants to complex diseases.** *W. Guan, L.J. Scott, M. Boehnke.* Dept Biostatistics and Center for Statistical Genetics, Univ Michigan, Ann Arbor, MI.

In the recent years, a large number of genome-wide association (GWA) studies have been conducted on multiple complex diseases. However, most of the common risk variants identified through these studies make only modest contributions to disease risk. The rapid advance in the next-generation sequencing technologies allows a near-to-complete survey of the genomic regions of interest and better studies of rare disease-predisposing variants. A common strategy to test the association of the rare variants with disease of interest is to pool the variants within a gene or a region as a single unit. With the availability of familial samples, an alternative approach is to look for co-transmission of disease with the possible linked rare alleles. Through analytically calculations and computer simulations, we have computed the power of such a linkage-based test given different numbers of linked disease-predisposing variants, assuming all these variants have very low allele frequencies but complete or high disease penetrance. We hope our results can help to understand the reality of the common diseases, rare variants assumption and its impact on the analyses of re-sequencing data that will be inexpensively available soon.

## 1939/T/Poster Board #488

**Developing SNP content for the Affymetrix next-generation whole-genome mapping panel.** *Y. Zhan, T. Asbury, S. Cawley, J. Gollub, E. Hubbell, G. Liu, R. Lu, Y. Lu, R. Mei, M. Moorhead, A. Pirani, M. Purdy, K. Schweighofer, A. Schweitzer, M. Shapero, T. Webster, L. Weng, A. Williams, J. Zheng, K. Jones.* Affymetrix, Inc. 3420 Central Expressway Santa Clara, CA 95051.

The flood of novel variants being discovered is fueling demand for an evolving suite of new genotyping panels to type these variants in a cost-efficient and high-throughput manner. In response to this need a new array-based system has been developed by Affymetrix, leveraging a new assay that combines the sensitivity of hybridization with the specificity of ligation and resulting in a high-performance next-generation genotyping technology.

This new assay has been used on a diverse panel of 1300 samples to screen variants from dbSNP build 129, including many variants from the 1000 genomes project and with good representation of rare alleles. The resulting database of validated variants is a resource from which multiple panels can be derived - either “top-up” panels to extend the coverage of previously-conducted Genome Wide Association Studies, or stand-alone panels optimizing coverage of common and rare variants for use in new studies.

We describe the process of the SNP screen and illustrate how an initial SNP panel is designed from the validated content using an algorithm that optimizes for a combination of technical performance and genetic coverage in a variety of populations and SNP categories.

## 1940/T/Poster Board #489

**Systematic comparison of haplotype inference error across diverse populations.** *K.A. Drake[1,2], J. Brooks[2], K. McKeown[2], A. Olson[2], E. Ruark[2], E.G. Burchard[1].* 1) University of California- San Francisco, San Francisco, CA; 2) University of California- Berkeley, Berkeley, CA.

Haplotypes are most commonly inferred using statistical methods. Each method has an intrinsic error rate. A systematic comparison of the method-specific error rate across populations has not been performed to date. Admixture and differences in patterns of linkage disequilibrium (LD) may cause haplotype inference error to vary across populations. We sought to determine how the error rate, the proportion of incorrectly inferred haplotypes out of all ambiguous haplotypes, differed across four HapMap populations and to identify unique characteristics of haplotype regions that were predictive of error. Trio data for each population was used to deduce true haplotypes in 100 haplotype regions of 20 kilobase pairs each that were randomly sampled from chromosome 1. Haplotypes were inferred separately on the unrelated founders and unrelated offspring using four methods (PHASE, Beagle, Shape-IT, and fastPHASE). The true haplotypes were compared to the inferred haplotypes to calculate the error rate for each population and method. The error rate was highest in the Maasai, followed by the Yoruba, Mexicans, and Europeans for all methods. Beagle had a much higher error rate than the other methods for all populations. This was expected because Beagle is sensitive to small sample sizes like those in the HapMap populations. Regions with high error were similar across both methods and populations. The error rate was compared in the founders and offspring for each population and method to examine the effect of sample size on error. The smaller offspring group had a higher error rate than the larger founder group for most of the populations and methods. However, in the Mexican population the error rate was similar in the founders and offspring for all of the methods except Beagle. Random forest identified LD and number of heterozygous SNPs as the most predictive factors for error, although several other features were also predictive of error. These results demonstrate that there are differences in haplotype inference error across populations with varying admixture and LD patterns. This difference in error may result in differences in the results of genetic association studies using haplotypes across populations.

## 1941/T/Poster Board #490

**Investigation of maternal effects, maternal-foetal interactions and parent-of-origin effects (imprinting), using mothers and their offspring.** *H. Cordell, H. Ainsworth, J. Unwin.* Newcastle University, Newcastle upon Tyne, United Kingdom.

Many complex genetic influences, including epigenetic effects, may be expected to operate via mechanisms in the inter-uterine environment. A popular design for the investigation of such effects, including effects of maternal genotype and maternal-foetal interactions, is to collect DNA from affected offspring and their mothers. This design can also be used for the investigation of parent-of-origin (imprinting) effects, although greater efficiency may be achievable via the additional collection of DNA from fathers, where possible. Here we propose a novel multinomial modelling approach that allows the estimation of complex genetic effects of this type using data from either case/mother duos or case/parent trios. Through the incorporation of additional assumptions (such as Hardy-Weinberg equilibrium, random mating and known allele frequencies) and/or the incorporation of additional control samples (such as unrelated controls, controls and their mothers, or both parents of controls), we show that the parameters of interest are identifiable and well-estimated by our method. We investigate the required sample sizes and data structures necessary to provide accurate estimation of such effects and high power for their detection. Our method is illustrated by application to data on several candidate genes involved in congenital cardiovascular malformation.

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## 1942/T/Poster Board #491

**Mining the Information Content of Natural Variation in Health Disparity Research.** *T.E. Mason[1], L. Ricks-Santi[1,2], J. Lindesay[3], P. Kurian[3], W. Hercules[3], G.M. Dunston[1,4].* 1) National Human Genome Center, Howard University, Washington, DC; 2) Howard University Cancer Center, Washington, DC; 3) Department of Physics, Howard University, Washington, DC; 4) Department of Microbiology, Howard University, Washington, DC.

Background: The most challenging problem confronting human geneticists today is deciphering how information is stored and communicated in DNA. The science of information theory developed by physicist Claude Shannon may be applicable to deciphering the "information content" (IC) encoded in the patterns of DNA sequence variation associated with disease in individuals and populations. Advances in sequencing the human genome, taken together with the construction of high density haplotype maps provide unique tools for testing a mathematical measure of IC in the human genome. Methods: We mined the IC of haplotypes associated with immunoresponsiveness in host adaptation to the environment by modifying Shannon's general equation for calculating entropy to measure the IC present in haplotype blocks of the human major histocompatibility complex (HLA-DR region). Since haplotype block composition varies in SNP count, we derived a normalization factor and the normalized information content (NIC) was calculated for the haplotype blocks constructed by Haploview v4.2. Genotype data was obtained from the Hap Map Phase 3 study population of African Americans from the southwest United States. The NIC scores were graphed to assess their distribution and outliers were identified. To determine the potential biomedical significance of IC we scanned the outliers for regulatory elements via ConSite. Results: The NIC scores for the region analyzed were uniformly distributed between zero and one, with a mean of 0.6133 (SD=0.1287; SE= 0.0094). Five blocks had NIC scores < 0.3624. Of the eleven SNPs in these blocks, two were in introns of the gene FOXC1 with the other nine in intergenic regions. Thirty-four putative transcription factor binding sites (TFBS) were identified and categorized for the five blocks, with the associated transcription factors having established correlations with known health disparities. Conclusion: The application of a mathematical method for measuring the information content of haplotype variation is intriguing and may provide a useful tool for understanding the role of biophysical information in dissecting the biology of disease.

## 1943/T/Poster Board #492

**Base calling for Resequencing Chips.** *W. Wang[1,2], P. Shen[1], M. Yu[1], S. Lin[3], T. Klopstock[4], R. Horvath[5], C. Palm[1], L. Pique[6], I. Schrijver[6], D. Cutler[7], M. Mindrinos[1], R. Davis[1], T. Speed[2], C. Scharfe[1].* 1) Stanford Genome Tech Ctr, Stanford Univ, Palo Alto, CA, USA; 2) Dept of Statistics, UC Berkeley, Berkeley, CA, USA; 3) Illumina Inc., San Diego, CA, USA; 4) Dept of Neurology, Ludwig Maximilians U., Munich, Germany; 5) Friedrich-Baur-Institute, Ludwig Maximilians U., Munich, Germany; 6) Dept of Pathology and Pediatrics, Stanford U., Stanford,CA, USA; 7) Dept of Human Genetics, Emory U., Atlanta, GA, USA.

Resequencing candidate genes for rare DNA variants with a minor allele frequency (MAF) of <0.05 across many medical cases is essential to identify causal variants for genetic disorders. Medical resequencing (MRS) arrays, preceded by multiplex target amplification, provide a rapid and cost-effective way of performing this task. Previously developed base-calling algorithms for resequencing arrays can only achieve high accuracy at a low call rate (90-95%). We developed SRMA -- Sequence Robust Multi-array Analysis, for MRS arrays, to achieve higher accuracy, while calling the remaining 5% of the sequence. This algorithm first determines the possible variant sites (observed occurrence <1 in 1kb) and the candidate variant alleles, and then performs variant calling for all samples at the variant sites that could be common or rare SNPs. We formalize the statistical framework and present a procedure to perform candidate gene resequencing. We specifically address three practical concerns for resequencing arrays: 1) quality assessment of multiplex target amplifications; 2) variations in discrimination of reference and variant signals caused by experiments (i.e. average intensity, lab effects) and chemistry (i.e. base pair composition, GC content and fragment length) that are genotype specific; 3) no training data is available to estimate variant-specific parameters at most loci. We build on robust linear mixture models to address these issues. We also provide a quality score to indicate high-confidence base-calls, and a reliability score to identify probes showing insufficient discrimination between reference and variant signals. We have used SRMA to resequence approximately 4Mb of DNA corresponding to the exons of 39 candidate nuclear genes for hereditary optic neuropathies (Mitochondrial diseases) in 40 individuals. As compared to the Sanger sequence, we obtained a false positive rate of approximately 1 in 50,000 bp and a false negative rate of approximately 10% (FDR=3%), with a 99.99% call rate. These efforts represent key steps towards our goal of performing effective candidate gene resequencing of human diseases.

## 1944/T/Poster Board #493

**An Epidemiological Study on Welders Exposed to Fumes Containing Nickel and Chromium to Evaluate Genotoxic Occupational Hazard of Welding.** *S.S. Chettiar, A.R. Patel, M.V. Rao.* Zoology Department, Human Cytogenetics Division, Gujarat University, Ahmedabad, Gujarat, India.

Purpose: Large occupational groups, such as welders are currently exposed equally to high levels of Nickel and Chromium and may therefore be at increased degree of health risk. A retrospective exposure assessment of welding fumes containing chromium and nickel was conducted at industrial development areas around Ahmedabad, India for a nested case-control study with welders. Methodology: Peripheral blood was collected from these individuals after obtaining written consent and subjected to DNA damage analysis. Chromosomal aberrations (CA), Cytoknesis blocked micronucleus assay and single cell gel electrophoresis assay (comet assay) were studied as biomarker for genotoxicity. Estimation of Cr and Ni content was done in whole blood by inductively coupled plasma atomic emission spectrometry (ICPAES), which revealed welders had higher Cr and Ni content when compared with controls. Complete blood profile was also undertaken in the welders. Chromosomal aberrations were scored by totaling the chromatid breaks and gaps as well as chromosomal break and gaps. The Comet assay was carried out to quantify basal DNA damage. The mean comet tail length was used to measure double strand breaks and tail moment was measured for single strand breaks. Results and Conclusion: The results indicated that the welders had a larger mean comet tail length than that of the controls ($p<0.001$) and increased CA. Frequency of micronucleus was significantly ($p<0.01$) increased in welders when compared to unexposed individuals. Analysis of variance revealed that occupational exposure ($P < 0.05$) had a significant effect on DNA damage, whereas smoking and age had no significant effect on DNA damage. The current study suggested that degree of DNA damage is directly dependent on duration of exposure to Cr and Ni in ambient air and drinking water at workplaces.

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## 1945/T/Poster Board #494

**Genome-wide Screening for alternative Otosclerosis Gene Loci.** *R. Birkenhäger, N. Lüblinghoff, K. Pohlmann, E. Prera, M. Petridi, R. Laszig.* Department of Oto-Rhino-Laryngology, Head and Neck Surgery, University Medical Center Freiburg, Killianstrasse 5, D-79106 Freiburg, Germany.

Background: Otosclerosis is one of the most frequent reason of hearing loss in adults and is caused by abnormal bone homeostasis of the otic capsule that leads to bony fixation of the stapedial footplate in the oval window. Impaired ossicular chain mobility results in a conductive hearing loss. In about 10% of all cases, an additional cochlear component develops, which represents most likely the encroachment of otosclerotic foci on the labyrinthine capsule that leads to a mixed or sensorineural hearing loss (SHL). The etiology of otosclerosis is unclear, although several hypotheses implicate genetic, viral, immunological, hormonal or endocrine factors. Epidemiological studies have indicated autosomal dominant inheritance with variable and reduced penetrance. So far seven loci (OTSC1-5, 6 & 7) for otosclerosis have been described, located on chromosomes 15q, 7q, 6p, 16q, 3q and 6q, but none of the genes have been identified. An alternative genetic approach, candidate-gene association studies, suggest modestly evidence that the genes COL1A1,COl1A2, COL2A1 and TGFβ1 contribute to disease development. Recently a genome-wide analysis identified single nucleotide polymorphisms (SNP) in the RELN gene associated with otosclerosis. In this study we performed a whole genome haplotype analysis of a large familiy to identify novel or verify known OTSC loci. Additionally we have accomplished SNP analysis in a German population at the TGFβ1 and RELN gene loci. Methods: All patients considered for this project had a proven otosclerosis and underwent stapes microsurgery. The familiy used for the genome-wide screen is a large South-East European family with 52 members (18 affected). The genome-wide haplotype analysis was applied using the Affymetrix 500 K SNP-Array. For SNP analysis at the TGFβ1 and RELN gene loci 329 patients, mainly of a German population, and a case control group were used. For statistical analysis the Bonferonni correction was used. Results: With the genome-wide haplotype analysis of a large family with 18 affected members it was possible to identified alternative gene loci for otosclerosis. SNP analysis in a German population showed in contrast to the literature no significant association to the TGFβ1 loci. Conclusion: Genetic factors play a significant role in the etiology of otosclerosis. Identification of these genes could lead to substantial improvements in the ability to diagnose and possibly even prevent this type of hearing impairment.

## 1946/T/Poster Board #495

**Auditory and Genetic Characterization of a Large American Pedigree with Inherited, Progressive, Post-lingual, Sensorineural Hearing Loss.** *V. Street[1], E. Thilo[2], P. Strombom[3].* 1) Bloedel Hearing Res Ctr, Univ Washington, Seattle, WA; 2) Neurobiology Program, Dept of Biology University of Washington Seattle,WA; 3) School of Medicine Tufts University Boston, MA.

Large families with monogenic non-syndromic hearing loss have allowed the mapping of at least 100 auditory related deafness (DFN) chromosomal loci. These loci have greatly enhanced our understanding of how the cochlea functions when receiving information during normal and pathogenic states. Here, we characterize the auditory and genetic features of a large American pedigree (referred to as the HL4 family, HL for hearing loss) segregating the autosomal dominant trait of progressive, post-lingual, sensorineural hearing loss that first impacts the higher auditory frequencies. Members of the HL4 family begin to notice hearing loss in their late teens to early twenties. In general, similarly-aged HL4 family members have comparable audiogram contours. To appreciate more fully the rate of hearing loss in the HL4 family with each decade of life, an audioprofile was constructed. Audiogram data for 21 affected individuals was plotted in Excel as level of hearing loss versus age with each frequency representing a unique data set. Using the trend line graph feature, best-fit regression lines were fit to the data. Equations of these regression lines were used to calculate expected hearing loss for ages 10-70 in ten year steps. These predictions were then plotted as hearing loss versus frequency with each line representing a different age group. The downward sloping audiogram configuration seen in affected HL4 family members shares features with audiograms for hearing impaired families with mutations in the DFNA2 (KCNQ4), DFNA5, and DFNA20/26 (ACTG1) loci/genes. Using 31 DNA samples from the HL4 family, linkage to DFNA 2, 5, and 20/26 were tested and excluded. Linkage has also been excluded to chromosomes 1, 5, 6, 7, 8, 17, and 18 representing approximately 36% of the genome.

## 1947/T/Poster Board #496

**SNPsniffer: Mining for gene candidates in the "genetic" vicinity of SNPs associated with disease.** *K. Elliott.* Wellcome Trust Centre Human Genetics, University of Oxford, United Kingdom.

The raft of recent genome wide association studies has identified hundreds of SNPs associated with common human diseases. However, only a small fraction of these have been proven to be the functional variants giving rise to disease. Many publications detailing potential candidate genes in the vicinity of these SNPs will report the most proximal gene which is not necessarily the best candidate or likely contain the associated functional variant. Given that linkage disequilibrium (LD) may extend for 100s of kb the functional variant giving rise to the association signal may affect a gene that is some distance away and several genes removed from the signal SNP. To complicate matters further functional elements controlling gene expression may be 100ks of kb away. An example is the region of LD surrounding the type 2 diabetes associated SNP rs5015480 which contains two compelling candidate genes, HHEX adjacent to the SNP and IDE which is 2 genes away. This type of scenario lead to the development of a tool, SNPsniffer, which provides an informed assessment of the LD structure of the region surrounding SNPs and the genes harboured within. This tool defines the region +/- 0.1 cM of the associated SNP which has an 85% likelihood of containing the linked functional variant (assuming the SNP follows HapMap reported patterns of LD). 50kb flanks are added to allow for close-by genes that may have regulatory variants within this region (eQTL studies suggest >90% of functional variants fall within the gene plus 50kb flanking regions). For each of the genes in the region the program interrogates information from NCBI's Gene, OMIM, PubMed, UniGene, HomoloGene, BLAST and Jackson's MGI databases, using a list of weighted disease-specific keywords, and a disease relevance score is generated. The output generated is in easily navigable HTML pages with positions of 0.1 cM boundaries and the SNP relative to surrounding genes. Of the 207 SNPs assessed, an average of 6.4 genes were found to be within the region defined by the tool and where there was more than one gene, 67% of genes flanking or spanning the SNP were not the best functional candidate as scored by the program. SNPsniffer provides a useful visualization of functional neighbourhood of the gene in the context of LD architecture and will provide researchers with important information in selecting SNPs for further study and reporting the functional candidacy of SNPs associated with disease.

## 1948/T/Poster Board #497

**A novel locus for autosomal dominant Retinitis Pigmentosa (RP35) in a family of German origin.** *Q. Prescott[1], C. Chakarova[1], A. Shah[1], N. Waseem[1], E. Zrenner[2], S. Bhattacharya[1].* 1) Dept of Genetics, Inst Ophthalmology, London, United Kingdom; 2) Molecular Genetics Laboratory, Institute for Ophthalmic Research, Centre for Ophthalmology, University Tübingen, Tübingen, Germany.

Purpose: To map a novel locus for autosomal dominant Retinitis Pigmentosa (adRP) and to identify the disease causing gene using a large three generation family of German origin. Methods: 17 individuals (7 affected, 9 unaffected members and one spouse) from the family underwent standard ophthalmological evaluations at the Institute for Ophthalmic Research, Tubingen, Germany. All previously known loci for adRP were excluded using two microsatellite markers flanking the disease gene at each locus. In parallel, a DNA of one affected individual was loaded on ASPER Chip for direct detection for known adRP mutations. Finally we undertook further linkage analysis by hybridizing all family DNAs to 50K Affymetrix SNP arrays. The genotypes were assigned after quality control screening with Affymetrix Genotyping Consol 3.0.2. Erroneous genotypes were cleaned with Alhomora and Pedcheck. Results: The data was analyzed with Genehunter using two point analysis between the disease and marker locus. Several regions with reasonably high LOD scores (approaching 3.00) were obtained. Given the size of the family a near maximum LOD score of 2.99 ($\theta$=0) was obtained at a novel locus in the human genome. This was confirmed with corresponding ABI Microsatellite markers from that region. Additional SNP analysis narrowed down the critical interval to 5.4 Mb. Additional families with adRP tested for linkage to this locus could not further refine the region. Conclusion: We have identified a family with autosomal dominant Retinitis Pigmentosa that clearly demonstrates the presence of a new RP locus in the human genome (RP35). Further investigation will use NimbleGen Sequence capture services where 5.4 Mb of selected region will be enriched for next generation sequencing on the ABI SOLiD sequencing platform. To evaluate this new approach a known TOPORS mutation (c.2474_2475insA) in a patient DNA will be used as a positive control. Detailed confirmation and characterization of any mutation found will be checked for segregation in this RP35 family and against ECCAC panels of normal controls.

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## 1949/T/Poster Board #498

**Mucolipidosis IIIA in a Chinese family associated with GNPTAB mutation.** *T. Zhan, X. Cui, Y. Liu, J. Zhang, Q. Wang, M. Liu.* Center for Human Genome Research and College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, Hubei, China.

ML III alpha/beta (ML IIIA, OMIM 252600), one of the lysosomal storage diseases, is also known as autosomal recessive inherited disease caused by absence or reduction of lysosomal enzyme N-acetylglucosamine-1-phosphotransferase (GlcNAc -phosphotransferase, GlcNAc-PT), result in the abnormal of the enzymes entry the lysosomal. GlcNac-1-phosphotransferase exists as a heterohexamer comprising three subunits, $\alpha/\beta$ encoded by GNPTAB and $\gamma$ encoded by GNPTAG, respectively. The $\alpha$ /$\beta$ subunits contain the catalytic site of the enzyme and are encoded by the GNPTAB gene. Mutations in the GNPTAB gene can cause ML IIIA. In this report, we identified and characterized a four-generation Chinese family, in which 4 patients display symptom of Mucolipidosis. Linkage and haplotype analysis mapped the disease gene of this Chinese family to chromosome 12 with a maximum LOD score of 2.64 at marker D20S112, where GNPTAB habored. DNA sequence for the whole coding region and exon-intron boundries of GNPTAB revealed compound heterozygous mutations in the family, a splicing mutation IVS13+1G>A (c.2715+1G>A), and a novel nonsense mutation p.R364X (c.1090C>T). All 4 patients in the family carry both of the GNPTAB mutations. The novel p.R364X mutation did not exist in unaffected family members and 226 normal controls. The IVS13+1G>A (c.2715+1G>A) mutation causes skipping of exon 12 directly onto exon 14 due to a deletion of 1103bp of exon 13 as previous reports, while p.R364X truncates the protein in the $\alpha$-subunit, yielding truncated $\alpha$-subunit and no $\beta$ -subunit of GNPTAB. This study is the first report that compound heterozygotes of mutations in GNPTAB may cause MLIIIA in Chinese and it expands the spectrum of mutations in GNPTAB causing MLIIIA. Further studies for the two mutations might help to understand the function of GNPTAB, and the relationship between GNPTAB mutations and the phenotypes of this disease.

## 1950/T/Poster Board #499

**Mapping a gene for Chiari I malformation in a single family: the identification of candidate haplotypes.** *D.A. Dyment[1], J. Parboosingh[1], L. Maclaren[1], J.N. Scott[2], B. Fernandez[3], A.M. Innes[1].* 1) University of Calgary, Calgary, Alberta, Canada; 2) Department of Diagnostic Imaging, University of Calgary, Calgary, Alberta; 3) Department of Medical Genetics, University of Memorial, St. Johns, Newfoundland.

Chiari I malformation (CM1) is the caudal displacement of the cerebellar tonsils >5mm past the foramen magnum. CM1 is thought to arise from a mesodermal defect during the development of the posterior fossa. There have been reports of families with multiple affected individuals as well as monozygotic twin pairs concordant for CM1 suggesting heritable forms of the condition. In addition, CM1 is seen repeatedly in association with several genetic syndromes including connective tissue disorders, disorders of the RAS pathway and craniosynostosis syndromes. Individuals with cytogenetic imbalances also have been reported with CM1. A naturally occurring animal model exists as CM1 is frequent in King Charles Cavalier spaniels. Despite this, no genes have yet been identified to cause non-syndromic CM1. We present a 3 generation family segregating isolated CM1 as an autosomal dominant disorder with incomplete penetrance. There are 10 individuals affected with CM1 (cerebellar tonsils >5mm) and another 3 cases with borderline CM1 (cerebellar tonsils 3-5mm). Twenty-three family members were genotyped for 6081 SNP's across the genome. A non-parametric analysis in a subset of the pedigree showed multiple regions with evidence for linkage LOD>2 and one region with significant linkage; LOD=3.55 at chromosome 19p12. The identification of the putative CM1 gene would further our understanding of the development of the posterior fossa and provide valuable information to CM1 patients and their families.

## 1951/T/Poster Board #500

**Genome-wide Linkage Analyses of Hereditary Prostate Cancer Families with One or More Members with Colon Cancer Provides Further Evidence for a Prostate Cancer Susceptibility Locus on 15q13-q14.** *L.M. FitzGerald[1], S.K. McDonnell[2], E.E. Carlson[2], W. Langeberg[1], L.M. McIntosh[1], K. Deutsch[3], L. Hood[3], E.A. Ostrander[4], D.J. Schaid[2], J.L. Stanford[1,5].* 1) Prostate Cancer Studies, Fred Hutchinson CRC, Seattle, WA; 2) Division of Biostatistics, Mayo Clinic, Rochester, 55905, USA; 3) Institute for Systems Biology, Seattle, 98103, USA; 4) Cancer Genetics Branch, NHGRI, National Institutes of Health, Bethesda, 20892, USA; 5) Department of Epidemiology, School of Public Health, University of Washington, Seattle, 98195, USA.

The search for susceptibility loci in hereditary prostate cancer (HPC) has proven challenging due to genetic and disease heterogeneity. One method used to increase genetic homogeneity is to stratify family collections based on the occurrence of other cancers. This method has the advantage of not only increasing the likelihood of detecting prostate cancer susceptibility loci, but may also identify loci with pleiotropic effects. We have identified and performed linkage analyses on members of 100 HPC families with one or more first-degree relatives with colon cancer. Pedigrees were stratified into families with one or more colon cancer case (n=100) and families with two or more colon cancer cases (n=28). These subsets were analyzed for 'prostate cancer' and 'prostate or colon cancer'. When analysed for 'prostate cancer', suggestive evidence for linkage (LOD≥1.86) was identified at 11q21, 18q21 and 19q13 in families with one or more colon cancer cases, and at 1p34, 15q13-q14 and 19q13 in families with two or more colon cancer cases. When analysed for 'prostate or colon cancer', suggestive evidence for linkage was identified at 11q21-q25, 15q14 and 18q21 in families with one or more colon cancer cases, and at 11q14, 13q32, 15q14 and 21q22 in families with two or more colon cancer cases. Stronger evidence for linkage was identified at 15q14 when analysing the 'prostate cancer' (recessive HLOD=3.33) and the 'prostate or colon cancer' (recessive HLOD=3.74) phenotypes in families with two or more colon cancer cases. The results presented here provide further suggestive evidence for prostate cancer susceptibility loci on chromosomes 11q14, 15q13-q14 and 19q13 and highlight loci at 11q21, 15q13-q14 and 18q21 as having possible pleiotropic effects. This study demonstrates the benefit of utilizing a comprehensive family history of other cancers to create more genetically homogenous collections of HPC pedigrees for linkage analyses.

## 1952/T/Poster Board #501

**Unraveling the LD Association Signal in a GWA Study of Lung Cancer.** *P. Scheet, M.R. Spitz, C.I. Amos.* Epidemiology, M. D. Anderson Cancer Center, Houston, TX.

Initial findings from genome-wide association (GWA) studies conducted in European samples may produce broad association signals, covering multiple major loci. Here we attempt to tease apart the linkage disequilibrium (LD) signal from one such study of lung cancer. The initial GWA study was conducted on 1,154 cases and 1,137 controls, all of European descent, from which an association peak was discovered on 15q25. To further refine the signal, 34 SNPs from this region were measured in 870 African American samples. A strongly associated SNP (rs16969968) in the region is fixed in African populations. One haplotype group strongly differentiates the cases and controls, and rs16969968 exists on 60% of these haplotypes. Here, we investigate the following potential sources of association signal: large segments of European ancestry in chromosomes from African American sample; a haplotype common in individuals of European ancestry; and additional SNPs in the region (typed or untyped). To do so, we use a model for haplotype variation and imputation-based LD mapping to condition on these various sources and look for residual effects.

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## 1953/T/Poster Board #502

**Genome-Wide Linkage Scan for Prostate Cancer Susceptibility in Finland: Evidence for Novel Loci on 4q13, 14q32 and 15q26 and confirmation of signal on 17q21-22.** *T. Wahlfors[1], A. George[2,3], H. Nati[1], C. Simpson[2], C.D. Cropp[2], T. Tammela[4], J. Bailey-Wilson[2], J. Schleutker[1].* 1) Institute of Medical Technology, University of Tampere and Tampere University Hospital, Tampere, Finland; 2) National Human Genome Research Institute, National Institutes of Health, Baltimore, Maryland; 3) Fox Chase Cancer Center, Philadelphia, Pennsylvania; 4) Department of Urology, Tampere University Hospital, University of Tampere, Tampere, Finland.

Prostate cancer (PRCA) genome-wide-linkage (GWL) studies have been used to localize rare and highly penetrant cancer susceptibility genes. Linkage studies performed in different ethnic backgrounds and populations have been somewhat disparate, resulting in multiple, often irreproducible signals because of genetic heterogeneity and high sporadic background of the disease. Our first GWL (GWL-I) study identified two novel loci with just 10 analyzed families. Here, we conducted a second genome-wide linkage (GWL-II) scan with 413 microsatellite markers in 44 new Finnish prostate cancer families. All GWL-II families had at least three affected individuals per family and had not been previously analyzed.. GENEHUNTER-PLUS was used for parametric and non-parametric GWL-II analyses. Additionally, to further dissect the linked loci, detailed clinical information was used in an Ordered Subset Analysis (OSA). The results of our GWL-II study provided evidence of linkage to sites on 15q26, 4q13, 17q21-22, and 14q32. OSA analyses provided increased evidence of linkage to 17q21-22, 15q26, and 4q24. Next we analyzed the combined GWL-I and GWL-II families. We also analysed a subset of families from GWL-I and GWL-II with at least two aggressive PRCA patients who were not a parent-offspring pair. The combined GWS-I/GWS-II analysis gave evidence of linkage to nine HPC linkage peaks; namely 17q21-22, 10q22, 14q32, 4q22-23, 4q25, 3q25-26.3, 15q26, 13q34, 6q12-16. Genome-wide multipoint analysis using GENEHUNTER-PLUS gave the highest HLOD score of 3.62 ($\alpha$=0.9191) at markers on 17q21-22 with a corresponding maximum NPL score of 2.92 (p=0.002) and homogeneity multipoint LOD = 3.61 at the same location. These results are noteworthy since linkage to this region has been previously observed and reported in studies of various populations. Subset results of “aggressive” pedigrees demonstrated lower evidence of linkage than when all families were analyzed together. Fine-mapping analysis of these linked regions is ongoing and will be performed using TaqMan® OpenArray Genotyping System. Furthermore, candidate genes in top ranked linked loci are currently under analysis.

## 1954/T/Poster Board #503

**A novel locus for autosomal dominant cone-rod dystrophy in a family of Gypsy origin.** *K. Kamenarova[1], S. Cherninkova[2], Q. Prescott[3], M. Romero Durán[1], A. Krishna[1], L. Valdés Sánchez[1], A. Oscar[2], R. Kaneva[4], I. Kremensky[4], C. Chakarova[3], I. Tournev[5], S. Bhattacharya[1].* 1) Department of Cellular Therapy, CSIC CABIMER, Seville, Seville, Spain; 2) Department of Neurology and Ophthalmology, University Alexandrovska Hospital, Sofia, Bulgaria; 3) Department of Genetics, Institute of Ophthalmology, UCL, London, UK; 4) Molecular Medicine Center, Medical University-Sofia, Sofia, Bulgaria; 5) Department of Neurology, University Alexandrovska Hospital, Sofia, Bulgaria.

**Methods:** 16 individuals (6 affected, 8 unaffected members and two spouses) from the family underwent standard ophthalmological evaluations at the Department of Neurology and Ophthalmology, University Alexandrovska Hospital, Sofia, Bulgaria. The phenotype was analyzed as an autosomal dominant trait with complete penetrance (0.9990) and a frequency of 0.0001. All previously known loci for adCRD were excluded using two microsatellite markers flanking the disease gene and at least one inner for each locus. This was followed by a genome-wide scan using microsatellite markers (ABI PRISM ® Linkage Mapping Sets V2.5). The data were used to calculate the LOD scores using the program MLINK for two point analysis. To create the haplotypes the data were converted for Genehunter and the erroneous genotypes were cleaned by Pedcheck. The software searched for the common regions shared between the affected individuals in the family and evidence for linkage to a genome interval was obtained. **Results:** The genome-wide search led to the identification of several regions with positive LOD scores approaching 1.5 and only one region gave a LOD score of 2.40 ($\theta$ = 0.0). All affected individuals shared a common haplotype when tested with a number of microsatellite markers spanning the linked region. This tentatively defines a novel locus for adCRD in the human genome. SNP analysis narrowed down the critical interval to 7.2 Mb. Several candidate genes within the tentatively linked region were screened for mutations by direct sequencing but so far no changes that segregate with the disease have been found. **Conclusion:** We have identified a family with autosomal dominant cone-rod dystrophy that is strongly suggestive for a new adCRD locus in the human genome, and is the only locus for adCRD known to map in this region. There are 45 genes in the critical interval that remain to be sequenced to identify the pathological change that cause this type of adCRD.

## 1955/T/Poster Board #504

**The Hunt for the Causative Gene in the *GLC1B* Locus.** *R. Sharafieh[1,2], A. Child[2], M. Sarfarazi[1].* 1) University of Connecticut Health Center, Farmington, CT; 2) St. George’s, University of London, London, U.K.

Purpose: The original *GLC1B* locus was mapped to the 2p11.2-q12.2 region, flanked by D2S2161 (2p11.2) and D2S176 (2q12.2) markers. Since then, other groups have confirmed and may have additionally reduced the area of interest to 2q11-q12, between markers D2S1381 and D2S176, a 6.66Mb region harboring a total of 37 genes. We utilized association studies to narrow down the number of genes to be screened in hopes of finding the putative gene(s) responsible for primary open-angle glaucoma (POAG) within the *GLC1B* region. Methods: Highly polymorphic SNP markers from the 37 candidate genes were selected using the Hapmap database. SNP genotyping was carried out with the ABI-SNaPshot Multiplex System and direct DNA sequencing was performed on an ABI-3100 machine employing 380 British subjects (190 POAG and 190 normal controls). Genotypic data was calculated for all subjects and statistically evaluated with SNP-STAT and PLINKS programs. Finally, genes with significant association were chosen for gene screening using sequence specific primers flanking the intron-exon boundaries for PCR amplification and direct DNA sequencing. Results: A two stage approach was implemented for gathering the genotypic data. The detection phase used 95 POAG subjects that included 30 probands from families with prior consistent linkage to the *GLC1B* locus. Our initial statistical analysis showed that genotypic or allelic association studies were significant for 10 of the 37 genes investigated. The authentication phase used an additional 95 POAG and a total of 190 matched normal control subjects. These 380 subjects were genotyped for SNP markers mapping to 6 of those genes (*RNF149, CREG2, IL1RL1, TBC1D8, C2orf29, PDCL3*). From this new round of case/control association study, rs13151 in the *RNF149* gene showed statistically significant (p=0.0077; OR=1.75). We sequenced this gene in 98 POAG families and observed 19 variations. The only sequence change in exon-1 (N139S) did not segregate in the two original families identified in. Selection of other SNP markers from other areas of these 37 genes and generation of their genotypic data in the same group of POAG and normal subjects are planned for the near future. Conclusions: Merging analysis of genetic linkage data with targeted gene association studies may accelerate in the identification of potential genes for a large candidate region. The application for this method in the *GLC1B* locus is being further investigated.

## 1956/T/Poster Board #505

**Replication of *MTNR1B* polymorphism with fasting glucose in Hong Kong Chinese population.** *C. Tam, J. Ho, W. So, R. Ma, J. Chan.* Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong, China.

***Background***: Recent genome-wide association studies in European populations suggest that melatonin receptor 1B (*MTNR1B*) gene located on chromosome 11q21-q22 is strongly associated with fasting glucose concentrations and glucose-stimulated insulin secretion, as well as predicts future type 2 diabetes (T2D). *MTNR1B* encodes one of the two high-affinity G-protein-coupled receptors for the pineal gland hormone melatonin which had previously been suggested to be involved in pathogenesis of T2D. In this study, we aim to replicate the association of the common *MTNR1B* rs10830963 polymorphism with extensive metabolic traits in Hong Kong Chinese population.

***Methods***: The single nucleotide polymorphism was genotyped in 583 adults [age mean ± SD = 41.4 ± 11 years, % males = 45] and 1065 adolescents [age mean ± SD = 15.4 ± 2 years, % males = 46] that participate in a health screening program. Associations of rs10830963 at additive model with metabolic traits including body mass index (BMI), systolic and diastolic blood pressure, lipids (total cholesterol, triglyceride, HDL, LDL) as well as fasting glucose were assessed by linear regression adjusted for covariates age and sex.

***Results***: Based on our findings, the reported risk allele G of rs10830963 is consistently and significantly associated with an increased concentration of fasting glucose (P = 0.004, mean ± SD = 4.80 ± 0.38 mmol/l for GG carriers, 4.79 ± 0.38 mmol/l for GC carriers, 4.74 ± 0.38 mmol/l for CC carriers). However, we did not observe any association between rs10830963 and other metabolic traits.

***Conclusions***: In summary, our study support *MTNR1B* as a susceptibility locus influencing fasting glucose in Chinese population.

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## 1957/T/Poster Board #506

**Analysis of copy number changes in Aicardi syndrome.** *N. Miyake[1], M. Kato[2], H. Saitsu[1], T. Mizuguchi[1], N. Matsumoto[1].* 1) Department of Human Genetics, Yokohama City University Graduate School of Medicine; 2) Department of Pediatrics, Yamagata University School of Medicine.

Aicardi syndrome [OMIM #304050] (AIC) is a congenital disorder characterized by the triads of agenesis of the corpus callosum, chorioretinal lacunae, and infantile spasms. The exact frequency of this disease is not known, but more than 450 cases have been already reported. AIC is considered as an X-linked dominant disorder, because almost all of AIC cases are female (supposedly male-lethal). It is very difficult to perform the linkage analysis which requires the pedigrees, because majority is sporadic and severely affected since birth with poor reproductive fitness. The causative gene has not been isolated even though the AIC gene was mapped to Xp22, based on a case showing de novo balanced 3/X translocation (46,X,t(X;3)(p22;q12)). Positional cloning from chromosomal abnormalities including copy number changes (CNCs) would be a practical approach for the AIC gene. In this study, we analyzed 23 Japanese AIC cases (2 male and 21 female) using Gene Chip Human Mapping 250K NSP1 Array (Affymetrics) to detect CNCs. CNCs were confirmed using FISH and /or quantitative PCR. Parental samples were also checked if available. We could not find any causative CNCs. Higher resolution arrays would be a next choice. All the following clinicians are highly appreciated for their participation to this work; Drs. Hirano S, Yoshikawa H, Abe S, Fujimoto S, Ando N, Shigetomo R, Suenaga N, Sugita K, Sudo A, Ono H, Akaboshi S, Tanaka R, Osaka H, Hirai S, Tokunaga Y, Inui T, Koshino S, Takahashi Y.

## 1958/T/Poster Board #507

**A genome-wide linkage scan for blood lipid phenotypes in the Khatri Sikh Diabetes Study (SDS).** *D.K. Sanghera[1,2], M.F. Begum[3], A. Mukerjee[4], S. Ralhan[5], G.S. Wander[5], N.K. Mehra[6], J.R. Singh[7], R.E. Ferrell[4], M.I. Kamboh[4], D.E. Weeks[3,4].* 1) Dept Pediatrics, Section of Genetics, College of Medicine, Univ Oklahoma HSC, Oklahoma, OK; 2) College of Pharmacy, Univ Oklahoma HSC, Oklahoma, OK; 3) Dept. Biostatistics, Univ Pittsburgh, PA; 4) Dept Human Genetics, Univ Pittsburgh, PA; 5) Section of Cardiology, Hero DMC Heart Instititute, Ludhiana, India; 6) Dept Transplant Immunology and Immuno-genetics, All India Institute of Medical Sciences and Research, New Delhi, India; 7) Center for Genetic Disorders, Guru Nanak Dev University, Amritsar, India.

In this investigation, we have carried out an autosomal genome-wide linkage analysis using robust score statistics to map genes for five quantitative traits of blood lipids including total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) cholesterol, and triglycerides in a unique family-based cohort from the Sikh Diabetes Study (SDS). A total of 870 individuals (526 male/344 female) from 321 pedigrees were successfully genotyped using 398 polymorphic microsatellite markers with an average spacing of 9.26 cM on the autosomes. A genome-wide linkage scan using QTL-ALL analysis revealed promising linkage signals with p-values ≤ 0.005 for total cholesterol, LDL cholesterol, and HDL cholesterol at chromosomes 5p15, 9q21, 10q21, 10p11, and 22q13. The most significant signal (p-value=0.0011) occurred at 10q21.2 for HDL cholesterol. We also observed linkage signals for total cholesterol at 22q13.32 (p-value=0.0016) and 5p15.33 (p-value=0.0031) and for LDL cholesterol at 10p11.23 (p-value=0.0045). Most of these identified regions in this Sikh population have been linked to lipid-related traits in previous studies and also contain other plausible candidate genes. Although these regions are not part of the loci detected for quantitative lipid traits by recent genome-wide association studies (GWAS) performed in Caucasians, convincing replication of these results from the findings of prior independent linkage studies suggests that these regions might contain novel susceptibility loci for serum lipids. Further denser and more informative genotyping in each of these regions would be important to discover functional loci influencing blood lipids. Our study represents the first large scale genome-wide effort to identify chromosomal regions with putative loci affecting lipid levels in a community of Khatri Sikhs from Northern India.

## 1959/T/Poster Board #508

**Linkage of epilepsy plus cleft lip syndrome to 14p11.2.** *D. Bai, J.N. Bailey, M. Tanaka, R.M. Durón, A.V. Delgado-Escueta.* Neurology, UCLA David Geffen School of Medicine & VA GLAHS, Los Angeles, CA.

Epilepsies are the most common neurological disorders worldwide, affecting approximately 50 to 100 million persons. Children of epileptic mother suffer from cleft lip with/without cleft palate (CL/P) more frequently than children of non-epileptic mother. Mothers of children with CL/P have a higher prevalence of epilepsy. Epilepsies associated with CL/P have been attributed mostly to teratogenic affects of antiepileptic drugs. We studied a four generation Caucasian family with both epilepsy and CL/P. Thirty family members (eight affected and 22 unaffected) participated in this study. Among eight affected members, five suffered from epilepsies, one was affected with both epilepsy and CL/P and two had only CL/P. Parents of both members with only CL/P had no epilepsy and no history of taking antiepileptic drugs. A genome wide linkage scan was performed with Marshfield standard 10 cM genome scan set 16 under an autosomal dominant model with 50 percent penetrance. We observed significant linkage to 14q11.2 (D14S742) with a LOD score of 3.36. Linkage was suggestive to 10p13 (D10S1430) with LOD score of 2.62, to 7p21.3 (GATA119B03, or D7S2200) with a LOD score of 2.21 and to15q12 (D15S1513) with a LOD score of 1.74. These findings provide evidence of significant linkage between epilepsy plus CL/P and 14q11.2. Results also support the hypothesis of epilepsy plus CL/P, as a syndrome, that shares common genetic susceptibility in early neuronal development. In clinical practice, in addition to looking for teratogenic affects of antiepileptic drugs, we should also pay attention to genetic factors underlying CL/P born from epileptic mother.

## 1960/T/Poster Board #509

**A novel locus for autosomal dominant non-syndromic hearing impairment, maps to chromosome 12q24.** *j. cheng, y. zhu, b. han, y. sun, j. li, d. han, p. dai, h. yuan.* Inst. Of Otolaryngology, Chinese PLA General Hospital, beijing, China.

Hereditary non-syndromic sensorineural hearing loss is a genetically highly heterogeneous group of disorders. To date, at least 60 loci for autosomal dominant non-syndromic sensorineural hearing loss (DFNA) have been identified by linkage analysis. Here we report the mapping of a novel autosomal dominant deafness locus at 12q24 by studying a large multi-generational Chinese family with post-lingual, high-frequency hearing loss that progresses to involve all frequencies. Onset of hearing loss in all affected subjects occurred in the second through fourth decade of life. Genomewide linkage analysis was then performed using Affymetrix Genome-Wide Human SNP Array 5.0. Linkage analysis was carried out under a fully penetrant autosomal dominant mode of inheritance with no phenocopies. Heterogeneity LOD (HLOD) analysis reveals the evidence of genetic heterogeneity for DFNA and evidence of linkage in a subset of the families to 12q24 (HLOD=5.656). Microsatellites markers was using for refined mapping and get a maximum two-point LOD score of 5.35 at theta=0 obtained for marker D12S76. Haplotype analysis placed the novel locus within a 5.63 cM genetic interval defined by markers D2S86 and D2S1612 without overlapping with the other identified DFNA loci. DNA sequencing of coding regions and exon/intron boundaries of candidate genes in this interval is being conducted to reveal disease-causing mutation in this family.

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## 1961/T/Poster Board #510

**Genome-wide linkage analysis of sister pairs affected with uterine leiomyomata reveals locus on chromosome 3.** *S. Eggert[1], K. Huyck[4], P. Somasundaram[3], E. Stewart[5], A. Schnell[6], K. T.Cuenco[6], C. Morton[1,2,3].* 1) Department of Pathology, Harvard Medical School, Boston, MA; 2) Department of Pathology, Brigham and Women's Hospital, Boston, MA; 3) Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Boston, MA; 4) Department of Pathology, University of Vermont, Burlington, VT; 5) Mayo Clinic, Rochester, MN; 6) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA.

Uterine leiomyomata (UL), commonly known as fibroids, are the most prevalent pelvic tumors in women. UL pose a major public health problem given their rate of prevalence (>70%) and symptoms (~20-25%) in women of reproductive age and their indication for >200,000 hysterectomies in the U.S. annually. A genetic basis for tumor development is supported by the observation of recurrent chromosomal aberrations. Further, analyses of ethnic predisposition, twin studies, and familial aggregation indicate a genetic component to UL predisposition, but no genome-wide study for heritable variants has been reported. A genome-wide SNP linkage panel was applied to 385 UL sister pair families, and a suggestive peak on chromosome 3 was detected by nonparametric linkage analyses (NPL = 3.08, rs954282). The genomic region of interest, as defined by NPL scores >2, extends 20 Mb (rs12629588 to rs11720298) and contains ~250 genes. This same region is also associated with height and age at first menarche, traits associated with UL. These findings concur with previous reports relating early menarche with shorter stature and increased risk of UL. Additional SNP fine mapping and sequencing across this region will be performed in cases and controls to identify possible causal variants associated with UL predisposition. Further understanding of genetic variants associated with UL will provide insight into the biology of tumor development and may lead to improved management or novel therapy.

## 1962/T/Poster Board #511

**Mapping of a novel locus for hereditary gingival overgrowth to chromosome 7.** *P.S. Hart[1], D. Pallos[4], S.T. Han[2], P. Sulima[2], T. Wu[3], R. Muralidharan[2], S.I. Jang[2], T.C. Hart[2].* 1) Office of the Clinical Director, NHGRI, NIH, Bethesda, MD; 2) Human Craniofacial Genetics Section, NIDCR, NIH, Bethesda MD; 3) CIT, NIH, Bethesda MD; 4) Department of Periodontology, University of Taubaté, São Paulo, Brazil.

Hereditary Gingival fibromatosis (HGF) is a benign overgrowth of the keratinized gingiva and can occur as an isolated or syndromic trait. Genetic heterogeneity has been documented for the isolated, non-syndromic (NS) forms, with at least 4 loci (mapping to 2p22, 2p23.3, 5q13-q22, 11p15) localized by linkage studies. Mutation of the SOS1 gene on 2p22 is the only causative gene identified to date for NS- HGF. The mutant SOS1 protein constitutively activates the MAP kinase signaling pathway, and is associated with increased fibroblast proliferation. A four generation Brazilian family segregating NS autosomal dominant HGF was ascertained in accordance with institutional review board approval from the corresponding university and the NIH. Diagnosis was based on the presence of isolated gingival overgrowth. A total of 64 members were available for this study, including 30 affected individuals. Sequence analysis of SOS1 revealed no mutations. Linkage analysis identified a 14 Mb region on chromosome 7q, bounded by D7S684 and D7S798, representing a novel locus. The maximum two-point LOD score of 8.44 was obtained at D7S688, while a multipoint LOD score of >10.0 was obtained for the interval. Several genes, including *RHEB, BRAF, CDK5* and *CSGlcA-T*, map to this interval and were selected as initial candidate genes based upon involvement in Ras signaling, cell growth or cell cycle regulation or transition. Sequence analysis of the full coding region, exon/intron junctions, and 500 bp of the promoter of more than 40 genes in the candidate region failed to reveal any mutations, but did reveal several novel sequence variants. For example, sequencing of *CSGlcA-T* revealed a novel SNP in the 5' UTR. This SNP segregated with the phenotype but was also found in 14% of ethnically matched controls, supporting its classification as a polymorphism. Subsequently, gene array and transcriptome profiling have indicated altered expression of several genes in the candidate interval, primarily related to regulation of cellular processes, particularly cellular adhesion and transporter activities. Sequencing analysis of the differently regulated genes in the interval is ongoing. This study identifies a new locus for HGF, further documenting genetic heterogeneity for this condition.

## 1963/T/Poster Board #512

**Heritability and Linkage of Serum Homocysteine in Caribbean Hispanics: The Family Study of Stroke Risk and Carotid Atherosclerosis.** *S. Slifer[1], D. Della-Morte[2], A. Beecham[1], B. Boden-Albala[3], M.S. McClendon[2], T. Rundek[2], S.H. Blanton[1], R.L. Sacco[2].* 1) Miami Inst for Human Genomics, Univ Miami, Miami, FL; 2) Dept of Neurology, Univ Miami, Miami, FL; 3) Division of Stroke, Dept of Neurology, Columbia Univ, New York, NY.

Elevated plasma total homocysteine(Hcy) levels are often associated with increased risk of ischemic heart disease and stroke. Since Hcy is a quantitative trait, variance components methods allow analysis of a more complete picture of the diagnostic spectrum than disease status alone. Importantly, although extensive candidate gene studies have identified several Hcy genetic variants such as MTHFR 677C-T, genes involved in Hcy have not been well characterized in the Caribbean Hispanic population.

Hispanic probands were selected from the Northern Manhattan Family Study (NOMAS). The resulting 100 families included 1362 individuals with all families having >80% of members of Dominican descent. A 10 cM genomic screen of 405 microsatellite markers was performed, using SOLAR to analyze Hcy as a quantitative trait while controlling for significant covariates in multipoint linkage analysis. One-third of the families had a majority of members enrolled in the Dominican Republic(DR), resulting in significant differences in diet and lifestyle. Such differences among individuals were accounted for with covariates in the linkage analysis while ordered subset analysis(OSA) was used to reduce possible phenotypic heterogeneity at a family level. After covariate adjustment, Hcy had an overall polygenic heritability of 0.44($p < 0.0001$). Ch 17 presented the most significant evidence for linkage at D17S2193(MLOD=2.66, empirical p=0.0005). OSA analysis based on families ranked by the proportion of individuals living in the DR gave significantly increased evidence for linkage(MLOD=3.92, p=0.0022) for 58 families with the highest DR enrollment. Interestingly, the second highest peak on ch 2 at D2S1356(MLOD=1.77, empirical p=0.0033) also increased after OSA analysis, this time after ranking families from lowest proportion of individuals enrolled in DR to highest(MLOD = 2.82, p = 0.0097) for 81 families with lowest DR enrollment. Analysis of Hcy as a quantitative trait may allow a more complete picture of cardiovascular risk. We identified two regions that showed suggestive linkage to Hcy levels in Caribbean Hispanic families from the DR. While individual environmental variation may be controlled by using covariates in the linkage analysis, further differences may persist at the family level, indicating possible heterogeneity of phenotypic Hcy levels.

## 1964/T/Poster Board #513

**Detection of a Hotspot for Mutations in the Third β-Strand of KITLG Responsible for Familial Progressive Hyperpigmentation.** *M. Amyere[1], T. Vogt[2], J. Hoo[3], F. Brandrup[4], M. Vikkula[1].* 1) Lab Human Molec Genetics, Christian de Duve Inst, Brussels, Belgium; 2) Department of Dermatology, University of Regensburg, Germany; 3) Department of Pediatrics, SUNY Upstate Medical University, Syracuse, New York; 4) Department of Dermatology, Odense University Hospital, Odense, Denmark.

Familial Progressive Hyperpigmentation (FPH) is an autosomal dominant disorder with reduced penetrance. The clinical signs consist of progressive diffuse, partly blotchy hyperpigmentation lesions, multiple café-au-lait spots, intermingled with scattered hypopigmented appearing maculae, and lentigenes. Histological and ultrastructural sections from the hyperpigmented lesions display strong basal hyperpigmentation of the epidermis with numerous melanophages containing large amounts of melanin. In contrast, the hypopigmented appearing macula show a slight basal hyperpigmentation of epidermis, but virtually no melanophages in the upper dermis (Zanardo et al., 2004). We performed a genome-wide linkage analysis in seven families using the Affymetrix SNP GeneChip Human Mapping Arrays, and identified significant linkage on 12q14.3-q22. Wang and collaborators reported a mutation in the KITLG gene on 12q in one Chinese family with FPH. We screened our affected family members and discovered a mutation in five of the families. The reported substitution Asn36Ser was found in three families living in the same area in Germany, and two novel substitutions, Val33Ala and Thr34Pro, in two other families. All the mutations cosegregated with the hyperpigmentation phenotype. Interestingly, all three mutations are located in the highly conserved third β-strand of the KITLG protein. This finding gives the first evidence for importance of this motif in the activation of the tyrosinase activity, which leads to increased melanin synthesis and FPH. (http://www.deduveinstitute.be/Vikkula) ; (miikka.vikkula@uclouvain.be).

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## 1965/T/Poster Board #514

**Molecular genetics study of anterior segment dysgeneses in Arab patients.** *M.A. Aldahmesh[1], A.O Khan[1,2], F. Alzahrani[1], F.S Alkuraya[1,3,4].* 1) Department of Genetics, Developmental genetics Unit, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia; 2) Department of Pediatric Ophthalmology, King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia; 3) Department of Pediatrics, King Khalid University Hospital and College of Medicine, King Saud University, Riyadh, Saudi Arabia; 4) Department of Anatomy and Cell Biology, College of Medicine, Alfaisal University, Riyadh, Saudi Arabia.

Anterior segment dysgenesis (ASD) (aniridia included) represents a heterogeneous group of ocular developmental abnormalities. The underlying genetic defect in the majority of ASD patients is still unknown. The purpose of this study is to systematically search for the underlying genetic defect in Saudi patients with ASD. We have enrolled a total of 36 individuals representing 21 families with phenotypes ranging from classical aniridia to the most complex forms of ASD. 4 approaches were used in this study: 1) Mutation analysis of genes known to cause ASD in humans 2) Mutation analysis of *CITED*-2 as a candidate gene based on the KO mouse phenotype 3) Genomewide analysis of copy number in all mutation-negative patients and 4) Homozygosity mapping, where applicable. Mutation analysis: 2 missense mutations in *PITX3* were identified in 2 sporadic patients with ASD phenotype. Although no point mutations in *PAX6* were identified, MLPA analysis revealed the presence of 2 deletion events (refined by Chip-based analysis, see below) involving the *PAX6* locus in 2 sporadic patients. No patients with *CITED*-2 mutations were identified. Copy number analysis: 250K SNP arrays revealed the presence of 1 duplication and 5 deletion events. 2 of the deletion events involved the PAX6 locus and were observed in patients with classical aniridia. Another deletion was also observed in association with classical aniridia that involves a genomic region 93Mb downstream of *PAX6*. A family of an affected mother and 3 affected children was found to cosegregate classical aniridia with a novel duplication in a dominant fashion. Finally, 2 deletion events involving chromosomes 2 and 9 were found to cosegregate with ASD in 2 families and they likely harbor novel ASD genes. Homozygosity Mapping: Using the same SNP arrays, we generated genomewide map of blocks of homozygosity in 2 consanguineous families. The first family with 9 affected individuals gave a sharp peak with high LOD score on chromosome 10 while the other family with 2 affected individuals revealed two peaks on chromosomes 2 and 12. Our analysis suggests that a more comprehensive genomic approach to patients with ASD is likely to increase the diagnostic yield. Using positional cloning (copy number analysis and linkage analysis), we have identified a number of candidate ASD intervals and work is underway to define the culprit genes/sequences within these loci.

## 1966/T/Poster Board #515

**Genome-wide Association in Model Organisms: Accounting for Relatedness.** *A. Skol[1], M. Abney[2], R. Cheng[2], A. Palmer[2].* 1) Med/Sec Gen Med, Univ Chicago, Chicago, IL; 2) Department of Human Genetics, Univ Chicago, Chicago, IL.

Advanced intercross lines (AIL) have been used to fine map quantitative trait loci (QTL) in a number of model organisms. We performed genome wide association analysis for sensitivity to methamphetamine using a combination of F2 and F34 mice. We used simulations to demonstrate that Type I (false positive) errors are dramatically inflated when the relationships among individuals are ignored; to address this concern we used statistical procedures that account for relatedness among individuals. We show that either permutation or gene dropping can be used to assess significance provided that relatedness is accounted for in the model. Our results identify multiple well localized genome-wide significant QTLs for sensitivity to methamphetamine using an analysis that combines data from the F2 and F34 populations. Our approach takes advantage of the greater power of F2 populations and the greater precision of F34 populations. We discuss how our methods might be extended to other laboratory populations (e.g. heterogenous stocks, outbred mice) where failure to account for genetic relatedness may also increase type I errors.

## 1967/T/Poster Board #516

**Fine-mapping of the FTO association signal in 3 thousand individuals of European and African descent.** *C. Dina[1,2], J.C. Chèvre[1], D. Meyre[1], D. Balding[4], P. Froguel[3], FTO group.* 1) UMR 8090, Lille Biol Inst, CNRS, Lille, France; 2) INSERM UMR915 CNRS ERL3147, Thorax Institute, Nantes, France; 3) Section of Genomic Medicine, Hammersmith Hospital, Imperial College London, Du Cane Road, London W12 0NN, United Kingdom; 4) Department of Epidemiology & Public Health Imperial College, St Mary's Campus.

Several association studies, either in extreme case-control samples or population-based have established very strong association of SNPs in intron 1 of the gene FTO with Body Mass Index and/or obesity. These SNPs are located within a 47 kb block overlapping exon 1, intron 1 and a portion of exon 2. We previously tested SNPs in the intronic highly conserved regions, and reported strongest association with SNP, rs1421085, but no putative functional polymorphism have been identified so far. While GWAS discovered numbers of genetic associations with various traits, the identification of the polymorphism(s) that cause this association is still uncommon. Furthermore, FTO locus showed only weak association in African descent populations. Using haplotype clustering methods, we first performed a fine-mapping analysis of the FTO locus to identify the underlying causal variant. Forty SNPs, spanning 100 kb which include the 47kb as well adjacent blocks were genotyped 2,446 controls and 1,935 obese adults and children. This design covers, with $r2 > 0.8$, all the HapMapSNPs displaying a MAF higher than 1% in this region. The distribution of posterior location probability, obtained using the HapCluster program, highlighted a 95% credible interval of 20 kb long. This region was sequenced in 90 individuals and 68 new unreported variants were discovered, along with 63, already described in databases. A total of 140 SNPs were selected for further genotyping in 9,000 individuals, either because of their high LD with associated SNP and to increase information on the haplotype structure. All individuals in this dataset were of European descent 220 of Senegalese origin and a selection of individuals of African descent in our different studies (196). We combined observed and imputed genotypes, inferred from the 90 individual panel, into an association study aimed at identifying variations displaying most significant association. A subset of highly significant SNPs was identified, including those already reported as well as newly identified SNPs. The results in the African descent samples were not significant and thus not conclusive to further select putatively causal SNPs. Nevertheless, the LD and allele frequency structure gives additional valuable information. In conclusion our fine mapping and deep sequencing approach identifed a subset of putatively functional SNPs located in FTO intron 1 was identified, whose evaluation of functional properties is in progress.

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## 1968/T/Poster Board #517

**Admixture Mapping of Sarcoidosis in African Americans.** *A.M. Levin[1], I. Datta[1], J. Yang[1], P.M. Mckeigue[2], C. Gray-Mcguire[3], M.C. Iannuzzi[4], B.A. Rybicki[1].* 1) Biostatistics and Research Epi, Henry Ford Health System, Detroit, MI; 2) MRC Human Genetics Unit, University of Edinburgh Western General Hospital, Edinburgh, UK; 3) Arthritis and Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK; 4) Department of Medicine, State University of New York Upstate Medical University, Syracuse, NY.

Sarcoidosis, a multi-organ granulomatous inflammatory disease, likely results from an exaggerated T cell response to an airborne antigen. A genetic predisposition has long been posited, and genome-wide linkage analyses and a recent genome-wide association study (GWAS) have identified regions of the genome harboring variants in specific candidate genes associated with susceptibility. With an incidence rate 3.7 fold higher than Caucasians, African Americans are more commonly affected by sarcoidosis, implying that African ancestry may play an important role in the etiology and pathogenesis of this disease. Therefore, we conducted the first sarcoidosis genome-wide scan to find regions of increased African origin that may harbor disease risk variants using a map of 1,231 single nucleotide polymorphisms (SNPs) highly informative for differentiating between African and Caucasian ancestry. We conducted our analysis using the admixture mapping program ADMIXMAP on genotypes of 1,301 unrelated individuals (1,014 cases and 287 controls). Overall, the mean estimates of African and European admixture proportions in this sample were 0.82 and 0.18, respectively. The global mean estimates of African ancestry were 0.83 in the cases and 0.81 in the controls (p=0.04). Our best evidence for African genomic regions associated with increased risk of sarcoidosis were found on chromosome 5p14.3-13.2 (SNP rs35397 p=0.005), 6p22.3-23.31 (SNP rs11962335 p=0.003), and 13q31.3-33.3 (SNP rs987531 p=0.004). Our best evidence for African genomic regions associated with decreased risk of sarcoidosis were found on chromosome 2p12-11.2 (SNP rs4852696 p=0.003), 2q35-37.1 (SNP rs6761192 p=0.006), 5q23.1-31.1 (SNP CV11532818 p=0.007), 6q23.2-25.1 (SNP rs1480642 p=0.001), and 8p21.3-p11.21(SNP rs1462906 p= 0.002). The ancestry-associated 5p locus fell within a region that we previously identified though linkage analysis. The 6p locus encompasses the HLA region, which contains genes previously shown to be associated with sarcoidosis risk. Further, both the 5p and 6q loci contain SNPs that were recently identified and validated to be associated with risk in a sarcoidosis GWAS of Caucasians. In summary, while none of our results achieved genome-wide significance, this first admixture mapping study of sarcoidosis in African Americans has corroborated prior evidence of loci associated with risk and may have pointed towards new sarcoidosis susceptibility regions.

## 1969/T/Poster Board #518

**MYH9 and non-diabetic kidney disease: narrowing the region of association by fine mapping.** *C. Winkler[1], G. Nelson[1], D. Bowden[2], B. Freedman[2], J. Kopp[3].* 1) Dept Molec Gen Epidemiology, SAIC, NCI-Frederick, Frederick, MD; 2) Wake Forest University, Raleigh, NC; 3) NIDDK, NIH, Bethesda, MD.

*MYH9* was previously identified as a major effect gene for HIV-associated nephropathy (HIVAN), idiopathic focal segmental glomerulosclerosis (I-FSGS) and as a risk factor for non-diabetic end stage renal disease (ESKD) in African Americans. The most informative risk alleles were contained within a haplotype spanning introns 12 and 23, (OR≈2-7; p<10-8). *MYH9* risk alleles are very frequent in African-Americans (≈60%) and much less European-Americans (<4%), thus explaining a major USA and global health disparity. To refine the region of association and identify potential causal SNPs, we performed a fine-mapping study of *MYH9* in I-FSGS and HIVAN cases and controls using 79 tagging or otherwise informative SNPs. The strongest associations are for two closely correlated SNPs in introns 13 and 14, (OR= 5.7, p<10-26 for combined HIVAN and I-FSGS, and OR = 2.8, p<10-22 for ESKD, recessive model). These SNPs also are common in African Americans (52 to 59%) and rare in European Americans (4 to 9%). The newly identified SNPs narrow the region of interest, point to alternative splicing as a possible mechanism for the observed associations with non-diabetic kidney disease, and enhance the power of *MYH9* genotyping for genetic screening and diagnosis.

## 1970/T/Poster Board #519

**Autosomal dominant distal motor neuropathy: an italian family not linked to known loci.** *M. Muglia[1], L. Citrigno[1,2], M. Pennisi[3], A. Patitucci[1], R. Barome[3], A. Magariello[1], A.L. Gabriele[1], G. Pennisi[3], R. Mazzei[1], F.L. Conforti[1], C. Ungaro[1], M. Zappia[3], A. Gambardella[1].* 1) ISN-CNR, Mangone Cosenza, Italy; 2) Department of Neurosciences, Psychiatric and Anesthesiological Sciences, University of Messina, Messina (Italy); 3) Department of Neurosciences, University of Catania, Catania (Italy).

The distal motor neuro(no)pathy (dHMN) is a heterogeneous group of disorders characterized by an exclusive involvement of the motor part of the peripheral nervous system. The overall clinical picture consists of progressive weakness and wasting of the extensor muscles of toes and feet. Later on, weakness and wasting also involves the distal upper limb muscles. Foot deformity is a common feature. Often, unusual or additional features are present in ‘complicated’ distal HMN, including predominance in the hands, vocal cord paralysis, diaphragm paralysis and pyramidal tract signs. The classification of the dHMN is complex and based on multiple factors such as age at onset, mode of inheritance and the presence/absence of additional and complicating features like vocal cord and diaphragmatic paralyses or pyramidal dysfunctions. Currently, autosomal dominant, autosomal recessive and X-linked forms have been described, 16 chromosomal loci have been mapped, and eight different genes have been detected. We studied a four generation Italian family where the dHMN phenotype segregated as an autosomal dominant trait. Eight subjects out of a total of 12 subjects ( 6 men and 2 women, aged 13-79 years) were affected. One subject in the upper first generation was assumed to have dHMN by history. Seven patients from three generations (II to IV) were studied. At the time of the study main clinical features included symmetrical progressive weakness and atrophy of the hand muscles and distal lower limb muscles. Electrophysiological findings of motor axonal polyneuropathy were found in 3 patients (aged 13 to 45 years) with variable clinical severity. We performed genetic linkage analysis with DNA markers from current-known dHMN loci. No support for linkage to any of the known loci was found in our family, confirming a genetic heterogeneity within autosomal dominant dHMN.

## 1971/T/Poster Board #520

**Genomewide identity by descent and homozygosity mapping reveals candidate regions for genetic susceptibility to obsessive-compulsive and tic disorders.** *E. Nurmi[1], B. Merriman[2], J. McCracken[1], S. Nelson[1,2], The OCD Collaborative Genetics Study.* 1) Department of Psychiatry, University of California at Los Angeles, Los Angeles, CA; 2) Department of Human Genetics, University of California at Los Angeles, Los Angeles, CA.

Obsessive-compulsive disorder (OCD) is a common psychiatric diagnosis producing significant psychological distress and social and occupational impairment. OCD is characterized by persistent, intrusive, disruptive thoughts and related repetitive rituals aimed at neutralizing these obsessions. While twin and family studies support a strong genetic etiology, risk alleles for OCD have yet to be identified. A highly familial subset of OCD is defined by an early age of onset and is associated with tics. In the OCD Collaborative Genetics Study (OCGS) sample, we observe a 5-fold increased rate of Tourette Syndrome (TS) and other tic disorders in relatives of female probands with early age of onset and comorbid tics. This subset of 32 families may represent a discrete population with greater genetic loading and homogeneity that is more amenable to genetic studies of OCD and tic susceptibility. We performed genomewide SNP genotyping in this sample using the 1M Illumina SNP chip. These data were analyzed for copy number variants (CNVs) and larger than expected regions of homozygosity and pairwise identity. Pedigree-free identity by descent (IBD) mapping can identify large genomic intervals inherited from a common ancestor in affected individuals of unknown relationship. The presence of large chromosomal haplotypes shared by multiple distantly-related affected individuals provides strong evidence for association with the selected phenotype. IBD mapping in our dataset revealed pairwise sharing of a 20 cM region of chromosome 2 mapping within a local female-specific linkage peak in the OCGS dataset. A 3 cM block of homozygosity across this region was identified in a third proband. Large blocks of IBD (5-14 cM) between 3 individuals were observed on chromosomes 8 and X. Two probands carried overlapping stretches of homozygosity on chromosome 2 that mapped to the only prominent linkage peak detected in genomewide screens for TS. Two regions of extended homozygosity encompassed candidate genes selected *a priori* on chromosomes 11 and 18. Several other promising candidate regions were identified. Genes mapping within these intervals were cataloged and prioritized for in-depth analysis. These data will compliment parallel association analyses, and will fuel and focus ongoing efforts to identify OCD and tic disorder susceptibility genes.

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## 1972/T/Poster Board #521

**Genome-wide Linkage Analysis for Genetic Causes of Macular Telangiectasia Type 2.** *N.L. Parmalee[1], K. Kiryluk[2], M. Gillies[5], R. Allikmets[3,4], The MacTel Project.* 1) Genetics and Development, Columbia University, New York, NY; 2) Department of Medicine, Nephrology, Columbia University, New York, NY; 3) Department of Ophthalmology, Columbia University, New York, NY; 4) Department of Pathology, Columbia University, New York, NY; 5) Clinical Ophthalmology and Eye Health, The University of Sydney, Sydney, Australia.

Macular Telangiectasia Type 2 is an adult onset disease that affects the macular region of the retina, resulting in reduction of central visual acuity in patients. Prevalence is approximated at 1:1000. While most cases appear to be sporadic, several families have been identified with multiple affected family members. The disease presents between the 3rd and 5th decade of life with changes in autofluorescence in the macula, decreased macular pigment, and neovascularization of the inner retina. Retinal thickening and the presence of a macular cyst in the juxtafoveolar region are frequently seen with optical coherence tomography. The disease is usually bilateral, and shows no difference in prevalence by gender. Because diagnosis depends on very subtle criteria, it is thought that macular telangiectasia may be underdiagnosed. The MacTel Project is an international consortium formed to study this disease. DNA samples have been collected by 22 centers in 8 countries. Retinal images are taken at the center of enrollment and sent to one reading center where are images are adjudicated to ensure that identical diagnostic criteria are applied across the cohort. At present we have DNA samples from 261 affected individuals, 143 relatives, and 63 unrelated controls. We identified 16 multiplex families and genotyped a total of 64 probands and family members on the Illumina 1M Duo chip for linkage and candidate gene analysis. Four families were later determined to be singleplex families and were removed from analysis; 12 families with 54 total individuals were analyzed for linkage. SNPs were selected for inclusion in linkage analysis based on: no missing calls, MAF greater than .25, cluster separation greater than .75, and GenTrain score greater than .80. This set of markers was then pruned for linkage disequilibrium in PLINK, removing markers with $R^2$ greater than .40. The remaining markers were partitioned into four independent sets of approximately 10,000 markers each, which were analyzed in MERLIN. Preliminary multipoint parametric and non-parametric analyses show peaks on chromosomes 1p and 10q, which are currently under analysis. Patients and families enrolled in the study are followed on a yearly basis to investigate the progression of the disease. We anticipate that the number of families available for linkage analysis will increase over the course of the study.

## 1973/T/Poster Board #522

**A Unified case-control association testing with unknown multilevel relationships.** *Y. Choi[1], T. Bhangale[1], S. Smith[2], L. Diatchenko[2], W. Maixner[2], J.M. Jordan[3], C.L. Hyde[4], S. John[5], B.S. Weir[1,6].* 1) Department of Biostatistics, University of Washington, Seattle, WA; 2) Algynomics Inc, Chapel Hill, NC; 3) Thurston Arthritis Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC; 4) Clinical Research Statistics, Pfizer Global Research & Development, New London, CT; 5) Molecular Medicine, Pfizer Global Research & Development, New London, CT; 6) Department of Genome Science, University of Washington, Seattle, WA.

Genome-wide association studies suffer from high rates of false-positive results when unknown population structures and cryptic relatedness exist. A number of methods have been proposed for testing association between markers and disease in structured populations and genomic control (GC) and principal component analysis (PCA) are most commonly used. We have developed a unified mixed-model approach to account for unrecognized population structures and familial-level relatedness for binary traits. We estimated unknown population structures and cryptic relatedness using maximum-likelihood methods and used logistic mixed model for testing association in multilevel structured populations. Our approach allows estimating relatedness in structured populations and adjusting the effect of potential confounders or environmental factors simultaneously. We compared a new approach with two previous methods, GC and PCA, and applied it to simulated data and to a real sample from Johnson County, North Carolina.

## 1974/T/Poster Board #523

**Genetic and Functional Analysis Suggest that FGFR1 Contributes to Human Obesity.** *H. Jiao[1,2], I. Dahlman[3], N. Mejhert[3], C. Henegar[4], A. Hinney[5], J. Hoffstedt[3], D. Brodin[1], B. Dubern[4], P. Galan[6], S. Czernichow[6], A. Silveira[7], F. Van't Hooft[7], T. Axelsson[8], M. Ryden[3], J. Hebebrand[5], J. Kere[1,2], K. Dahlman-Wright[1], A. Hamsten[7], K. Clement[4], P. Arner[3].* 1) Department of Biosciences and Nutrition, Karolinska Institutet, SE-141 57 Stockholm, Sweden; 2) Clinical Research Centre, Karolinska University Hospital, SE-141 57 Stockholm, Sweden; 3) Department of Medicine at Karolinska Institutet and Karolinska University Hospital, SE-141 86 Stockholm, Sweden; 4) INSERM, U-872, Nutriomique (team 7) 75006 Paris, France; University Pierre and Marie Curie-Paris 6, Cordeliers Research Center, 75006 Paris, France; AP-HP, Pitié-Salpétrière Hospital, 75013 Paris, France; 5) Department of Child and Adolescent Psychiatry of the University of Duisburg-Essen, Essen, Germany; 6) INSERM, U-557/INRA U-1125, CNAM, UP13, CRNH-IdF, 93017 Bobigny, France; University Paris 13, 93017, Bobigny, France; AP-HP, Avicenne Hospital, 93017 Bobigny, France; 7) Cardiovascular Genetics Group, Atherosclerosis Research Unit, Department of Medicine, Solna, Karolinska Institutet, SE-17176 Stockholm, Sweden; 8) Molecular Medicine, Department of Medical Sciences, Uppsala University, Uppsala, Sweden.

Objective: The aim of this study was to identify susceptibility genes for obesity by focusing on subjects with extreme obesity, and to investigate pathophysiological mechanisms linking these genes to adiposity. Research Design and Methods: A genome-wide association analysis was performed on subjects with early onset or morbid obesity [N=164] and elderly that had always been lean [N=164]. Results were replicated in cohort 2-5 comprising a total of 4674 severely obese subjects and 5663 lean or population-based controls. Results: rs7012413 (MAF~30%) in the fibroblast growth factor receptor 1 (FGFR1) gene associated with obesity in each of five investigated cohorts (N=10665) with an over all odds ratio of 1.20 [1.09 - 1.33] and P 1.8x10 -6 which was 7.0x10-8 in a recessive model. FGFR1 and one of its ligands, fibroblast growth factor-1 (FGF-1), regulate adipogenesis. In studies of human abdominal subcutaneous adipose tissue we observed increased FGFR1 levels in obese versus lean subjects. rs7012413 CC genotype associated with lower adipose tissue FGFR1 levels than CT or TT. FGF-1 was secreted within adipose tissue with increased rates in obesity. In conclusion a polymorphism in FGFR1 associates with obesity and may affect body fat accumulation through FGF-1/FGFR1 mediated paracrine regulation of adipogenesis. This study is the first to imply adipose tissue as a primary organ regulating common obesity.

## 1975/T/Poster Board #524

**Genome-wide association analysis of Body Mass Index in Pima Indians.** *A. Malhotra, S. Kobes, W.C. Knowler, L.J. Baier, R.L. Hanson.* NIDDK, Phoenix, AZ.

Obesity is an increasing health problem in the United States and worldwide. Numerous studies have been done to understand the genetic contributors to Body Mass Index (BMI), a measure of obesity, but only a limited number of susceptibility variants have been identified. In the current study, genome-wide association (GWA) analysis was performed for BMI in 1149 Pima Indians (526 males and 623 females; age ≥15 years) using single nucleotide polymorphisms (SNPs) from the 1 million Affymetrix SNP panel. Of the genotyped SNPs, 454,194 passed quality control procedures and had minor allele frequency >0.05; these were analyzed for association with BMI. Phenotypic data included BMI at multiple examinations (range: 1-15 examinations for a given individual) collected from 1965-2004. Average BMI from all 6,079 exams in these individuals was 33.5 kg/m2. Within-family association tests were performed for each SNP using a maximum-likelihood based mixed model procedure, as implemented in the Statistical Analysis System (SAS) software. Variance components representing family membership and repeated measures within an individual (assuming an autoregressive correlation structure) were included in the analysis and the BMI measurements were adjusted for sex, birth year, and age. The strongest associations were with 6 adjacent SNPs on chromosome 6 (located at 53,493,043bp-53,509,958bp with p-value range: 1.62x10-6 to 3.81x10-6). In addition, 3 SNPs (located at 20,863,634bp-20,881,374bp) on chromosome 4 were associated with BMI (p=1.71x10-6 to 2.68x10-6). While these specific regions do not contain known obesity genes, they should be further explored for novel causal variants. We also assessed evidence for association for SNPs near previously identified susceptibility genes. SNPs near several genes including NEGR1 (p=0.0214), RASAL2 (p=0.0006), DGKG (p= 0.0420), FTO (p=0.0122), and MCR4 (p=0.0033) were associated with BMI in the present study. In conclusion, SNPs on chromosomes 6 and 4 were associated with BMI in Pima Indians. Further analysis of these regions is particularly important since a majority of previous studies have been done in Caucasian populations, whereas genetic susceptibility factors may differ by ethnicity.

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## 1976/T/Poster Board #525

**Ethnic diversity in fine mapping of the susceptibility loci *G6PC2-ABCB11* for fasting glucose and *CDKAL1* for type 2 diabetes.** *F. Takeuchi[1], T. Katsuya[2], S. Chakrewarthy[3], K. Yamamoto[4], T. Fujisawa[2], E. Nakashima[5], K. Ohnaka[6], H. Ikegami[7], T. Sugiyama[8], T. Nabika[9], A. Kasturiratne[3], S. Yamaguchi[9], S. Kono[6], R. Takayanagi[6], Y. Yamori[10], S. Kobayashi[9], T. Ogihara[11], A. de Silva[3], R. Wickremasinghe[3], N. Kato[1] for the NIBIO GWA Study.* 1) Research Institute, International Medical Center of Japan; 2) Osaka University Graduate School of Medicine; 3) University of Kelaniya; 4) Medical Institute of Bioregulation, Kyushu University; 5) Nagoya University Graduate School of Medicine; 6) Graduate School of Medical Sciences, Kyushu University; 7) Kinki University School of Medicine; 8) Institute for Adult Diseases, Asahi Life Foundation; 9) Shimane University School of Medicine; 10) Research Institute for Production Development; 11) Osaka General Medical Center.

**Objective.** Recently, genome-wide association (GWA) studies have successfully identified numerous loci influencing disease susceptibility or quantitative traits. To discern the causal variant(s), we need to enumerate potential causal variants and narrow them down in the region of interest. Along this line, we compared populations of European and Asian descent to refine association signals at two susceptibility loci *G6PC2-ABCB11* and *CDKAL1* for glycemic traits previously identified by GWA studies of Europeans.

**Methods.** In the *G6PC2-ABCB11* region, we examined fasting glucose association in 776 non-diabetic Japanese samples who are part of our ongoing GWA study, and chose index SNPs for further genotyping in the general populations of Japanese ($N$ = 4,813) and Sri Lankans ($N$ = 2,319). In the *CDKAL1* region, based on our GWA study in the Japanese, we compared haplotypes and their association with type 2 diabetes (T2D) between the Japanese and Europeans. SNPs representing the associations were tested in case-control samples of Japanese ($N$ = 2,774) and Sri Lankans ($N$ = 1,114).

**Results.** We could confirm the association of fasting glucose with variants at *G6PC2-ABCB11*. In addition to the reported SNP rs560887, we identified significant associations at a novel variant rs3755157 in both the Japanese ($\beta$ = 0.057 mmol/l, $P = 2.6 \times 10^{-8}$) and Sri Lankans ($\beta$ = 0.069 mmol/l, $P$ = 0.001), with allelic heterogeneity implicated between the two SNPs. In the *CDKAL1* region, the use of ethnic diversity in linkage disequilibrium (LD) pattern helped to refine T2D association signals to an index SNP rs9368222. Furthermore, cross-population filtering could appreciably reduce potential causal variants; i.e., from 79 to 7 in the *G6PC2-ABCB11* region and from 69 to 3 in the *CDKAL1* region.

**Conclusions.** It has been argued that populations of African descent may be advantageous for fine mapping but statistical power could be limiting due to the unavailability of sufficiently large samples and/or a smaller effect size. Instead, our data demonstrate that ethnic diversity in the pattern and strength of LD between populations of European and Asian descent can allow us to appreciably reduce potential causal variants and also to focus on most promising variants for functional follow-up.

## 1977/T/Poster Board #526

**Multi-stage genome-wide association study uncovers genetic factors influencing the P300 event-related brain potential.** *M. Zlojutro[1], N. Manz[2], M. Rangaswamy[2], X. Xuei[3], L. Bierut[4], H. Edenberg[3], T. Foroud[3], A. Goate[4], V. Hesselbrock[5], S. Kuperman[6], J. Nurnberger Jr.[3], J. Rice[4], M. Schuckit[7], B. Porjesz[2], L. Almasy[1], COGA Collaborators.* 1) Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Department of Psychiatry, SUNY Health Science Center, Brooklyn, NY; 3) Indiana University School of Medicine, Indianapolis, IN; 4) Department of Psychiatry, Washington University School of Medicine, St. Louis, MO; 5) Department of Psychiatry, University of Connecticut, Farmington, CT; 6) University of Iowa, Psychiatry Research, Iowa City, IA; 7) University of California, San Diego, CA.

Event-related brain potentials (ERPs) are measurements of neuroelectric activity that are altered in patients with various psychiatric disorders and may serve as quantitative correlates of disease liability. In particular, alcoholic subjects and their offspring exhibit a reduction in the amplitude of the P300 component, a positive oscillatory wave occurring approximately 300-500 milliseconds after a target stimulus during a cognitive task. Results of a genome-wide association study (GWAS) based on the Illumina HumanHap 1 million SNP array are presented for the P300 component at the Pz scalp position for 1,123 unrelated case-control samples ascertained from the Collaborative Study on the Genetics of Alcoholism (COGA). Although the most statistically significant SNPs did not achieve genome-wide significant association after adjustment for multiple-testing (smallest P = 2.8 x 10-6 for rs12043526), several new genomic regions not previously identified through linkage analyses were implicated, with 50 top-ranking SNPs selected for follow-up genotyping in 1,374 family-based COGA samples. The most compelling association obtained in the follow-up data was for marker rs2148682, located within the evolutionary conserved gene DNAJC6, a regulator of molecular chaperone activity on chromosome 1p31 (Bonferroni corrected P = 8.7 x 10-3; two-stage combined P = 1.3 x 10-4). Other convincing associations were discovered for markers in the forkhead box transcription factor FOXP4 on chromosome 6p21 (combined P = 3.0 x 10-6) and in FRMD4A on chromosome 10p13 (combined P = 3.7 x 10-5). This large-scale GWAS highlights previously unknown biological pathways contributing to the P300 brain potential in individuals with alcohol dependence.

## 1978/T/Poster Board #527

**Recombination Intensity and Hot Spot Distribution across Major Histocompatibility Complex Based on Preimplantation HLA Haplotyping Data.** *T. Sharapova, S. Rechitsky, O. Verlinsky, A. Kuliev, Y. Verlinsky.* Reproductive Genetics Institute, Chicago, IL.

The fine scale distribution of meiotic recombination in humans can be inferred from the population SNP genotyping data, assessed by linkage studies or measured by high-resolution sperm typing. Each approach has its limitations, lowering the accuracy of the existing recombination maps. Major Histocompatibility Complex (MHC) is one of the most extensively studied regions of human genome. Genotyping of 20,000 single sperms has been previously performed to build the recombination map of human MHC. The method infers several biases due to reported significant individual and gender dependent variation in recombination rate. We present estimation of recombination rate and its distribution within MHC region based on the analysis of preimplantation HLA haplotyping data comprising both male and female meioses. Preimplantation haplotyping has been performed for 110 couples in a need of HLA matching stem cell donor for an affected sibling. STR genotyping of 5.4Mb region encompassing MHC has been performed in single blastomeres and polar bodies using nested multiplex PCR. A panel of 24 STR markers was applied, dividing the MHC region into 23 segments. In total 1787 male and 1930 female meioses received from 245 IVF/PGD cycles have been studied resulting in the identification and mapping of 33 and 50 recombinant chromosomes respectively. The estimated comprehensive recombination intensity within 5.4Mb segment (D6S248-D6S1618) was 0.37cM/Mb for males and 0.53cM/Mb for females with female: male ratio of 1.5. Eleven out of 23 segments showed 1.8-8 fold difference in crossover intensity between males and females supporting gender related variation in recombination activity. In females 6 segments located within HLA class II region displayed a 1.6-4.8 fold increase in recombination activity compared to the expected rate of 0.53cM/Mb. Three of them (D6S2447-G51152, TAP1-Ring3 and Ring3-D6S1560) correspond to the previously described hotspots detected by sperm typing in males (DQB1-DQB3, DMB and Ring3-DPB1). One segment D6S1629 to D6S1568 showed a significantly higher recombination intensity (P<0.05) representing a hotspot of 268kb which has not been previously described. The study represents the first extensive direct measurement of recombination rate within MHC region in females using preimplantation haplotyping data. The results support the heterogeneity in recombination intensity within MHC and indicate the presence of novel hotspot adjacent to HLA class II region.

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## 1979/T/Poster Board #528

**Identification of Genes Involved in the Regulation of Hematopoiesis.** *M. Varney, V. Sollars.* Marshall University Joan C. Edwards School of Medicine, Huntington, WV.

Proper regulation of gene expression, protein expression, and protein activity is vital to the development of blood cells. When the expression of particular genes and proteins involved in the differentiation of hematopoietic stem and progenitor cells is altered from normal patterns, differentiation can be blocked and progenitor cell frequency can be increased significantly, contributing to hematopoietic malignancies. One such malignancy, acute myelogenous leukemia (AML) is characterized by the expansion of the myeloid progenitor cell compartment. To identify genes that control the regulation of hematopoiesis, we studied the size of the myeloid progenitor cell compartment in 27 strains of inbred mice. Phenotypic information was collected by performing hematopoietic progenitor cell assays called colony forming cell (CFC) assays. By comparing phenotypic similarities and differences among the strains to genotypic similarities and differences among the strains via in silico mapping, we were able to identify 2 genes, and 1 additional chromosomal region of interest in controlling hematopoiesis. More specifically, these genes were implicated in the phenotypes associated with the development of macrophages and granulocytes. In silico mapping is a bioinformatics technique that is based on single nucleotide polymorphism (SNP) markers. It generates information that can be used to identify chromosomal regions containing genes that are responsible for the phenotype studied. Both genes implicated in this study, Slit3 and Foxo1, have been shown to play roles in other cancers. Further investigation into these genes and the additional chromosomal region of interest may yield new information about them as possible oncogenes or tumor suppressors in AML.

## 1980/T/Poster Board #529

**A novel role for the non-receptor protein tyrosine phosphatase PTPN14 in lymphangiogenesis.** *A.C. Au[1], P.A. Hernandez[1], E. Lieber[3], B.D. Gelb[1, 2], G.A. Diaz[1, 2].* 1) Department of Genetics & Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) Department of Pediatrics, Mount Sinai School of Medicine, New York, NY; 3) Department of Pediatrics, Lincoln Hospital and Mental Health Center, Bronx, NY.

The lymphatic system serves as a unidirectional recovery system that transports fluid, cells and macromolecules from interstitial spaces back into the central circulation. Perturbations in lymphatic vasculature development, maintenance or function lead to a variety of pathological disorders, including lymphedema. Through genome-wide mapping of a multi-generational consanguineous kindred with autosomal recessive inheritance of choanal atresia and lymphedema, we have mapped the trait to chromosome 1q32-41. Further analysis of the 13.2-cM critical region spanning 8.6 Mb led to the identification of a loss-of-function mutation in the non-receptor protein tyrosine phosphatase gene *PTPN14*. Despite extensive effort, other kindreds with choanal atresia and lymphedema were not identified. To prove causality between *PTPN14* mutation and lymphedema, we have generated a mouse model of *PTPN14* deficiency from an ES cell line in which the *Ptpn14* locus was disrupted by a gene trap. Mice homozygous for a *Ptpn14* trap allele recapitulated the lymphedema phenotype in the form of hindlimb and forelimb swelling. Immunofluorescence staining of ear sections from symptomatic mice revealed a hyperplastic dermal lymphatic network, whereas the blood vasculature was apparently unaffected. Although high expression of PTPN14 in human umbilical vein endothelial cells suggested an important role in endothelial cell regulation, no role in lymphatic development or function has been described. In order to elucidate the physiological role of PTPN14 in lymphangiogenesis, we tested the hypothesis that PTPN14 functioned as a regulator of the lymphangiogenic factor vascular endothelial growth factor receptor-3 (VEGFR-3). The effect of PTPN14 expression on VEGFR-3 phosphorylation status after ligand activation and the ability of the proteins to form a complex formation were tested in transiently transfected cells. We observed that tyrosine phosphorylation of VEGFR-3 receptors was diminished in cells co-expressing the wild type, but not the mutant, PTPN14. In addition, the proteins were found to form a complex as detected by co-immunoprecipitation assays. Our cumulative data are consistent with an inhibitory functional interaction between PTPN14 and VEGFR-3, potentially through a direct physical complex. Based on these findings, we propose that PTPN14 functions as a regulator of lymphatic development in mammals and is a novel candidate molecule for the modulation of lymphangiogenesis *in vivo*.

## 1981/T/Poster Board #530

**A genome-wide association scan localizes a QTL influencing Epstein-Barr virus infection to the major histocompatibility complex region.** *R. Rubicz[1], R. Yolken[2], L. Bauman[1], M. Carless[1], E.I. Drigalenko[1], J.E. Curran[1], M.P. Johnson[1], S.E. Cole[1], T.D. Dyer[1], L. Almasy[1], M.C. Mahaney[1], E. Kraig[3], E.K. Moses[1], C.T. Leach[4], J. Blangero[1], H.H.H. Göring[1].* 1) Dept. of Genetics, Southwest Foundation for Biomedical Resesearch, San Antonio, TX; 2) Dept. of Pediatrics, Johns Hopkins School of Medicine, Baltimore, MD; 3) Dept. of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX; 4) Dept. of Pediatrics, University of Texas Health Science Center at San Antonio, San Antonio, TX.

To better understand the role of infections with common pathogens in chronic inflammation, we measured IgG antibodies to the Epstein-Barr virus (EBV) nuclear antigen (EBNA) in >1,000 randomly ascertained Mexican American participants (age range 15-94 years) in the San Antonio Family Heart Study. EBV is a common herpes virus. Many infections are asymptomatic but primary infections in adolescents and young adults often lead to infectious mononucleosis. In some individuals, infection leads to more serious diseases including cancers such as Burkitt's Lymphoma and nasopharyngeal carcinoma. Reasons for the observed differential response to EBV infection are little known but likely include genetic susceptibility to infection. Anti-EBNA IgG antibody titer was measured in plasma samples using solid phase enzyme immunoassay methods. In our sample, 48% of the individuals had definitive levels of antibodies, 24% had intermediate, suggestive levels, and 8% did not have detectable levels. No significant differences were observed by sex or age. Under the assumption that the IgG antibody level to EBNA is a meaningful reflection of infection, we sought to localize genetic factors that influence the serological phenotypes. Both the quantitative antibody titer and the discrete serostatus phenotype were found to be significantly heritable (with estimates of 40% and 63%, respectively). Shared environmental factors, modeled via co-habitation, were non-significant. We performed a genome-wide association scan on 856 individuals with phenotype and genotype data (generated using Illumina's HumanHap 550 BeadChip) under an additive measured genotype association model, accounting for the non-independence of family members using a random effects kinship model. We detected genome-wide significant associations on chromosome 6 in the human leukocyte antigen (HLA) region. The most significant association was obtained with SNP rs4248166 in *BTNL2*, a gene which is associated with T-cell function ($p = 9.0*10^{-9}$ and $2.8*10^{-9}$ for the quantitative and the qualitative phenotype, respectively). Multiple SNPs in the region support this finding. Our results indicate that EBV infection is regulated by genetic determinants within the HLA complex. Further analysis of this finding might lead to an increased understanding of the role of EBV in human disease as well as better methods for predicting individual response to EBV infection.

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## 1982/T/Poster Board #531

**Admixture mapping for fetal hemoglobin QTLs in Jamaica: prospects and challenges.** *C.A. McKenzie[1], J. Butler[2,10], L. Creary[3], N. Hanchard[4], S.G. Yi[5], X. Zhu[5], I. Hambleton[6], V. Taylor[7], J. Hirschhorn[2,9,10,11], S. Menzel[8], T.E. Forrester[1], S.L. Thein[8].* 1) Tropical Metabolism Research Unit, University of the West Indies, Kingston, Jamaica; 2) Division of Genetics, Boston Children's Hospital, Boston, USA; 3) NHS Blood and Transplant, London, UK; 4) Department of Pediatric and Adolescent Medicine, Mayo Clinic, Rochester, USA; 5) Department of Epidemiology and Biostatistics, School of Medicine, Case Western Reserve University, Cleveland, USA; 6) Chronic Disease Research Centre, University of the West Indies, Cave Hill, Barbados; 7) National Blood Transfusion Service, Kingston, Jamaica; 8) King's College London School of Medicine, Division of Gene and Cell Based Therapy, London, UK; 9) Division of Endocrinology, Boston Children's Hosp, Boston, USA; 10) Broad Institute, Cambridge, USA; 11) Department of Genetics, Harvard Medical School, Boston, USA.

An elevated level of fetal hemoglobin (HbF) provides significant amelioration of the severity of hemoglobinopathies such as β-thalassemia and sickle cell disease (SCD). Clinical trials demonstrate that medications such as hydroxyurea, which act in part by raising HbF levels, provide important clinical benefits; identifying determinants of HbF is thus of considerable interest. HbF levels display strong genetic determination and 3 quantitative trait loci (QTLs) have been identified so far. We and others have shown that there are significant differences in HbF levels between white European- and African-descent populations. Admixture mapping of novel QTLs in appropriate populations might be possible if ethnic differences are related to genetic ancestry. In order to assess the prospects for admixture mapping in Jamaica we have genotyped 72 SNPs in samples obtained from our previous study of healthy African-descent participants (n = 189) from the main blood donation center in the capital Kingston (A/C) and from a rural enclave (n = 641), founded in the early 1800s by German immigrants (A/G). The markers include 20 SNPs on chromosomes 2, 6, and 11 that were previously reported to be associated with HbF (HbFSNPs), 40 ancestry informative markers from across the genome (G-AIMs) and 12 AIMs from HbF-associated regions (R-AIMs). HbF was represented by F cell (FC) levels measured using flow cytometry. The average percentage of European ancestry (P_ceu) was significantly higher and more variable ($P < 0.001$) in the A/G group (23.8 ± 27.4%) than in the A/C group (7.5 ± 6.0%). P_ceu was significantly associated ($P < 0.05$) with FC after adjustment for age, sex, sickle carrier status, and C-158T HBG2 genotype. We added each of the markers in turn to this base model to test for FC-marker association. At least one HbFSNP from each of the previously-reported HbF-associated chromosomal regions was significantly associated with FC. One G-AIM (on chr. 14) was marginally associated with FC. None of the R-AIMs was associated with FC. A review of the distribution of P_ceu and pairwise disequilibrium between unlinked and linked markers indicates that there is important stratification within and between the A/C and A/G samples. These results extend previous results (to a non-anemic African-descent group) and also provide important information for the design of genome-wide admixture mapping studies for novel HbF QTLs and other traits of medical importance in Jamaica.

## 1983/T/Poster Board #532

**Linkage analysis using 370K chips in two sets of Retinitis Pigmentosa pedigree samples.** *F. Cheng[1,2], C. Li[1], L. Wang[3], X. Zhang[1,2], B. Liu[1,2], G. Li[3], C. Zeng[1].* 1) Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, China; 2) Graduate School of Chinese Academy of Sciences, Beijing, China; 3) Beijing Institute of Ophthalmology, Beijing Tongren Hospital, Capital University of Medical Science, Beijing, China.

Using whole genome chip for linkage analysis has been shown as an efficient strategy to map disease related genes in comparison with application of STR probes. Here we report the mapping of retinitis pigmentosa (RP) susceptible genes by chip analysis in two Chinese RP families. RP shows considerable symptomatic and genetic heterozygosity in different cases. Both pedigrees, each with 7 and 8 patients respectively, showed incomplete and dominant inheritance model. All infected ones showed RP symptoms before 30 years old and a few had RP even before 10.

DNA samples were hybridized to Illumina 370K CNV-quad chips and SNPs with poor call rates were removed for further analysis. Large families were divided into small nuclear trio families for relationship test. After exclusion of a few SNPs showing Mendelian errors, Merlin package was used to do the Linkage study. Chromosome regions with positive LOD scores were further subjected to haplotype analysis. According to the relationships of nuclear families, parental haplotypes of each trio were deduced from genotype data to determine the transmission. Finally, four different haplotypes showing strong RP co-segregation were identified. With about 2Mb in each, two regions in chromosome 1 and 8 were obtained from family 1, and two separate regions in chromosome 14 were resolved in family 2, respectively.

To verify above results, STR markers near these four regions were then chosen to type in related samples. Mlink package was used for two-point linkage analysis. At recombination rate $\theta=0$, LOD score were 3.160 and 2.600 for D14S258 and D14S68 in family 1, and LOD scores were 1.852 and 1.710 for D1S425 and D8S1771 in family 2, respectively. Four candidate genes in these regions have been selected for re-sequencing based on gene annotation and function related studies. A two-nucleotide insertion (CT) in the third exon of RD3 (retinal degeneration 3) was identified in RP affected members but not in healthy ones in family 1. No mutation was found in candidate gene NRG1 (neuregulin 1). Sequence analysis of RDH12 (retinol dehydrogenase 12), and ABCD4 (ATP-binding cassette, sub-family D, member 4) was in the process.

## 1984/T/Poster Board #533

**Identification of 11 novel mutations in 8 BBS genes via high-resolution homozygosity mapping.** *H.M. Harville[1], S. Held[1], A. Diaz-Font[2], E.E. Davis[3], B.H Diplas[3], J. MacDonald[1], W. Zhou[1], M. Chaki[1], P.L. Beales[2], N. Katsanis[3], E. Otto[1], F. Hildebrandt[1].* 1) Howard Hughes Medical Institute and Departments of Pediatrics and of Human Genetics, University of Michigan Health System, Ann Arbor, MI, USA; 2) Molecular Medicine Unit, UCL Institute of Child Health, London, UK; 3) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Bardet-Biedl syndrome (BBS) is primarily an autosomal recessive disorder characterized by rod-cone dystrophy, obesity, mental retardation, hypogonadism, post-axial polydactyly, renal cysts, and other anomalies of the kidney and urinary tract. To date, mutations in 14 genes have been identified as causing BBS. The vast genetic heterogeneity of BBS renders diagnosis difficult in terms of both the time and cost required to screen all 204 coding exons. Here, we report the use of genome-wide homozygosity mapping as a tool to identify homozygous segments in BBS patients at known BBS loci. In a worldwide cohort of 45 families, we identified, via direct exon sequencing, causative mutations in 20 families. Eleven mutations were novel, thereby increasing the number of known BBS mutations by 5% (11/218). Thus, in the presence of extreme genetic locus heterogeneity, homozygosity mapping provides a valuable approach to the molecular genetic diagnosis of BBS and the discovery of novel pathogenic mutations.

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## 1985/T/Poster Board #534

**Brain arteriovenous malformation maps to 5p13-q14, 15q11-q13 and 18p11: Linkage analysis with clipped fingernail DNA on SNP array.** *H. Kuniba[1,2,6], M. Oikawa[1,3,6], T. Kondoh[4], A. Kinoshita[1,6], H. Moriuchi[2], T. Nagayasu[3], N. Niikawa[5,6], K. Yoshiura[1,6].* 1) Dept Hum Genet, Nagasaki University, Nagasaki, Japan; 2) Dept Pediatrics, Nagasaki University, Nagasaki, Japan; 3) Dept Surgical Oncology, Nagasaki University, Nagasaki, Japan; 4) Misakaenosono Mutsumi, The institute for persons with severe intellectual/motor disabilities, Konagai, Japan; 5) Research Institute of Personalized Health Sciences, Health Sciences University of Hokkaido, Tobetsu, Japan; 6) Solution Oriented Research for Science and Technology (SORST), Japan Science and Technology Agency (JST), Tokyo, Japan.

Familial arteriovenous malformations (AVM) in the brain is a very rare disease, which is defined as the occurrence of AVM in two or more relatives (up to third-degree relatives) in a family without any associated disorders such as a hereditary hemorrhagic telangiectasia. We encountered a Japanese family, including four affected members in four successive generations, with brain AVM. A genome-wide linkage analysis using Affymetrix GeneChip 10K 2.0 Xba Array was carried out on the pedigree. While blood samples for DNA extraction were provided by only the proband, clipped fingernails were obtained for DNA extraction from the other ten individuals in the family with informed consent. SNP genotyping from the fingernail samples were successful, showing the mean SNP call rate of 92.49%. Multipoint LOD scores were calculated using MERLIN software and we identified 18 regions with positive LOD scores. Haplotype and linkage analyses with microsatellite markers in these regions confirmed three possible disease-responsible loci, i.e., 5p13.2-q14.1, 15q11.2-q13.1 and 18p11.32-p11.22. Ten selected genes within 5p13.2-q14.1 were directly sequenced. The candidate genes consisted of *MAP3K1*, *DAB2*, *OCLN*, *FGF10*, *ESM1*, *ITGA1*, *ITGA2*, *EGFLAM*, *ERBB2IP*, and *PIK3R1*. Although no causative genetic alteration was found in these genes, we cannot rule out the possibility that the loci are responsible for familial brain AVM. This is the first report of adoption of fingernail DNA for SNP array analysis to detect susceptible regions to brain AVM.

## 1986/T/Poster Board #535

**Quantitative Trait Linkage Analysis of Gamma-Band Response in Over 600 Individuals in a Nepalese Population Genetic Isolate.** *S.L. Santangelo[1,2,3], T. Sitnikova[1,2], M.H. Hall[1,4], D. Yu[2], J. Subedi[5], S. Ojha[6], P. Shrestha[7], P. Shrestha[7], S. Tamang[7], J.L. VandeBerg[8], J. Blangero[8], S. Williams-Blangero[8].* 1) Harvard Medical School, Dept Psychiatry, Boston, MA, USA; 2) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA; 3) Harvard School of Public Health, Boston, MA, USA; 4) Psychology Research Laboratory, McLean Hospital, Belmont, MA, USA; 5) Miami University, Dept. Sociology, Gerontology, Oxford, OH, USA; 6) Tribhuvan University Institute of Medicine, Dept. Psychiatry, Kathmandu, Nepal; 7) Nepal Biomedical Research Center, Kathmandu, Nepal; 8) Southwest Foundation for Biomedical Research, San Antonio, TX, USA.

In an effort to map genes influencing schizophrenia, we measured schizophrenia endophenotypes in over 600 members of a genetic isolate from Jiri, Nepal. The sampled individuals are from a single pedigree in the Jirel population comprising 2500 individuals who participated in a genome-wide linkage scan. One of the traits we measured is oscillatory brain activity in the gamma-band range (35-45Hz), which may arise from synchronized output of parvalbumin-expressing, fast spiking GABA neurons to the pyramidal cell assemblies. Abnormalities in this neural mechanism have been documented in schizophrenia patients and their first-degree relatives. We measured induced gamma-band activity between 250-550 msec after infrequent stimuli onset in the classic auditory oddball paradigm. The power of the event-related induced activity was calculated at the the PZ, P3 and P4 scalp locations in 614 individuals via the Instantaneous Frequency Analysis module of Neuroscan Scan 4.0 software based on the method of complex demodulation. Gamma-band response was evaluated in a quantitative trait locus (QTL) genome-wide linkage analysis after estimating heritability. Heritabilities for gamma band activity at all three scalp locations were significant, controlling for age and sex, and ranged from 0.19-0.22 (p=0.002-0.006). QTL linkage analysis for gamma response, adjusted for age and sex, yielded peak LOD scores of 1.63 on chromosome 6 (182 cM) for PZ; 1.42 on chromosome 2 (98 cM) for P3, and 1.84 on chromosome 2 (98 cM) for P4. There was also a LOD of 1.31 on chromosome 2 (98 cM) for PZ. For this Nepalese pedigree and marker set, evidence of suggestive linkage (likely to happen ≤ once by chance in a genome scan) is obtained for LOD scores ≥ 1.65. Therefore, we obtained suggestive linkage (maxLOD = 1.84) at chromosome 2p13 at the P4 electrode, with similar signals at the same chromosomal location at the PZ and P3 electrodes. Linkage to this same region was previously found for schizophrenia in another population isolate in Palau, Micronesia. Genes in this region include: transforming growth factor alpha (TGFA), implicated in circadian sleep-wake cycles, SPRED2, a member of the Sprouty family of proteins that regulate growth factor-induced activation of the MAP kinase cascade, and sepiapterin reductase (SPR) an enzyme that catalyzes the final step in the synthesis of tetrahydrobiopterin (BH4), an essential cofactor for synthesis of many neurotransmitters, including serotonin.

## 1987/T/Poster Board #536

**Chromosome 3q22 and BMPR2 mutations interact epistatically to cause familial pulmonary arterial hypertension.** *R. Subaran[1], L. Rodgriguez[1], W.C.L. Stewart[1], S. Marathe[1], S. Pramanik[2], R.J. Barst[2], W.K. Chung[2], D.A. Greenberg[1].* 1) Biostatistics, Columbia Univ, New York, NY; 2) Columbia University Medical Center, New York, NY.

A heritable trait with low penetrance, familial pulmonary arterial hypertension (FPAH) presents a tractable model for understanding complex diseases. The most extensively characterized determinant of FPAH expression is the BMPR2 gene. BMPR2 encodes the bone morphogenetic protein type II receptor and regulates proliferation of the pulmonary vasculature. Though nearly all FPAH cases carry heterozygous, loss-of-function BMPR2 mutations, over 80% of individuals as a whole with these mutations never express disease. Therefore, BMPR2 mutation status alone is an unreliable predictor of FPAH. We explore the possibility that additional genetic factors influence FPAH expression among BMPR2 carriers. By performing genome-wide linkage analysis on 15 FPAH families, all segregating BMPR2 mutations, we find 4 previously unreported loci linked (LOD > 2.0) to FPAH: 2p22, 3p12, 3q22 and 13q21. One of these loci, 3q22, shows strong evidence for linkage (LOD = 3.43). Imputation of trait model by maximizing the LOD score at 3q22 with respect to penetrance suggests 65-70% penetrance at this locus. Therefore, examining co-segregation of both 3q22 and the BMPR2 locus can be a far better predictor of FPAH than checking BMPR2-mutation status alone. While outlining a paradigm for understanding diseases of even greater complexity, our studies also uncover a novel locus interacting epistatically with BMPR2, allowing better prediction and treatment of FPAH.

## 1988/T/Poster Board #537

**A QTL on chromosome 7p influences the timing of the pubertal growth spurt in healthy children from the Fels Longitudinal Study.** *B. Towne[1], J. Blangero[2], E.W. Demerath[3], K.D. Williams[4], T.D. Dyer[2], S.A. Cole[2], W.C. Chumlea[1], R.M. Siervogel[1], S.A. Czerwinski[1].* 1) Wright State University, Dayton, OH; 2) Southwest Foundation for Biomedical Research, San Antonio, TX; 3) University of Minnesota, Minneapolis, MN; 4) Temple University, Philadelphia, PA.

Adult stature, and stature measured at any age during childhood, is highly heritable, and recent genome-wide analyses have identified QTL and SNPs linked or associated with adult or childhood stature. Very little, however, is known of genetic influences on specific features of individual growth patterns during childhood. In this study, we applied a triple logistic curve-fitting method to extensive serial height data from 475 healthy children aged 2 years to early adulthood (233 boys and 242 girls) in the Fels Longitudinal Study to identify in each of them the timing of the initiation of the pubertal growth spurt (age at pre-pubertal minimum growth velocity or "age at take-off" - ATO) and the timing of the pubertal growth spurt while at its most intense (age at peak height velocity - APHV). Mean ATO and APHV in these boys and girls was 10.68 and 8.72 years, and 13.75 and 11.53 years, respectively. The heritability of ATO was high in magnitude and significance ($h^2$ = 0.69 ± 0.10; p < 0.0001), as was the heritability of APHV ($h^2$ = 0.72 ± 0.10; p < 0.0001). Each of these children has been genotyped for ~400 STR markers spanning the autosomal genome. In genotype-by-sex (GxS) interaction models incorporating sex-specific variance components, significant linkage of ATO to 7p21.1-15.3 at 37 cM between markers D7S507 and D7S493 was found (LOD = 4.12), as was significant linkage of APHV to this exact same chromosomal region (LOD = 4.21). The effect of this 7p QTL on the timing of the pubertal growth spurt was more pronounced in females than in males. Contained within the support interval of this 7p QTL for the timing of the pubertal growth spurt is the growth hormone releasing hormone receptor gene (GHRHR). Johansson et al. (2009) recently reported significant linkage results in this same region of chromosome 7p for height in two samples of primarily adults from Sweden, and they found tagSNP haplotypes in GHRHR to be associated with height in their study samples. Other positional candidate genes in this region of 7p and known to regulate aspects of skeletal growth and development include the HOXA1-13 gene cluster at 7p15.2. Together, these findings suggest the presence of a gene or genes on chromosome 7p that influence the timing of pubertal growth, as well as other aspects of growth including height status. Supported by NIH grants R01HD12252, R01HD40377, F32HD053206, and R37MH59490.

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## 1989/T/Poster Board #538

**Genome-wide Linkage Analysis Using SNP Chip on a Korean Family with Late-Onset Nonsyndromic Hearing Loss.** *H.-H. Won[1,2], H.-J. Kim[3], S.H. Hong[4], J.-W. Kim[3].* 1) Samsung Biomedical Research Institute, Seoul, Korea; 2) Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Korea; 3) Department of Laboratory Medicine and Genetics, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea; 4) Department of Otorhinolaryngology and Head and Neck Surgery, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea.

Hereditary hearing loss (HL) represents the most extreme example of genetic heterogeneity, and a growing list of chromosomal loci and genes have been uncovered to underlie the condition. Linkage analysis is one of the powerful approaches for the identification of disease-causing genes, and the typical polymorphic markers used for the approach have been mutiallelic microsatellite markers (or short tandem repeats). However, single nucleotide polymorphisms (SNPs), bi-allelic markers, have advantages including its abundance in the human genome and ease of typing. In this study, we tried to localize the disease gene for nonsyndromic late-onset HL in a Korean family through a genome-wide linkage analysis using a set of SNP markers. Blood samples were collected from 21 individuals in the pedigree, of which the pattern of disease running was suggestive of autosomal dominance inheritance. The Illumina 6K SNP linkage panel was used as the markers, the MERLIN program was used for the calculation of linkage scores. Mutipoint parametric and nonparametric analyses were performed to yield LOD sores and NPL scores, respectively. We found a chromosomal locus on the band 5q31 to have a significantly high linkage scores both on multipoint parametric and non-parametric analyses. Haplotype analyses also revealed the heterozygous allele disease haplotype in affected individuals. The chromosome band 5q31 is one of the gene-rich genomic segments, and several known disease loci (genes) have been previously identified in the chromosome band 5q31 in linkage with hereditary HL including DFNA1 (*DIAPH1*), DFNA15 (*POU4F3*), DFNA42, and DFNA54. We performed direct sequencing of the two known HL genes (*DIAPH1* and *POU4F3*) within our locus and found no mutations, suggesting there would be a novel HL gene in the region. This study demonstrated that linkage analysis using biallelic, SNP markers can successfully identify the disease locus for HL. Identification of a novel HL gene by the candidate gene study within our locus on the chromosome band 5q31 will further extend the genetic heterogeneity of hereditary HL in human.

## 1990/T/Poster Board #539

**Genetic linkage evidence for distinct subtypes of schizophrenia characterized by age at onset and neurocognitive deficits.** *Y.J. Lien[1,2], P.C. Hsiao[2], C.M. Liu[3,4], S.V. Faraone[5], M.T. Tsuang[6,7], H.G. Hwu[1,3,4], W.J. Chen[1,2,3,4].* 1) Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan; 2) Genetic Epidemiology Core Laboratory, Division of Genomic Medicine, Research Center for Medical Excellence, National Taiwan University, Taipei, Taiwan; 3) The Department of Psychiatry, College of Medicine, National Taiwan University, Taipei, Taiwan; 4) Department of Psychiatry, National Taiwan University Hospital, Taipei, Taiwan; 5) The Genetics Research Program and the Department of Psychiatry and Behavioral Sciences, SUNY Upstate Medical University, Syracuse, NY; 6) The Department of Psychiatry and the Center for Behavioral Genomics, University of California, San Diego, CA; 7) Harvard Institute of Psychiatric Epidemiology and Genetics, Harvard Departments of Epidemiology and Psychiatry, Boston, MA.

As schizophrenia is genetically and phenotypically heterogeneous, targeting subphenotypes with possible greater genetic loadings may help reveal a more homogeneous subset of families with greater linkage signals. This study aimed to evaluate the genetic linkage evidence for schizophrenia in subsets of families using earlier age at onset or greater neurocognitive deficits as subphenotypes. Participants of this study included patients with schizophrenia and their first-degree relatives recruited from six data collection research center throughout Taiwan. The sample comprised 1,207 affected individuals and 1,035 unaffected individuals of Han Chinese ethnicity from 557 sib-pair families co-affected with DSM-IV schizophrenia. Subjects completed a face-to-face semi-structured interview, the Continuous Performance Test (CPT), the Wisconsin Card Sorting Test, and were genotyped with 386 microsatellite markers across the genome. A series of nested ordered subset genome-wide linkage analyses were conducted and the statistical significance for a subset-derived increase in linkage signal was evaluated using permutations to obtain a chromosome-wide p value. Five chromosomal regions (2q, 7q, 8q, 9p, and 12q) had significant increases in maximum nonparametric linkage z (NPL-Z) scores in subsets of families of patients with schizophrenia characterized by age at onset or neurocognitive deficits compared with those obtained in initial linkage analyses using all available families. A maximum NPL-Z score of 4.12 (empirical $p = 0.007$) at 2q22.1 was found in 295 families ranked by increasing age at onset. Based on this subset, a further subsetting by false alarm rate on the undegraded and degraded CPT obtained further increase in the nested subset-based NPL-Z on 2q22.1, with a score of 5.36 (empirical $p = 0.004$) in 228 families and 5.50 (empirical $p = 0.001$) in 243 families, respectively. We found strong evidence of linkage on chromosome 2q22.1 in families of schizophrenia patients with younger age at onset and more CPT false alarm rates. We also found evidence of linkage on 7q, 8q, 9p, and 12q in families with particular neurocognitive deficits. These results highlight the importance of incorporating genetic-related subphenotypes in unraveling the complex genetics of schizophrenia.

## 1991/T/Poster Board #540

**Anthropometric traits for eyes and nose show evidence of genetic effect in Mongolian individuals.** *D.W. Suh[2,3], S.W. LiM[1,2,4], H.J. Kim[2,3], J.H. Yi[2], J.I. Kim[1,2,4], J.S. Seo[1,2,3,5].* 1) Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine, Seoul 110-799, Korea; 2) ILCHUN Genomic Medicine Insititute, Medical Research Center, Seoul National University, Seoul 110-799, Korea; 3) Department of Biomedical Sciences, Seoul National University Graduate School, Seoul 110-799, Korea; 4) Psoma Therapeutics, Inc., Seoul 110-799, Korea; 5) Macrogen Inc., Seoul 153-023, Korea.

The anthropometric traits for eyes and nose are complex quantitative traits influenced by many factors including genetic and environmental input. There have been only a handful of reports regarding the genetic effect and the genes linked to phenotypes of eyes and nose. The aim of this study is to determine the amount of genetic effect and quantitative trait locus (QTL) of 7 eyes and nose anthropometric traits in an isolated Mongolian population, using the data collected from 1,014 individuals (434 male and 580 female) of Mongolian origin. Frontal and dextrolateral photograph of each subject were taken, and 7 eyes and nose anthropometric traits were recorded. 349 short tandem repeat (STR) microsatellite markers on 22 autosomes of each subject were also genotyped. Heritability estimates of 7 eyes and nose size adjusted by significant covariates among age, sex, age*sex, age^2 and age^2*sex were ranged between 0.48 to 0.84. With variance-component linkage analysis, we found evidence of linkage (multipoint LOD score ≥2.0) on chromosome 1, 2, 4, 11 and 13. The peaks on chromosome 2 and 4 were found to overlap in more than one trait. Implementing 4 additional covariates (height, weight, BMI and body fat) yielded increase in the heritability estimates (h2=0.48-0.90), and evidences of linkage (multipoint LOD score ≥2.0) could be detected on chromosome 4, 5, 9, 13, 15, 17 and 18. Highest multipoint LOD score was 3.9 in bilateral eye fissure length on chromosome 5, where the region included additional linkage peak in biocular width. Further efforts will assist in our understanding of the link between genetic factors and the human anthropometric traits.

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## 1992/T/Poster Board #541

**A novel locus for autosomal recessive non-syndromic mental rentardation (NSMR) maps to 13q12.3-q13.2.** *A. Noor[1], A. Mir[2], A. Mikhailov[1], A. Fennell[1], M. Ayub[3], J.B. Vincent[1].* 1) Neurogenetics Section, Center For Addiction and Mental Health (CAMH), Toronto, ON, Canada; 2) Department of Bio-Sciences, COMSATS Institute of Information Technology (CIIT), Islamabad, Pakistan; 3) St. Luke's Hospital, Middlesborough, UK.

BACKGROUND: Mental retardation (MR) is defined by an intelligence quotient (IQ) of less than 70 associated with functional deficits in adaptive behavior, and has a prevalence of 1-3% in the population. Although non syndromal autosomal recessive forms of MR (NS-ARMR) are believed to be relatively more common, only five genes have been reported so far. The objective of the present study was to identify a new locusgene for ARMR. METHODS: We ascertained a consanguineous family affected with non-syndromic autosomal recessive mental retardation. The phenotype was present in 4 individuals from two branches of the family. To map the chromosomal location of the causative gene we undertook Affymetrix 5.0 gene chip SNP analyses of all affected individuals and two unaffected individuals, assuming that a founder mutation was responsible. RESULTS: All affected individuals shared a 4.9 Mb haplo-identical region of homozygosity located on chromosome 13q12.3-q13.2, defined by SNP markers rs9506126 and rs9598929. The physical location of the critical region is 28,727 Mb to 33,621 Mb (UCSC, March 2006) and it contains ~30 Refseq genes. We have identified several candidate genes within this locus including UBL3, KATNAL1, FRY, PDS5B and KIAA0774. Sequencing of candidate genes to identify the causative mutation is in process. CONCLUSION: We have mapped the chromosomal location of a novel gene responsible for autosomal recessive non-syndromic mental retardation.

## 1993/T/Poster Board #542

**A novel autosomal recessive syndrome of mental retardation, kyphoscoliosis and congenital heart disease maps to chromosome 11q14-q23.** *A. Tzschach[1], M. Garshasbi[1,2], P. Nekooee[2], R. Kariminejad[3], H. Ropers[1], A. Kuss[1], H. Najmabadi[2,3], K. Kahrizi[2].* 1) Human Molecular Genetics, Max Planck Institute for Molecular Genetics, Berlin, Germany; 2) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; 3) Kariminejad/Najmabadi Pathology & Genetics Center, Tehran, Iran.

We report on three siblings - two brothers and a sister born to healthy consanguineous Iranian parents - with a novel mental retardation/multiple congenital anomalies (MR/MCA) syndrome. All three patients suffered from moderate to severe mental retardation, kyphoscoliosis which became apparent at the age of approximately 6 years and congenital heart disease. Body measurements were within the normal range, and metabolic tests and neurological investigations were normal in all patients. Chromosome analysis including array CGH revealed no abnormalities. The patients had three healthy siblings; their parents were first cousins. Assuming an autosomal recessive gene defect in this family, we performed genome-wide linkage analysis (homozygosity mapping) using a 10K SNP array (Affymetrix) and identified a single linkage interval on the long arm of chromosome 11. The homozygous interval was flanked by SNPs rs1391221 and rs1880206 and spans 28 Mb in 11q14.2-q23.2. The LOD score was 2.65. The similarity of the clinical features, the pedigree structure and the linkage data suggest that the patients in this family suffer from a novel autosomal recessive MR/MCA syndrome. Mutation analysis in candidate genes within the homozygous interval will eventually disclose the underlying genetic defect in this family.

## 1994/T/Poster Board #543

**Genome-wide association study of cataract in the Marshfield Personalized Medicine Research Project as part of the eMERGE network.** *E.S. Torstenson[1], C.A. McCarty[2], S.D. Turner[1], Y. Bradford[1], D. Berg[3], P. Peissig[3], J. Linneman[3], J. Starren[3], C. Waudby[2], L. Chen[4], M.D. Ritchie[1].* 1) Center Human Genetics Research, Vanderbilt Univ, Nashville, TN; 2) The Center for Human Genetics , Marshfield Clinic Research Foundation; 3) Biomedical Informatics Research Center, Marshfield Clinic Research Foundation; 4) Department of Opthalmology, Marshfield Clinic Research Foundation.

The Electronic Medical Records and Genomics (eMERGE) network is an NHGRI funded initiative with the goal of testing whether electronic medical record (EMR) systems can serve as a resource for collecting phenotypic information for genomic studies. To test this hypothesis, five groups around the United States are using natural language processing algorithms to derive phenotypes in patients who have DNA samples in the institution's biobank. The Marshfield Clinic in Central Wisconsin represents one such institution and our phenotypes are cataract and high-density lipoprotein (HDL) cholesterol (U01-HG004608). Here we will discuss our genome-wide association study for cataract. Cataract is the leading cause of vision loss in the US and blindness in the world. Summary prevalence estimates indicate that 17.2% of Americans aged 40 years and older have cataract in either eye and 5.1% have pseudophakia/aphakia (previous cataract surgery). With increased life expectancy, the number of cataract cases and cataract surgeries is expected to increase dramatically unless primary prevention strategies can be developed and successfully implemented. Known environmental risk factors for cataract include cigarette smoking, ultraviolet light and chronic steroid use. It has been suggested that as many as 40 genes may be involved in age-related cataract. Evidence for a major gene has been identified for cortical and nuclear cataract, with heritability estimates of 58% and 48%, respectively. To date, no genome-wide association studies of age-related cataract in unrelated individuals have been reported in the medical literature. Presence or absence of cataract (through surgery records, diagnosis codes, and natural language processing) in addition to cataract type (cortical and nuclear) was electronically harvested from subject EMRs in 3900 individuals (2600 cases and 1300 controls). All samples were genotyped with the Illumina 660-Quad platform. Each SNP passing rigorous quality control measures was tested for association with cataract using logistic regression, assuming an additive model. Here we report several highly significant findings, shedding some light on the genetic contribution to cataract. Finally, we demonstrate the success of using an EMR-derived phenotype to perform genetic analysis of complex disease. This study along with the other eMERGE projects provide evidence supporting the use of biobanks linked to EMRs for genomic studies.

## 1995/T/Poster Board #544

**Novel compound heterozygous mutations in CERKL cause autosomal recessive retinitis pigmentosa in a non-consanguineous Chinese family.** *L. Wang, Z.H. Tang, T. Ke, Q. Wang, M.G. Liu.* Huazhong University of Science and Technology, Wuhan, Hubei, China.

Objective: To identify the genetic basis of severe autosomal recessive retinitis pigmentosa in a non-consanguineous Chinese family. Methods: Linkage and halpotype analyses were used to identify chromosomal location of the pathogenic gene in the arRP family. Direct DNA sequence analysis was used to identify the disease-causing mutation, and to demonstrate if the mutations co-segregate with the disease in the family. Results: The disease gene in the Chinese arRP family was linked to the RP26 locus. DNA sequence analysis revealed two compound heterozygous mutations in the newly identified RP26 disease-causing gene, CERKL. One mutation is c.156_157insT in exon 2, and the other is c.758delT in exon 5. The affected individuals in the family inherited both mutations, whereas two unaffected sisters inherited normal alleles from their parents. The two mutations were not detected in 100 unrelated normal controls. Conclusions: There are only three mutations have been identified in CERKL, and all of them were detected in consanguineous families. Here we report two novel CERKL mutations, c.156_157insT in exon2, and c.758delT in exon5, in a non-consanguineous Chinese arRP family. It is the first report that compound heterozygous mutations of CERKL may cause severe RP. These studies expand the spectrum of CERKL mutations causing arRP. Clinical Relevance: Clinical and genetic characterization of retinitis pigmentosa may provide more accurate prognosis in RP patients, and help to understand the pathology of this important disease.

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## 1996/T/Poster Board #545

**Predicting the functional importance of SNPs to improve the power of genome-wide association studies.** *R.J. Klein, M.A. Levenstien.* Program in Cancer Biology and Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA.

Over the last several years, genome-wide association studies (GWAS) have successfully identified numerous alleles associated with common disease. Despite these successes, it is clear that these variants do not explain the full heritability of these diseases. One problem with GWAS is that the large number of single nucleotide polymorphisms (SNPs) tested require a severe multiple testing correction. To have the power to detect a genome-wide significance level of $10^{-7}$ requires extremely large studies, especially for alleles of modest effect. If we could predict *a priori* which SNPs are most likely to have some functional consequence, we may be able to reduce the number of tests and thereby increase the power of GWAS without increasing the study size. To begin to identify those sequence features most likely to contain functionally important variants, we cross-referenced bioinformatic annotation of genomic sequence features with minimally biased genome-wide data on SNP allele frequencies. Using the annotations available in the Ensembl database, we categorize SNPs in the human genome into classes related to regulatory features, such as epigenetic modifications and transcription factor binding sites in addition to classes related to gene structure and cross-species conservation. Based on population genetic theory, we expect the dereived allele frequencies to trend lower in functional classes of elements than in those that are neutrally evolving. We assess the strength of natural selection for each class relative to the genome as a whole using the distribution of derived allele frequencies (DAF) within each class. We apply this DAF analysis to Perlegen resequenced SNPs genome-wide. Groups of regulatory elements annotated by Ensmbl as well as some individual elements, such as PolII binding sites, DNase I hypersensitivity sites, and several histone methylation sites, show negative selection in comparison to the genome as a whole. In fact, some histone modification sites in addition to constrained elements outside of genes appear to have derived allele frequencies comparable with those of the coding regions. We estimate that if one were to restrict analysis of GWAS to SNPs that fall in the functional classes we identify, the number of SNPs to be tested would be reduced by two orders of magnitude, thereby yielding increased power.

## 1997/T/Poster Board #546

**The tumor suppressor LKB1 kinase gene associated with type 2 diabetes.** *P. Keshavarz[1], H. Inoue[2].* 1) Department of Genetics, Guilan University of Medical Science, Rasht, Iran; 2) Institute for Genome Reseach, The University of Tolkushima.

Inactivating germ line mutations in the LKB1 gene underlie Peutz-Jeghers syndrome characterized by hamartomatous polyps and an elevated risk for cancer. The tumor suppressor LKB1 is an upstream kinase in the AMP-activated protein kinase (AMPK) cascade provided a molecular link between energy metabolism and cancer. A recent study by Shaw and colleagues elucidated the role of LKB1 in type 2 diabetes. To assess whether the LKB1 variants has any impact to type 2 diabetes in Japanese, we screened all ten exons, their exon-intron boundaries, and the 5' and 3' flanking regions of LKB1 to identify single nucleotide polymorphisms (SNPs), and we genotyped 911 unrelated Japanese type 2 diabetic patients and 876 control subjects to show possible associations between genotypes or haplotypes and type 2 diabetes. We observed associations of nominal significance with an intronic SNP in the LKB1 (rs741765; OR 1.33, 95% CI 1.05-1.67, p = 0.017, under a recessive genetic model with type 2 diabetes. In the haplotype analysis, consisting of six representative SNPs, revealed one haplotype, to be associated with type 2 diabetes (P = 0.03). We speculate that the LKB1 gene influences insulin resistance and susceptibility to type 2 diabetes in the Japanese population.

## 1998/T/Poster Board #547

**A whole genome-wide association study of psoriasis within the Japanese population using 26,061 microsatellite markers.** *A. Oka[1], T. Mabuchi[2], H. Hayashi[1], K. Yamaguchi[1], E. Matsushita[1], Y. Keisuke[3], S. Mano[4], A. Ozawa[2], T. Gozyobori[5], H. Inoko[1].* 1) Division of Basic Medical Science and Molecular Medicine, Department of Molecular Life Science, Tokai University School of Medicine, Isehara, Kanagawa 259-1193, Japan; 2) Departments of Dermatology, Tokai University School of Medicine, Isehara, Kanagawa 259-1193, Japan; 3) Department of Cardiorenal and Health Medicine, Yokohama City University School of Medicine, Yokohama Kanagawa 236-0004, Japan; 4) Institute of Natural Sciences, Nagoya City University, Nagoya 467-8501, Japan; 5) Center for Information Biology and DNA Data Bank of Japan, National Institute of Genetics, Shizuoka 411-8540, Japan.

Psoriasis is a common skin disorder characterized by inflammatory cell infiltration and hyperproliferation of epidermal cells. The familial nature of this disease, which affects almost 2% of Caucasian populations, has long been recognized on the basis of twin studies and linkage analysis. However, in the Japanese population, a lower incidence (0.1%) has been observed with most psoriasis cases being sporadic. These facts define psoriasis as a multifactorial disease triggered by the involvement of some environmental factors in individuals with a particular genetic background. We have recently tested and established the use of approximately 26,000 microsatellite markers for genome-wide association studies of common diseases (http://www.jbirc.aist.go.jp/gdbs/). Our aim now was to apply the same collection of microsatellite markers to identify the susceptibility loci and the pathogenic genes responsible for psoriasis in a case-control association study of the Japanese population. An initial set of 375 psoriasis patients and an identical number of control samples, all of Japanese descent, were applied by pooled DNA genotyping in the three-step genomic screen. Microsatellites that had remained statistically different between cases and controls in all three screening steps were confirmed by individual genotyping using the same set of 375 patients and 375 controls and the additional set of 186 patients and 186 controls. Accordingly, we found significant association for one microsatellite as assessed by the Fisher's exact test, except for psoriasis-susceptibility locus (PSORS1) of the HLA region, when we applied a p-value 2.6x10^-7 for significance after Bonferroni's correction to account for multiple comparisons. This microsatellite was located at 5'-flanking region of one gene of the GPCR family. Moreover the expression level in peripheral blood mononuclear cell was the correlation with the susceptibility allele, however the function of this gene was not clear so far.

## 1999/T/Poster Board #548

**Genetic study of otosclerosis in NL founder population.** *N. Abdelfatah[1], S. Moore[3], T. Batten[4], K. Hodgkinson[1], A. Griffin[2], D. Galaturia[1], C. Negrijn[1], T. Young[1].* 1) Memorial University of NL, St John's, NL, Canada; 2) Central Regional Integrated Health Authority, Grand Falls-Windsor, NL A2A 2E1; 3) Department of Pediatrics, Memorial University of Newfoundland, St. John's, NL, Canada; 4) ENT Consultants, 1Paton St, St John'S, NL A1B 4S8.

Introduction Otosclerosis is a common form of hearing loss in the Caucasian population with a prevalence of 0.3-0.4 worldwide. Histological studies indicates that sclerotic foci in the temporal bone invade the stapedio-vestibular joint that leads to fixation of the stapes bone in to the oval window and cause the development of conductive hearing loss. The etiology of otosclerosis is unknown, but genes and environmental factors with possible interaction between them are believed to cause otosclerosis. Since 1998, seven loci (OTSC1, OTSC2, OTSC3, OTSC4, OTSC5, OTSC7, OTSC8) segregating with autosomal dominant form of otosclerosis have been mapped; none of the genes have been identified yet. Also, several association studies including several genes (COL1A1, COL1A2, NOG and RELN genes) have been carried out with sporadic forms of otosclerosis. Seven unrelated otosclerosis families were ascertained from Newfoundland, a genetically isolated population. Purpose of the study: To identify the disease-causing gene(s) for otosclerosis in families with autosomal dominant form of inheritance, and understand the mechanism underlying disease development. Methods: Two large multiplex NL families (2114, 2108) and five other probands were genotyped for microsatellite markers span the intervals of the seven known loci, the collagen genes COL1A1, COL1A2 and NOG genes. Haplotypes across the ten loci were constructed manually using the least number of recombination. Results: Haplotypes analysis demonstrate that all affected 2114 family members share a region of 10.7Mb that overlaps with the original 17.9 Mb region at OTSC3 and a region of 53.0 Mb at chr7 including OTSC2 and COL1A2 and RELN, genes were previously associated with otosclerosis. Haplotypes analysis for family 2081 exclude the linkage to OTSC1, OTSC2, OTSC3, OTSC5, OTSC8, and COL1A1, NOG gene. Further genotyping and haplotype construction for the remaining loci and genes are in progress. Summary: A five generation family (2114) appears to be linked to OTSC3 and OTSC2. Also, all affected members share a large haplotype that includes COL1A2 and RELN genes. A four generation family (2081) has been excluded from the linkage to six of the mapped otosclerosis loci and two associated genes. Comparing haplotype of potentially linked locus at OTSC3 among seven probands, three of the seven probands share a portion the linked region. Further studies are in progress to confirm the linkage.

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## 2000/T/Poster Board #549

**A Fine Mapping Theorem to Refine Results from Association Genetic Studies.** *S.J. Schrodi, V.E. Garcia, C.M. Rowland.* Dept Statistical Genetics, Celera, Alameda, CA.

Much remains to be explained about human genetic architecture and specific variants underlying important traits such as disease phenotypes - both critical to successful fine mapping following GWAS. High density mapping and inference of susceptibility variants is highly reliant upon the positional pattern of disease association peaks. In this work we describe the nature of the decay curve of association patterns due to declining LD from a causative site. Under a variety of disease models, we show that the central tendency approximation $\chi^2_M \sim r^2 \chi^2_D$ holds, where $\chi^2_M$ and $\chi^2_D$ are the chi-sq statistics at a marker and disease-causing site, respectively; and $r^2$ is the standard measure of LD between the two sites. We use the phrase "fine mapping theorem" for this approx to underscore its potential utility in discovering specific variants underlying traits studied in very high density mapping studies. Monte Carlo simulations were used to characterize the amount of error in the approximation. These results showed that the maximum mean squared error is a concave function of $r^2$ peaking at intermediate levels of r2 across all the disease models screened. Next, given a potential causative polymorphism and several closely-linked sites with disease association data, a method was developed to quantify the departure from the fine mapping theorem. Calculating this departure metric for all SNPs in an associated region will give a measure of correspondence with the fine mapping theorem for each polymorphism, and enable one to determine the most likely (i.e. those with the smallest departure metric) disease-causing variants under a theoretical model (i.e. a single disease-predisposing variant and numerous closely-linked markers associated with disease solely through LD). Lastly, we applied these approaches to previously-published fine mapping datasets for type 1 diabetes (*IL2RA* region consisting of 305 SNPs) and rheumatoid arthritis (*TRAF1* region consisting of 138 SNPs). In both datasets, single SNPs with the highest correspondence to the theoretical association decay patterns were identified. Conversely, SNPs deviating from their chi-sq values expected under the theorem may constitute additional susceptibility polymorphisms in the region studied. Similar applications of this fine mapping theorem may prove to be a pragmatic approach to delineate genes, gene regions, or functional motifs responsible for disease etiology subsequent to initial genetic results from GWAS.

## 2001/T/Poster Board #550

**Homologous Genomic Loci Can Cause Genotyping Errors and can be Overrepresented in SNP Panels Selected according to Linkage Disequilibrium Tagging Methods.** *A.L. Guiney, J.A. Fagerness, A. W. Kirby, J. W. Smoller.* Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA.

Many commonly assayed single nucleotide polymorphisms (SNPs) occur within sequences that share high homology to regions elsewhere in the genome. Assays of these SNPs, which commonly use relatively short target sequences, will not always be specific to the single desired locus. Assays that interrogate more than one genomic locus can result in false genotypes that appear to be properly assayed SNPs, and can pass standard quality control filters. Many of these incorrectly genotyped polymorphisms appear in commonly used SNP databases. Because their apparent genotypes are not the result of one locus' genetic variability these SNPs will be out of linkage disequilibrium (LD) with nearby polymorphisms and will break up otherwise contiguous blocks of (LD). As a result they will be overrepresented in SNP panels selected according to LD tagging methods. In addition, they can show false associations to studied phenotypes.

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## 2002/T/Poster Board #551

**Mutations in the Integrin responsible for a congenital muscular dystrophy with hyperlaxicity and their impact on normal cellular adhesion.** *M. Tetreault[1,2], I. Thiffault[1,2], L. Loisel[1], J. Mathieu[3], Y. Robitaille[4], M. Vanasse[5], B. Brais[1,2].* 1) Laboratoire de Neurogenetique et motricite, CRCHUM Hopital Notre-Dame, Montreal, QC, Canada; 2) Neuromics Center for Excellence of Universite de Montreal, Universite de Montreal, CRCHUM Hopital Notre-Dame, Montreal, QC, Canada; 3) Carrefour de la sante de Jonquiere, Saguenay, QC, Canada; 4) Departement de pathologie, Hopital Sainte-Justine, Montreal, QC, Canada; 5) Clinique des maladies neuromusculaires, Centre de readaptation Marie-Enfant, Hopital Sainte-Justine, Montreal, QC, Canada.

Congenital muscular dystrophies (CMD) are a heterogeneous group of disorders. A growing number of CMD have been found to be associated with joint hyperlaxicity. We recruited 16 French-Canadian cases belonging to 13 families from Southwestern Quebec affected by an autosomal recessive congenital muscular dystrophy with hyperlaxicity (CMDH). All patients present muscle weakness, proximal contractures coexisting with distal joint hyperlaxicity. They have a more benign course than most described CMD, with preservation of walking into adult age in most cases. Mutations were uncovered in an integrin. To date, deletion, complex insertions (Alu, LTR) and partial duplications of the gene were uncovered in the patients. Considering that integrins are transmembrane proteins that interact with extracellular matrix proteins we performed cell adhesion assays using cell lines from patients with different mutations. Comparing controls and patients cell lines; we found that these mutations modified the binding ability to certain extracellular partners. The identification of mutations in a second integrin gene causing a congenital muscular dystrophy confirms the importance of this family of proteins in ensuring the proper interactions between the extracellular matrix and the membrane essential to normal muscle development and function.

## 2003/T/Poster Board #552

**Replication of synergy between genetic variants in TF and HFE as risk factors for Alzheimer's Disease.** *J.S.K. Kauwe[1,2], S. Bertelsen[2], K. Mayo[2], C. Cruchaga[2], R. Abraham[3], P. Hollingworth[3], D. Harold[3], M.J. Owen[3], J. Williams[3], S. Lovestone[4], J.C. Morris[2], A.M. Goate[2].* 1) Department of Biology, Brigham Young University, Provo, UT; 2) Department of Psychiatry, Washington University School of Medicine, St. Louis, MO; 3) MRC Centre for Neuropsychiatric Genetics and Genomics, Department of Psychological Medicine, School of Medicine, Cardiff University, Cardiff, UK; 4) Department of Neuroscience, Institute of Psychiatry, Kings College, London, UK.

Alzheimer's disease (AD) is a complex disease that is likely influenced by many genetic and environmental factors. Evidence from the Alzgene meta-analyses and several recent studies suggests rs1049296 in transferrin (TF) is associated with risk for AD. However, as is the case with many reported associations, replication has been difficult and often yields inconsistent results. It has been suggested that this lack of replication may be a "signature of epistasis". Citing evidence that iron may play a role in AD pathology, Robson et al. (2004) reported that epistatic interaction between rs1049296 (P589S) and rs1800562 (C282Y) in the hemochromatosis gene (HFE) results in significant association with risk for AD. In this study we attempted to replicate their findings in a total of 1166 cases and 1404 controls from three European and European American populations. We observed significant association between bi-carriers of the minor alleles of rs1049296 and rs1800562 in the combined sample using synergy factor (p=0.0016, OR= 2.71). These results validate those of the previous report and suggest that TF and HFE modulate risk for AD.

## 2004/T/Poster Board #553

**Schizophrenia and cancer gene networks: a comparative study and their applications.** *Z. Zhao[1,2,3], J. Sun[1,2].* 1) Biomedical Informatics, Vanderbilt University, Nashville, TN; 2) Department of Psychiatry, Vanderbilt University, Nashville, TN; 3) Vanderbilt-Ingram Cancer Center (VICC), Nashville, TN.

Schizophrenia is a heritable and major psychiatric disorder whose pathogenesis likely involves multiple genes interacting themselves or with environment. Cancers have been found to be caused by many genes and their interactions in the cellular systems. In this study, we first compiled the schizophrenia candidate genes and general cancer genes. The schizophrenia candidate genes were selected based on a multi-dimensional evidence-based approach using genes from association studies, linkage scans, gene expression, and literature search. We used independent unbiased p values in the genome-wide associate studies (GWAS) to search for the optimal weight matrix, which was subsequently applied to prioritize schizophrenia candidate genes (SZGenes). In addition to schizophrenia and cancer genes, we also selected neurodevelopment related genes, essential genes and non-disease genes for comparison. Next, we extracted the subnetworks in the whole human protein-protein interaction (PPI) network. Our comparative analysis demonstrated that SZGenes tend to have intermediate connectivity and intermediate efficiency with which a perturbation can spread throughout the network relative to cancer genes, essential genes and non-essential genes. Furthermore, we found that SZGenes do not have a strong trend on interacting with each other or clustering compared to cancer genes. This difference suggests that schizophrenia and cancer might have different pathological mechanisms even though both have been considered as complex disease. Finally, we identified a few novel schizophrenia candidate genes from some schizophrenia small subnetworks that are enriched with small GWAS p values. Our follow up experiments verified them being associated with schizophrenia. This study represents the first systematic gene ranking and network analysis for schizophrenia and comparison with cancer. The extracted disease-specific networks also provided an opportunity for identifying novel candidate genes.

## 2005/T/Poster Board #554

**Does copy number variation influence personality?** *A.T. Bagshaw[1], P.R. Joyce[2], M.A. Kennedy[1].* 1) Department of Pathology, University of Otago, Christchurch, New Zealand; 2) Department of Psychological Medicine, University of Otago, Christchurch, New Zealand.

It has recently emerged that copy number variants (CNVs) consisting of insertions and deletions kilo bases or mega bases in size are a very common and potentially important source of genetic variation. Investigation of the phenotypic effects of this variation is still in its infancy, but several studies have reported associations between CNV and neuro-psychiatric phenotypes including schizophrenia and autism. To further our understanding of this link we are studying the relationship between CNV and personality traits, some of which show significant heritability and appear to act as risk factors for various mental disorders. Our initial work used publicly available data to determine whether any candidate genes for personality are located within known CNV regions. Out of 196 candidate genes considered, we found 136 that overlap with CNVs larger than 1 kb listed in the Database of Genomic Variants [1]. However, almost all particular CNVs are rare, and none of our candidate genes are in CNV with a population frequency greater than 4 percent, based on the most comprehensive dataset available [2]. We have therefore taken the approach of genotyping the ten most commonly occurring known CNVs, anticipating an effect of CNV on the expression of both participant and distal genes, for which there is reasonable evidence [3]. Our test subjects include several cohorts (over 1000 individuals), for which we have personality data assessed using Cloninger's Temperament and Character Inventory (TCI) questionnaire [4]. We will report association data from these common CNVs with novelty seeking and other specific heritable components of personality. 1. Iafrate, A.J., et al., Detection of large-scale variation in the human genome. Nat Genet, 2004. 36(9): p. 949-51. 2. Itsara, A., et al., Population analysis of large copy number variants and hotspots of human genetic disease. Am J Hum Genet, 2009. 84(2): p. 148-61. 3. Stranger, B.E., et al., Relative impact of nucleotide and copy number variation on gene expression phenotypes. Science, 2007. 315(5813): p. 848-53. 4. Cloninger, C., D. Svrakic, and T. Przybeck, A psychobiological model of temperament and character. Arch Gen Psychiatry, 1993. 50(12): p. 975-990.

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## 2006/T/Poster Board #555

**Deletion of *CHRNA7* suggests that this gene mediates part or all of the phenotype for deletion 15q13.3.** *A. Beaudet[1], C. Schaaf[1], S.-W. Cheung[1], A. Patel[1], P. Stankiewicz[1], B. Lanpher[2], L. Immken[3], G. Simpson Patel[3], M. Shinawi[1].* 1) Dept Molec & Human Gen, Baylor Col Med, Houston, TX; 2) Div of Medical Genetics, Vanderbilt Univ, Franklin, TN; 3) Medical Genetics, Specially for Children, Austin, Tx.

Deletion of chromosome 15q13.3 has been associated with mental retardation, idiopathic epilepsy, autism, schizophrenia, and bipolar disorder. The majority of patients have one or more of these findings, but some individuals have a normal or near normal phenotype indicating that the penetrance may be 70-90%. The typical deletion interval includes six genes: *OTUD7A, KLF13, TRPM1, MTMR10, MTMR15,* and *CHRNA7*. The CHRNA7 gene encodes the $\alpha$7 subunit of the neuronal nicotinic acetylcholine receptor, which functions as a homopentameric synaptic ion channel. *CHRNA7* has been suggested as a candidate gene in schizophrenia and has been considered to be the most likely gene to be contributing to the epilepsy and neurobehavioral abnormalities associated with deletion 15q13.3. We have identified three children with deletions of CHRNA7 and a portion of *OTUD7A* which encodes a deubiquitinating enzyme, but leaving the other four genes intact. The propositii were 14 months, 20 months, and 15 years of age when studied and had combinations of failure to thrive, hypotonia, developmental delay, and mental retardation. Parents were studied in one family, and the deletion was inherited from the mother. These data strengthen the possibility that *CHRNA7* mediates part or all of the phenotypic effects seen with deletion 15q13.3, but a contribution from *OTUD7A* remains a possibility. We also have identified 30 families with the reciprocal duplication which was inherited in most cases rather than de novo. Extended families with the duplication are being evaluated to determine if there is any evidence for phenotypic effects. We are sequencing the *CHRNA7* gene searching for point mutations in patients with various developmental disabilities.

## 2007/T/Poster Board #556

**SNPs in the regulatory subunit of calcineurin are associated with CSF phospho-tau protein levels, and risk for Alzheimer's Disease.** *C. Cruchaga[1], J.S Kauwe[1], K. Mayo[1], S. Bertelsen[1], P. Nowotny[1], A.R. Shah[2], A.M. Fagan[2], D.M. Holtzman[2], J.C. Morris[2], A. Goate[1,2].* 1) Dept Psychiatry, Washington Univ, St Louis, MO; 2) Department of Neurology, Washington University, St. Louis, MO, USA.

Using cerebrospinal fluid (CSF) tau and A$\beta$42 levels as endophenotypes for Alzheimer's disease (AD) provides biological models for the effects of disease-associated genetic variation. We have previously shown that genetic variation within MAPT influences CSF ptau181 levels in AD subjects and is associated with age at onset. To identify additional genetic variation influencing CSF ptau181 levels, we selected 384 single nucleotide polymorphisms (SNPs) in 34 genes related to tau posttranslational modification and tested for association with CSF ptau181 levels in 374 individuals. Follow-up of the significant SNPs in a second independent CSF series replicated the associations for SNPs located in genes encoding the regulatory (PPP3R1; rs1868402; p=6.28x10$^{-05}$) and in the catalytic (PPP3CA; rs17030739 p= 2.05x10$^{-04}$) subunits of the phosphatase PP2B (calcineurin). To further investigate these associations and their interaction with A$\beta$ pathology we used CSF A$\beta$42 levels to stratify the sample into those with and without likely A$\beta$ deposition in brain. The association with CSF ptau181 levels is driven by cognitively normal individuals with likely A$\beta$ deposition. Rs1868402 also showed association with PPP3R1 mRNA levels and plaque pathology in the same strata and with risk for AD (p=0.01, OR=1.19, 95%CI = 1.01-1.39). Following cerebral deposition of A$\beta$, rs1868402 or another highly correlated functional SNP increases risk for AD by regulating PPP3R1 expression, leading to increased tau phosphorylation and pathology. Using CSF tau and A$\beta$42 levels as endophenotypes provides a biological model of rs18684102 and calcineurin and identifies an interaction between A$\beta$ and tau pathology wherein tau events occur after A$\beta$ deposition.

## 2008/T/Poster Board #557

**Screening of genes that have an important role in the development of motor neurons in ALS patients.** *H. Daoud, P. N. Valdmanis, F. Gros-Louis, P. A. Dion, G. A. Rouleau.* Center of excellence in neuromics, University of Montreal, Montreal, Quebec, Canada.

Background: SOD1 is a highly-expressed gene in the brain that, when mutated, yields a remarkably selective phenotype only in motor neurons. This may be due to genetic or physical interactions with genes specific to this population of neurons. A set of genes specifically expressed at different stages of development in mouse corticospinal motor neurons (CSMN) has been recently identified, including 29 statistically significant and biologically relevant genes. Several of these key genes are potentially instructive for CSMN development while others are exclusively expressed within the CSMN. We hypothesized that these genes represent good candidates which might be mutated in ALS patients. Interactions of these genes with SOD1 could help explain the specificity of neuron-targeting in ALS. Objectives: To screen ALS patients in order to identify novel mutations in a subset of genes specifically expressed in CSMN. This may help to implicate genes crucial to motor neuron development as causative in ALS. Methods: Following a complete annotation of 29 CSMN-specific genes, the entire exonic sequence of these genes were sequenced in a cohort of 190 (80 SOD1-negative familial ALS and 110 sporadic ALS) individuals. Non-disease causative polymorphisms were eliminated following a search for them in SNP databases and by sequencing 190 ethnically matched controls. Results: A total of 322 variants were identified, including 175 intronics, 8 UTRs, 69 silents, and 69 missenses and 1 nonsense mutation. Of the missense changes, 20 are known SNPs, 36 are frequent changes, and 33 are rare variants. Of the rare variants, 16 are predicted to potentially harm the function of the protein. Follow-up examination of these genes in additional ALS and control samples will help to determine which one(s) are significant to ALS. Conclusions: We identified several potential causative variants in a set of 29 genes that had never been studied before. Genes with excess of mutations will be screened in additional familial and sporadic cases. In the future, we plan to functionally characterize these mutations in in vitro and in vivo models.

## 2009/T/Poster Board #558

**Association of the polymorphism -308 G/A of the gene TNF-$\alpha$ in Mexican Parkinson`s patients.** *M. Garza[1], M.C.E. Vargas[2], E. Martinez[3], F.J. Jimenez[4], A.M. Puebla[5], M.P. Gallegos[2].* 1) Neuroscience, Universidad Autonoma de Guadalajara, Zapopan, Zapopan, Mexico; 2) Laboratorio de Genetica Molecular, CIBO, IMSS; 3) Centro Universitario de los Lagos, Universidad de Guadalajara; 4) Hospital de Especialidades, CMNO, IMSS; 5) Laboratorio de Inmunofarmacologia, CIBO, IMSS.

Parkinson disease (PD) is the second most common neurodegenerative disease that affects elderly people over 65 years old, some present genetic factors could be responsible for the development of this disease. The gene TNF-$\alpha$ (tumor necrosis factor - alpha) codes for a proinflammatory cytokine segregated by activated macrophages. This cytokine is involved in the regulation of different biological processes that includes neuroprotective or neurodeleterious effects depending on the stage of the disease. In our case-control association study we analyzed 71 patients with PD, and 197 controls in the general population of the Occident of Mexico, we amplify a fragment of 107 bp of the gene TNF-$\alpha$ by polymerase chain reaction (PCR), this fragment contents a recognition site for the restriction enzyme NcoI for the wild type allele (G) that generates two fragments of 78 bp and 20 bp. The polymorphic allele (A) is free of this recognition site for NcoI. The allele A for the polymorphism -308 G/A of the gene TNF-$\alpha$ is present in 15% (21/139) of the patients with PD and in 9% (34/393) of the controls, when we compare this data our findings are stadistically significant OR 1.88 (CI 0.99-3.48), p<0.05. In conclusion, the allele A shown a significant association with PD in the Occident of Mexico.

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## 2010/T/Poster Board #559

**Association Analysis of Adenosine A1 receptor (ADORA1) and Dopamine D1 receptor (DRD1) genes with schizophrenia in the Japanese population.** *L. Gotoh[1], H. Mitsuyasu[1], A. Takata[1], Y. Kobayashi[1], K. Yamada[2], T. Yoshikawa[2], H. Kawasaki[1], S. Kanba[1].* 1) Dept Neuropsychiatry, Kyushu Univ, Fukuoka, Japan; 2) Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, Saitama, Japan.

Antipsychotic agents used for the treatment of schizophrenia affect dopamine D2 receptor (DRD2) mediated neurotransmissions, suggesting that dopaminergic dysfunction plays an important role in the pathophysiology of schizophrenia. On the other hands, it is shown that SCH23390, a selective DRD1 antagonist, inhibited PCP-induced schizophrenia-like behaviors. It is also reported that adenosine neurotransmitter system has functional interaction with dopaminergic neurotransmission. It is also known that N6-cyclopentyladenosine, ADORA1 agonist functionally involved in inhibition of PCP-induced behavior of schizophrenia model rats, as well as DRD1 antagonist. Therefore, it is possible to hypothesize that the functions of ADORA1 and DRD1 could be some part of the pathophysiology of schizophrenia. We reported that ADORA1 gene polymorphisms are involved in pathophysiology of schizophrenia using the Japanese population in Kyushu region in our previous association study. In this study, we carried out the replication study using the schizophrenia samples in Kanto region in Japan. In addition, we also investigated the association between DRD1 gene polymorphisms and schizophrenia phenotype in the same population. The case-control samples consisted of 570 unrelated schizophrenia patients (285 men, 285 women; mean age 47.0 ± 11.4 years) and 570 age- and sex-matched controls (285 men, 285 women; mean age 46.7 ± 11.1 years). Eight SNPs (5 SNPs of ADORA1 gene which indicated statistical significant tendency in previous study and 3 SNPs of DRD1 gene which are polymorphic in Japanese based on the database of HapMap project) were genotyped by Taqman real-time PCR method. Association analysis of each polymorphism was performed between schizophrenia patients and controls. The result showed that one SNP of DRD1 gene indicated statistically significant difference between the two populations (P<0.05), but no SNPs of ADORA1 gene indicated significant difference. However, after Bonferroni correction, this statistical difference of DRD1 gene polymorphism was disappeared. Further analysis such as haplotype prediction, linkage disequilibrium calculation, sliding window analysis and multi-variate statistical analysis are in progress. All subjects were given informed consent before blood collection strictly based on the Ethics Committees of the Nagoya University Graduate School of Medicine, Fujita Health University, and the RIKEN Brain Science Institute.

## 2011/T/Poster Board #560

**RGS4 gene in Schizophrenia: case-control and family-based studies, drug response and Tardive Dyskinesia.** *Y. Hirata[1], RP. Souza[1], N. King[1], JA. Lieberman[2], HY. Meltzer[3], G. Remington[1], JL. Kennedy[1].* 1) Neurogenetics Section, CAMH, Univ Toronto, Toronto, ON, Canada; 2) Department of Psychiatry, Columbia University, NY, USA; 3) Departments of Psychiatry and Pharmacology, Vanderbilt University, Nashville, TN, USA.

Purpose: The regulator of G-protein signalling 4 (RGS4) is a key player in dopamine action in the brain, and thus represents a candidate gene for schizophrenia (SCZ). The RGS4 gene and SCZ has shown mixed association results. Recently, a few studies have investigated the association of RGS4 polymorphisms with sub-phenotypes of SCZ. To further clarify the role of RGS4 in this disease, we examined the association of RGS4 with the diagnosis of SCZ in both case-control and family-based samples, and with clozapine response as well as tardive dyskinesia (TD), a potentially irreversible movement disorder associated with chronic antipsychotic exposure. Methods: We examined 4 tagged SNPs across the RGS4 gene (7 kb) in a sample of 182 case-control pairs and 104 small nuclear familes. Genotyping was done using an Illumina platform. SPSS 13.0 and Haploview 4.0 were used for genotype and allelic analyses. Haplotype analyses were performed using UNPHASED 3.0.13 and Haploview 4.0. For the family sample FBAT1.0 was used. Multiple test correction was performed using permutation testing. Results: No marker was out of HW equilibrium in either the case or control sample. We did not observe any association with single marker or haplotype analyses in the case-controls. In the families, nominal significance was observed for SNPs rs2661319 (p=0.034) and rs2842030 (p=0.049), but these did not remain significant after permutation tests. Haplotype analyses showed nominal associations with SCZ, but after permutation, the T-G haplotype (rs2842030-rs10799897) remained significant (p=0.049). When the two sample sets were combined, the G allele of rs2661319 had overall risk significance for SCZ (p=0.009). For analysis of clozapine response (N=139) we found significant association for rs10799897 (genotype: p=0.035, allele: p=0.041). The haplotype A-C (rs10799897-rs10759) was also significant after permutation (p=0.027). For analysis of TD (N= 106) we found no single marker association. The A-G haplotype (rs2661319-rs2842030) remained significant after permutation (p=0.038). Conclusion: We found moderate association between RGS4 and SCZ in our SCZ samples and the sub-phenotypes of clozapine response and TD. Given the important role of RGS4 in dopamine and other neurotransmitter signalling in the brain, further work on larger samples is warranted.

## 2012/T/Poster Board #561

**Genome-Wide Association points to the *NLGN4X*, *CTNND2*, and *DAB1* genes as candidates genes for Autism Spectrum Disorders.** *J. Holden[1,2,3], X. Liu[1,2], M.L. Hudson[1,2], Y. Qiao[5], N. Riendeau[5], I.L. Cohen[6], A.E. Chudley[7], C. Forster-Gibson[4], E. Rajcan-Separovic[5], M.E.S. Lewis[5], ASD-CARC.* 1) Autism Research Program, Ongwanada Resource Ctr, Kingston, ON, Canada; 2) Dept. Psychiatry, Queen's University, Kingston, ON, Canada; 3) Dept. Physiology, Queen's University, Kingston, ON, Canada; 4) Dept. Family Medicine, Queen's University, Kingston, ON, Canada; 5) Dept. Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 6) Dept. Psychology, Institute for Basic Research in Developmental Disabilities, Staten Island, NY, USA; 7) Dept. Human Genetics, University of Manitoba, Winnipeg, MB, Canada.

Autism Spectrum Disorders (ASDs) are the most common childhood developmental disorder. They are defined by significant deficits in communication, social reciprocity and behaviour and include Autistic Disorder, Asperger syndrome and Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS). Several chromosomal regions have been implicated as harbouring autism-risk genes, based on candidate gene, linkage and copy number variant studies. More recently, genome-wide association and genome-wide linkage studies are being applied to this etiologically heterogeneous group. We performed a family based GWA study on 255 affected individuals and their parents from 150 simplex and 48 multiplex families using the Affymetrix 250k *Nsp* SNP array. Our strongest findings were for three SNPs within or flanking the *NLGN4X* gene on the X-chromosome at Xp22.31-32 (rs7881412: $p = 6.66 \times 10^{-16}$; rs4593704: $p = 2.03 \times 10^{-7}$; rs5916143: $p = 1.68 \times 10^{-13}$). NLGN4X plays a role in the maturation and function of neuronal synapses and previous studies have found mutations in this gene in several persons with autism, Asperger syndrome and intellectual disability. SNPs in two other genes showed marginally significant association: two SNPs within the *CTNND2* gene at 5p15.2 (rs258841: $p = 9.03 \times 10^{-8}$ and rs10065669: $p = 8.94 \times 10^{-7}$) and one SNP in the *DAB1* gene at 1p32.2 (rs17115767: $p = 5.61 \times 10^{-8}$). The *CTNND2* gene encodes catenin delta-2, and is within a CNV we reported in a patient with ASD without the classical Cri-du-Chat cry. This gene was recently found to be disrupted in a subject with schizophrenia (Vrijenhoek et al, 2008). The DAB1 (disabled 1 homolog of Drosophila) protein is required for neuronal layering in the cerebral cortex and cerebellum and is part of the Reelin-Dab1 signaling pathway. Given the association of the *RELN* gene with ASDs in previous studies, *DAB1* is also an excellent candidate gene for autism.

## 2013/T/Poster Board #562

**Association Analysis of Adenosine A1 receptor (ADORA1) and Dopamine D1 receptor (DRD1) genes with bipolar disorder in the Japanese population.** *H. Kawasaki[1], L. Gotoh[1], H. Mitsuyasu[1], A. Takata[1], Y. Kobayashi[1], K. Yamada[2], T. Yoshikawa[2], S. Kanba[1].* 1) Dept Neuropsychiatry, Kyushu Univ, Fukuoka, Japan; 2) Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, Saitama, Japan.

Bipolar disorder (BD), known as manic-depressive illness, is a frequent and severe psychiatric disorder. There have been various studies regarding the pathophysiological mechanisms of BD, although the etiology is still unknown. One of the several candidate genes involved in the pathogenesis of BD is dopamine D1 receptor (DRD1), especially its function in the prefrontal cortex. In our previous studies, we reported that the adenosine A1 receptor (ADORA1) may be genetically associated with schizophrenia, which may share the same molecular basis with BD. It is reported that the adenosine neurotransmission systems affect the functions of dopaminergic neurotransmission systems. Therefore, it is possible to hypothesize that the functions of ADORA1 and DRD1 are involved in the pathophysiology of the BD. To clarify the genetic relationship between these receptors and BD, we carried out the association study using the Japanese population. The subjects for the case-control analysis consisted of 366 patients with BD (181 males and 185 females; 50.1±13.4 years old), and 370 control subjects (185 males and 185 females; 50.6±12.6 years old). Eight SNPs (5 SNPs of ADORA1 gene which indicated statistical significant tendency in our previous study of schizophrenia and 3 SNPs of DRD1 gene which are polymorphic in Japanese based on the database of HapMap project) were genotyped by Taqman real-time PCR method. Association analysis of each polymorphism was performed between BD patients and controls. The result showed that one SNP of ADORA1 gene and two SNPs of DRD1 gene indicated statistically significant difference between the two populations (P<0.05). However, after Bonferroni correction, these statistical differences of ADORA1 and DRD1 gene polymorphisms were disappeared. Further analysis such as haplotype prediction, linkage disequilibrium calculation, sliding window analysis and multi-variate statistical analysis are in progress. All subjects were given informed consent before blood collection strictly based on the Ethics Committees of the Nagoya University Graduate School of Medicine, Fujita Health University, and the RIKEN Brain Science Institute.

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## 2014/T/Poster Board #563

**LINGO1 polymorphisms are associated with essential tremor in Europeans.** *S. Klebe[1], S. Thier[1], D. Lorenz[1], M. Nothnagel[2], G. Stevanin[3,4,5], A. Durr[3,4,5], A. Nebel[6], S. Schreiber[6], G. Kuhlenbäumer[7], G. Deuschl[1].* 1) Department of Neurology, University Hospital Schleswig-Holstein, Kiel, Germany; 2) Institute of Medical Informatics and Statistics, Kiel, Germany; 3) AP-HP, Pitié-Salpêtrière Hospital Department of Genetics and Cytogenetics, Paris, France; 4) INSERM, U975, Paris, France; 5) Université Pierre et Marie Curie-Paris 6, UMR_S975, CNRS7225, Centre de Recherche Insitut du Cerveau et de la Moelle, Pitié-Salpêtrière Hospital, Paris, France; 6) Institute of Clinical Molecular Biology, Christian-Albrechts-University Kiel, Germany; 7) Institute of Experimental Medicine, Christian-Albrechts-University, Kiel, Germany.

Essential Tremor (ET) is one of the most common movement disorders. Former association studies focussing on candidate genes in ET found a number of risk variants but most of them were not replicated. Very recently, a genome wide association study revealed two intronic sequence variants in the LINGO1 gene associated with ET. Here, we have confirmed for the first time association between sequence variants in the LINGO1 gene and the ET phenotype in independent German and French ET samples. The ORs for the identified intronic markers rs8030859 (P = 1.0x10-4), rs9652490 (P = 9.1x10-4) and rs11856808 (P = 3.6x10-2) were 1.72 (C.I.: 1.31-2.26), 1.61 (C.I.: 1.21-2.14) and 1.30 (C.I.: 1.02-1.66), respectively, in our German sample. LINGO1 is an interesting candidate gene because it plays a key role in the CNS biology, is selectively expressed in the nervous system and is an inhibitor of oligodendrocyte differentiation and neuronal myelination. Our study gives further evidence that LINGO1 acts as a susceptibility gene for ET.

## 2015/T/Poster Board #564

**Genetic Analysis of Monozygotic Twins Discordant for Cognitive Abilities.** *K. Kobayashi[1], M. Furukawa[1], C. Yu[1], C. Shikishima[2], J. Ando[2], T. Toda[1,3].* 1) Division of Molecular Brain Science, Department of Physiology and Cell Biology, Kobe University Graduate School of Medicine, Kobe, Japan; 2) Department of Education, Faculty of Letters, Keio University, Tokyo, Japan; 3) Division of Neurology, Kobe University Graduate School of Medicine, Kobe, Japan.

Recent study showed that copy-number variations (CNVs) exist within even monozygotic twins and that CNV analysis in phenotypically discordant monozygotic twins may provide a powerful tool for identifying disease-predisposition loci. In order to try to identify the genes which are associated with intelligence, we studied DNAs from lymphocytes of eight pairs of monozygotic twins with discordant intelligence quotient (IQ) by using the Affymetrix 500K SNP array for genome-wide CNV analysis. Almost no CNV differences were seen in the twin pairs, but several CNVs were found on chromosomes 4q22.1, 7q22.1, 19q13.3, and others, in which some interesting genes reside. Further studies will be conducted to identify genes associated with intelligence.

## 2016/T/Poster Board #565

**PPopulation- and Family-Based Studies Confirm A Role for Functional Polymorphisms of the *MTHFR* Gene in the Etiology of Autism.** *X. Liu[1,4], F. Solehdin[2], I. Cohen[3,4], S. Lewis[2,4], J. Holden[1,4].* 1) Dept Psychiatry, Queen's Univ, Kingston, ON, Canada; 2) Dept Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 3) Dept Psychology, NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY USA; 4) The Autism Spectrum Disorders - Canadian American Research Consortium (ASD-CARC; www.autismresearch.ca).

Autism spectrum disorders (ASDs) are a group of debilitating neurodevelopmental conditions characterized by impairments in verbal and non-verbal communication, social interaction and the presence of repetitive behaviours and restricted interests. Despite a general lack of success in identifying genes that are responsible for a majority of ASD cases, there are some striking observations that suggest that changes in both a wide variety of genes and epigenetic modifications of the genome by methylation of CpG islands that are important for normal brain development and growth, cognitive function and behaviour are involved in their etiology. For example, the risk for ASD is very high for persons with the fragile X, Prader Willi, and Rett syndromes, all of which are associated with abnormal methylation. Two variants in the methylenetetrahydrofolate reductase (MTHFR) gene have been shown to affect DNA methylation levels, and one of these has been shown to be associated with autism susceptibility. We examined these two functional SNPs in the MTHFR gene, C677T and A1298C, in a cohort of Caucasian families with a single child diagnosed with an ASD in both case-control and family-based studies. (We cannot rule out that some of these families would not be multiplex families.) The SNPs were in moderately strong linkage disequilibrium. Population-based case-control comparisons revealed a higher frequency of the low-activity 677T allele (*P=0.0004*), higher prevalence of 677TT genotypes (*P=0.0016*), marginally higher frequency of the 1298A allele (*P=0.056*) and a trend towards increased prevalence of the 1298AA genotype (*P=0.124*) in autistic probands versus controls. Analysis of combined genotypes revealed a significantly higher frequency of the double homozygous 677TT/1298AA genotype (*P=0.007*) and the 677T-1298A haplotype in affected individuals relative to controls (*P=0.0004*). Family-Based Association Testing (FBAT) demonstrated significant transmission disequilibrium for both C677T and A1298C, with an excess transmission of the 677T (*P=0.000065*) and 1298A (*P=0.015*) alleles and the 677T-1298A haplotype (*P=0.000091*) from parents to affected offspring. These results suggest that reduced MTHFR activity may serve as an epigenetic risk factor for autism in some families.

## 2017/T/Poster Board #566

**A familial duplication affecting the RORA gene co-segregates with visuo-neuropsychological impairment and focal glucose metabolism decrease.** *S. Markx[1], T. Vrijenhoek[2], J.I. Friedman[3], I.M. Janssen[2], W.A. van der Vliet[2], L. Edelmann[3], K.L. Davis[3], J.M. Silverman[3], H.G. Brunner[2], A. Geurts van Kessel[2], J.A. Veltman[2].* 1) Department of Psychiatry, Columbia University, New York; 2) Department of Human Genetics, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 3) Departments of Psychiatry and Human Genetics, Mount Sinai School of Medicine, New York, USA.

Recent genomic research has revealed many genomic rearrangements - predominantly copy number variation (CNV) - underlying mental disorders. Sometimes the phenotypic consequences of such a rearrangement are immediately evident, but much more often this is not the case, especially in more complex phenotypes. One solution would be to improve phenotypic analysis by applying high-resolution imaging techniques such as magnetic resonance imaging (MRI) and positron emission topography (PET) to detect clinical subtleties that are usually not detected in routine diagnosis. We exemplify the importance of this approach in our study on the role of a rare CNV in a family of which members had variable psychiatric phenotypes. We identified a partial duplication of a brain-specific isoform of the RORA gene in three family members with psychosis-related clinical phenotype. Routine diagnosis and assessment of intellectual abilities based on the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III) initially gave inconsistent results for the three members: the mother suffered from major depression episodes, but had normal IQ; her two daughters were diagnosed for schizophrenia and severe mental retardation. Detailed MRI and PET-based brain analysis demonstrated the co-segregation of the duplication with neuropsychological impairment - specifically visuo-constructive and visuo-analytic aspects - and decreased glucose metabolism in the thalamus and peri-ventricular white matter. While homozygous loss-of-function deletion of RORA in mice results in a *staggerer* phenotype, characterized by severe ataxia and cerebellar atrophy, the current study is the first to show implications of variation in RORA with neuropsychiatric phenotype in humans. Interestingly, the RORA gene is located on 15q22, a genomic region previously linked to the quantitative trait visual attention in schizophrenia patients. Our study highlights the importance of performing both detailed genotypic and phenotypic studies in complex traits, and indicated that RORA plays an important role in visual attention defects.

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## 2018/T/Poster Board #567

**Association analysis between HTR2A and prepulse inhibition with normal controls and schizophrenia in the Japanese population.** *M. Moriwaki[1,2], T. Kishi[1], T. Kitajima[1], M. Ikeda[1,3], Y. Yamanouchi[1], Y. Kinoshita[1], K. Kawashima[1], T. Okochi[1], T. Okumura[1], T. Tsunoka[1], O. Furukawa[2], K. Fujita[2], N. Iwata[1].* 1) Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japanpsychiatry, Fujita Health University, Toyoake, Aichi, Japan; 2) Okehazama Hospital, Toyoake, Aichi, 470-1168, Japan; 3) Department of Psychological Medicine, School of Medicine, Cardiff University, Heath Park, Cardiff, CF14 4XN, United Kingdom.

Impairment of the prepulse inhibition (PPI) deficit and the acoustic startle reflex (ASR) habituation is considered to be one of the endophenotypes for schizophrenia. A recent our study has showed that a PPI deficit was detected in Japanese schizophrenic patients. Also, other study reported that PPI and habituation of the ASR are modulated by polymorphisms in serotonin 2A receptor gene (HTR2A) in schizophrenia. Therefore, we investigated the association between the two functional SNPs in HTR2A prepulse inhibition with normal controls and schizophrenia in the Japanese population. We evaluated the startle measures of mean magnitude of ASR, habituation (HAB), and PPI at prepulse sound pressure intensities of 82 dB (PPI82), 86 dB (PPI86), and 90 dB (PPI90). ASR was significantly different between schizophrenic patients and controls. HAB and all PPI session data from schizophrenic patients were significantly lower than in controls. In addition, we detected significant differences for ASR, habituation and each PPI (82, 86 and 90dB) between schizophrenic patients and controls with the use of multiple regression analysis. HTR2A genotypes, the gender and smoking state were not correlated with ASR, habituation or any PPI in multiple regression analysis. In conclusion, we suggested that functional SNPs in HTR2A did not play a major role of pathophysiology for PPI with schizophrenia and controls in the Japanese population.

## 2019/T/Poster Board #568

**Homozygosity mapping in 4 unrelated Iranian families with autosomal recessive mental retardation identifies overlapping linkage intervals on chromosome 1p34: a frequent cause of ARMR?** *H. Najmabadi[1,3], M. Garshasbi[2], I. Bahman[1], S. Ghadami[1], M.M. Motazacker[2], L. Abbasi Moheb[2], S. Esmaeeli Nieh[2], L. Puettmann[2], M. Mohseni[1], M.J. Soltani Banavandi[1], H. Darvish[1], P. Jamali[4], P. Nikoui[5], F. Soleimani[1], H. Hu[2], W. Chen[2], K. Kahrizi[1], A. Tzschach[2], A.W. Kuss[2], H.H. Ropers[2].* 1) University of Social Welfare and Rehabilitation Sc, Tehran, Iran; 2) Max-Planck-Institute for Molecular Genetics, Berlin, Germany; 3) Kariminejad-Najmabadi Pathology and Genetics Center, Tehran, Iran; 4) Shahroud Welfare Institution, Shahroud, Iran; 5) Bandar Abbas Welfare Institution, Bandar Abbas, Iran.

Other than X-linked mental retardation, autosomal recessive mental retardation (ARMR) has so far received very little attention. This holds particularly for non-syndromic forms (NS-ARMR). To date, only 13 loci for NS-ARMR and no more than 5 genes for this condition have been published. Recently, autozygosity mapping in 78 families with NS-ARMR has revealed that the molecular basis of NS-ARMR (Najmabadi et al., 2007, Hum. Genet. 121(1):43-8) is extremely heterozygous. Indeed, two recurrent mutations have only been observed in a single gene (Garshasbi et al., 2008, Am. J. Hum. Genet. 82(5):1158-64; Molinari et al., 2008, Am J Hum Genet. 82(5):1158-64), suggesting that there are no frequent genetic causes of NS-ARMR. Still, in the course of an ongoing large-scale investigation into the molecular causes of ARMR, we have now identified four consanguineous families, three with non-syndromic and one with syndromic ARMR. In each of the families the analysis yielded a single, almost identical homozygous interval on chromosome 1p34. This is the most conspicuous clustering of linkage intervals observed in our entire cohort of more than 250 consanguineous Iranian families that have undergone autozygosity mapping so far. Therefore, it is tempting to speculate that the underlying defect in three or even all four of these families involves the same gene locus, MRT4 (Najmabadi et al., 2007, Hum. Genet. 121(1):43-8), which may well account for several percent of the ARMR causing mutations in the Iranian population. The genomic region that is common between these intervals comprises 7.7 Mb and encompasses 128 genes. Mutations in the coding regions of several genes, including STIL and POMGNT1, two candidate genes for syndromic forms of MR, were excluded by Sanger sequencing. Chromosome sorting and next generation sequencing, a novel strategy recently pioneered in our laboratories, is now in progress to speed up the search for the underlying gene defect or defects. Results of these studies will be reported.

## 2020/T/Poster Board #569

**Impact of *DRD2* on ADHD-Type Behavior in a Population-Based Birth Cohort: Effect on Women's Temperament.** *E. Nyman[1,2], A. Loukola[1], T. Varilo[1,2,3], A. Taanila[4,5], T. Hurtig[4,6], I. Moilanen[6], J. McGough[7], M.-R. Järvelin[4,8,9], S. Smalley[7], S.F. Nelson[7,10], L. Peltonen[1,2,3,11].* 1) Public Health Genomics Unit, Institute for Molecular Medicine Finland FIMM, Helsinki, Finland; 2) Department of Medical Genetics, University of Helsinki, Finland; 3) Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA, USA; 4) Institute of Health Sciences, University of Oulu, Finland; 5) Unit of General Practice, University Hospital of Oulu, Finland; 6) Clinic of Child Psychiatry, University and University Hospital of Oulu, Finland; 7) Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles, USA; 8) Department of Epidemiology and Public Health, Imperial College London, UK; 9) Department of Child and Adolescent Health, National Institute for Health and Welfare, Helsinki, Finland; 10) Department of Human Genetics, University of California, Los Angeles, USA; 11) 11 Wellcome Trust Sanger Institute, Cambridge, UK.

Attention-deficit/hyperactivity disorder (ADHD) is a common childhood-onset disorder with a significant impact on public health and strong evidence for genetic contribution to disease risk. Previously we have reported results of a candidate gene study in a population-based birth cohort, The Northern Finland 1986 Birth Cohort (NFBC 1986), showing association of a variant of *Dopamine receptor D2 (DRD2)* with ADHD in males but not in females. The current study tests the potential impact of dopamine receptor gene variants on Cloninger temperament traits in the ADHD study sample from NFBC 1986. The NFBC 1986 cohort population of over 9000 individuals is characterized by founder effect, isolation and minimized genetic heterogeneity. We utilized a unique population sample ascertained from this cohort. The systematically ascertained subjects had stringent inclusion criteria for lifetime diagnosis of ADHD using a best estimate procedure and DSM-IV criteria, resulting in 178 definitive or probable (falling 1 symptom short) ADHD cases and 157 symptom free controls. In this sample we genotyped markers in all the known genes of the dopamine receptor gene family and tested them against temperament traits. We found evidence for association of *DRD2* with low Persistence among ADHD females, but not in males. The *DRD2* variant associating with low Persistence in females is the same variant we previously found associating with ADHD in males of this same cohort. Furthermore, we have previously reported an association of the same *DRD2* variant with low Persistence in a similar, older birth cohort, The Northern Finland Birth Cohort 1966. The current study adds further support to our previous results on the involvement of *DRD2* in the etiology of ADHD-type temperament. Together the results of these two investigations suggest that *DRD2* may have an impact on ADHD-type temperament in both males and females, but the particular outcome of its effect appears to be gender-specific, manifesting as a disorder in males and as ADHD-type temperament in females. Furthermore, these findings propose that the putative role of low Persistence as a possible endophenotype for ADHD deserves more attention in further studies.

## 2021/T/Poster Board #570

**Association study of serotonin transporter polymorphism with bipolar disorder, schizophrenia and TCI scale in the Japanese.** *N. Oribe, H. Mitsuyasu, L. Gotoh, H. Kawasaki, S. Kanba.* Dept Neuropsychiatry, Kyushu Univ, Fukuoka, Japan.

Since serotonin transporter (SERT) is one of the sites of action of antidepressants, this gene has been indicated to be related to the pathophysiological mechanisms of mood and anxiety disorders. Differences in SERT expression and function produced by gene polymorphisms are associated with several psychiatric diseases. Two polymorphic regions of SERT gene, a 44-base-pair (bp) insertion / deletion polymorphism in the promoter region (SERTPR) and variable number of tandem repeats (VNTR) in second intron (SERT-in2), have been characterized. SERT-in2 is reported to be associated with unipolar depression, bipolar depression, schizophrenia, and anxiety disorders. SERTPR is also shown to be associated with unipolar and bipolar depression. However, the results from various sources are inconsistent. In this study, we investigated the frequency distribution of polymorphic variants of short (S, s) and long (L, l) alleles, genotypes and haplotypes of SERTPR, and SERTin2, in patients with bipolar disorder (BD) and schizophrenia (SZ) and compared them with those obtained from the Japanese normal controls (NC). Twenty four BD patients and 191 SZ patients diagnosed using the Structured Clinical Interview for DSM-IV (SCID) and 77 NCs were included in this study. The SERTin2 and SERTPR were genotyped by electrophoresis and association analyses were performed. As a result, SERTPR indicated statistically significant differences between BD and NC, and SZ and NC, whereas SERTin2 showed no difference. Additionally, several studies suggested that the function of the serotonin neurotransmission systems associate with some psychological tendencies in human although it is still controversial. Thus, we investigated the associations of SERTPR and SERTin2 polymorphisms with Temperament and Character Inventory (TCI) scales. We genotyped 56 Japanese (16 BD patients and 40 NCs) with SERT polymorphisms and also carried out the TCI tests. However the results showed no significant association. Further analysis with more samples is now in progress. All subjects were given informed consent before blood collection strictly based on the ethical regulations of Kyushu University.

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## 2022/T/Poster Board #571

**Evidence for epistasis between AKT and FOXO3A as risk factors in bipolar disorder.** *M.A. Romano-Silva[1,4], L.A. Magno[4], D.M. Miranda[3,4], A.A. Ferreira[1], F.S. Neves[1], L.A. De Marco[2,4], H. Correa[1,4].* 1) Saude Mental, Faculdade de Medicina, Universidade Federal Minas Gerais, Belo Horizonte, MG, Brazil; 2) Cirurgia, Faculdade de Medicina, Universidade Federal de Minas Gerais; 3) Hospital Julia Kubitschek, FHEMIG, MG, Brazil; 4) INCT de Medicina Molecular, Faculdade de Medicina, Universidade Federal de Minas Gerais, MG, Brazil.

Bipolar Disorder (BD) is one of the leading causes of disability amongst all medical and psychiatric conditions, affecting approximately 1-3% of the worldwide population. In this study, using a case-control approach in a Brazilian population, we aimed to investigate whether AKTIP, AKT and FOXO3A polymorphisms, candidate genes that encode key proteins in the signaling of dopaminergic and serotonergic systems, were associated with BD. In addition, we analyzed the role of selected SNPs conferring vulnerability to suicidal behavior and explored the epistasis among FOXO3A, AKT and AKTIP genes. Subjects were diagnosed with Bipolar Disorder I or II (age = 41.4±12.9). TaqMan SNP genotyping assays were used. We found that demographic and clinical data were associated with lifetime history of suicide attempt. Bipolar Disorder was associated with two FOXO3A SNPs in both allele and genotype distribution (SNP1, genotype: p=0.0109, allele: p=0.0280; SNP2, genotype: p=0.0072, allele: p=0.0068) which remained significant after $1X10^5$ permutations. AKT1 polymorphisms showed association with general suicide attempt (rs1130214, p=0.0471) and especially violent attempt (rs2494746, p= 0.039). Strong LD was observed in FOXO3A (block 1: D'= 1; block 2: D' 0.98) and AKT1 gene (block 1: D'= 1). Significant differences in both FOXO3A and AKT1 haplotypes frequencies between bipolar patients and control was detected. The combined effects of the polymorphisms of AKT1, AKTIP and FOXO3A, in gene-gene interaction analysis, were associated with BD and this data indicated that a synergistic interaction increases the susceptibility to BD. (cross-validation consistency: 10/10; testing balanced accuracy: 0.7530; OR= 12.449 and p= 0.0063). Our data suggest that demographic and clinical characteristics and genetic variations in the AKT1, AKTIP and FOXO3A pathway are associated with increased risk for BD in a Brazilian sample.

## 2023/T/Poster Board #572

**The role of common and rare variants in NOTCH3 Gene in age associated cerebral small vessel disease.** *H. Schmidt[1,2], M. Tscherner[1], R. Schmidt[2].* 1) Molecular Biology, Medical University Graz, Graz, Austria; 2) Department of Neurology, Medical University Graz, Graz, Austria.

Background: Cerebral small vessel disease detected by brain MRI as white matter lesions (WML) and/ or lacunes is present in >0.20; of the population aged over 65. WML progress rapidly and lead to cognitive decline, gait disturbances as well as stroke. Risk factors for WML are age and hypertension, its heritability is 0.55-0.70. CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukencephalopathy) represents a monogenetic form of cerebral small vessel disease. It is caused by mutations in NOTCH3 gene. The aim of the study was to describe the distribution of genetic variants in the NOTCH3 gene in the elderly and evaluate their roles in age-associated WML. Methods: The study was conducted in the cohort of the Austrian Stroke Prevention Study (ASPS) a single centre, prospective population based study in the normal elderly in Graz, Austria. WML was defined by brain T2-weighted MRI images and wml volume was measured by a semi-automated algorithm. The NOTCH3 gene including the 5`and 3`-regions was screened for sequence variants by dHPLC and by direct sequencing in 88 ASPS participants with and in 82 without WML. Common SNPs which showed different frequency between the 2 screening subsets, were genotyped using the TaqMan technology in the whole ASPS cohort (N=923). We used the additive genetic model to test association between these SNPs and WML volume. Adjustment was done for age and hypertension. Results: We detected 35 SNPs in the NOTCH3 gene, 23 SNPs were located in exons, 4 in introns, 3 in the promoter and 2 in the 3' UTR. In total 10 SNPs were novel. Among the 35 SNPs 10 SNPs had a minor allele frequency of >0.05;. Three of these SNPs, rs7257550 (beta= -0,114, p= 0,0496796) rs10423702 (beta= -0,116, p= 0,044) and rs1044009 (beta= -0,116, p= 0,048) were significantly associated with WML volume before correction for multiple testing. We detected 25 rare SNPs (MAF<0.05), 12 were missense, 5 silent mutations, 2 were in introns, 2 in the promoter and 2 in the 3'-UTR. Discussion: This is the first study investigating genetic variations in NOTCH3 gene in the normal population and their relation to age associated cerebral small vessel disease. We found that both common and rare variants are frequent in NOTCH3. Common SNPs might enhance the risk for WML in the elderly, but larger studies are needed to test if this association is real. The role of rare SNPs in the development of WML have to be further evaluated.

## 2024/T/Poster Board #573

**Integrated next-generation sequencing and array based analysis of genetic modifiers in Velocardiofacial Syndrome (VCFS).** *A. Urban[1,2], Y. Zhang[1], D. Palejev[1], R. Haraksingh[2], J.O. Korbel[3], M. Snyder[2], S. Weissman[1].* 1) Genetics Department, Yale Univ, New Haven, CT; 2) MCDB Department, Yale University, New Haven, CT; 3) European Molecular Biology Laboratory, EMBL, Heidelberg, Germany.

Schizophrenia is a major public health concern (affecting 1% of the population) and a complex disease with a strong genetic component. Velocardiofacial Syndrome (VCFS) is typically caused by a 3 Mbp heterozygous deletion on chromosome 22q11 and up to 30% of patients develop schizophrenia or similar symptoms. This makes VCFS a prime point of entry to understand the complex genetic etiology of schizophrenia. However, the search for the modifying genetic variants needed to differentiate the 30% of the patients with mental disease from the remaining 70%, with both groups carrying the 3 Mbp main deletion, has so far stayed incomplete. Earlier we used High-Resolution CGH (HR-CGH) on NimbleGen 385K custom arrays to show that the main deletion can substantially vary in size between patients [Urban, Korbel et al., PNAS 06] and that there are additional, smaller, CNVs to be found within the 3 Mbp deletion interval on the non-deleted chromosome [Korbel, Urban et al., PNAS 07]. Then we developed HR-PEM (paired-end mapping) [Korbel, Urban, Affourtit et al., Science 07] (also [Korbel et al., Genome Biol., 09]) for sequencing based high-resolution CNV analysis, and identified 22q11 as a hotspot for small CNV/SV. Here we report on the integrated genome-wide search for modifiers in VCFS using HR-CGH and HR-PEM in conjunction with array capture resequencing (ACRes). We are using a custom designed 385K NimbleGen longmer array to capture a 5 Mbp locus containing the VCFS region on chromosome 22q11 and a NimbleGen 2.1M longmer array to capture all exons in the human genome. We sequence the captured fractions on the Illumina Genome Analyzer 2, but using the paired-end protocol to enhance the power of detection and to reach out from the exons into their regulatory regions. Through this we can detect any type of genetic variant in the target regions, from single nucleotide changes to large CNV/SV, and we correlate and validate the results with HR-CGH data for the same samples. We have applied this approach to a panel of VCFS patients, unaffected relatives and controls and have detected several hundred genetic variants. We also analyze genome-wide transcriptional activity in cell lines from the same VCFS patients, using Illumina GA2 based RNA-Seq to be able to detect intensity of expression as well as variable splicing, allele specific transcription and novel and non-exonic transcripts, and can now correlate the RNA-Seq data with the ACRes data on genetic variation.

## 2025/T/Poster Board #574

**Common Variant (CV) and Rare Deletion in the Tyrosine Hydroxylase Gene Contribute to Parkinson Disease Risk.** *L. Wang, T. Edwards, G. Daffu, A. Burt, I. Konidari, W. Scott, S. Zuchner, E. Martin, J. Vance.* Miami Institute for Human Genomics and Dr. John T. Macdonald Foundation Department of Human Genetics, Miller School of Medicine, University of Miami, Miami FL.

Parkinson disease (PD) is characterized by a progressive loss of dopaminergic neurons in the substantia nigra. The tyrosine hydroxylase (TH) gene encodes a key enzyme for the biosynthesis of dopamine. Mutations in TH have been previously described as an etiology for an autosomal recessive, dopamine responsive dystonia (Segawa syndrome) and infantile Parkinsonism. Previous association studies of TH did not find evidence for association with PD, which could be attributed to the small sample sizes (N≤430) and limited number of variants (N≤2) examined. To thoroughly examine TH, we evaluated 12 tagSNPs surrounding TH in 697 PD cases and 213 unrelated controls. Initial analysis showed no association (p>0.05). However, tagSNP rs2070782 was also genotyped in two previous large scale studies of PD: a genome wide association study (GWAS) on the NINDS Neurogenetics repository samples (N=537), and a candidate gene study of 268 SNPs from 121 candidate genes in a Japanese cohort (N=380). This SNP was not significant in either study. To increase statistical power, we performed a joint analysis of the three studies (N=1827 total) using publicly available data. Indeed, this analysis found evidence for association at rs2070282 with PD risk (p=0.008, OR=1.2). In all studies, the C allele is more frequent in cases (0.48 to 0.56) than in controls (0.44 to 0.48). Recently, we completed a GWAS of PD using the Illumina Human610-Quad chip. Copy number variation (CNV) analysis using the SNP array found a novel deletion over entire TH gene in one PD patient, which was confirmed by quantitative PCR (qPCR). Patient's age-of-onset was 54 years. Her clinical presentation was mild and typical for dopamine responsive PD, with no evidence of dystonia. We are the first to provide evidence for both association at a CV and existence of a rare CNV in TH for PD risk. The pattern described here for TH mirrors that found in $\alpha$-synuclein gene, where point mutations, CNVs, and CVs all are associated with risk of PD. Our study highlights challenges in genetic studies of complex diseases: the effect size of CVs is usually small and the low prevalence of rare variants (RVs, including deletions) makes them hard to identify. Sharing data and performing joint analysis is instrumental to detect CVs with small effect sizes. Both CVs and RVs need to be considered for a more complete and comprehensive understanding of the genetic architecture of complex phenotypes.

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## 2026/T/Poster Board #575

**Genome-wide homozygous haplotype mapping to identify Autism Spectrum Disorder candidate genes.** 
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Autism spectrum disorder (ASD) is a severe neurodevelopmental disorder of complex and heterogeneous aetiology. ASD is characterised by altered cognitive ability including impaired language and communication skills, deficits in social reciprocity and repetitive and restricted patterns of behaviour and interests. Evidence to support a strong genetic component in the aetiology of ASD stems largely from twin studies which demonstrate a much higher heritability in monozygotic (92%) compared to dizygotic (2-10%) twins. Recent studies offer support for the hypothesis that rare genetic variants contribute to the disorder. However the systematic detection of susceptibility genes containing low-frequency mutations in the genome is technically challenging. Large tracts of extended homozygosity represent a newly explored form of genetic variation and may be highly relevant in disease gene discovery. The mapping of homozygous regions of matching haplotype on a genome-wide scale could help to identify low-frequency potentially disease-liable candidate genes associated with complex disorders. A cohort of ~1500 ASD trios was genotyped for 1 million SNPs allowing adequate coverage for detection of extended runs of homozygosity. The 4000 sample cohort was clustered and separated into analysis groups based on common population ancestry. For each group we applied a whole-genome homozygous haplotype (HH) mapping approach to identify HH that are statistically more prevalent in ASD probands compared to parental controls. Such regions may facilitate the identification of novel risk genes contributing to this complex disorder. The genes within the significant HH were assessed in terms of their biological relevance to the ASD phenotype. Our research identified a number of novel candidate ASD genes of functional and positional relevance. We also replicated significant findings across multiple population analysis groups and the main findings will be presented at the ASHG 2009. Furthermore an unexpectedly high proportion of published ASD genes are located within HH that are significantly more common in ASD patients. Pathway analysis of the significant candidates showed an excess of genes involved in axon guidance, an interesting observation given the recent implication of this gene family in ASD. Our study highlights the utility of HH mapping as a candidate-gene identification strategy and may improve our understanding of the molecular network underlying this complex disorder.

## 2027/T/Poster Board #576

**Contactin 4 as an Autism Susceptibility Locus.** 
*C.E. Cottrell[1], N. Bir[2], E. Varga[2], D. Cunningham[2,4], R. Zernzach[3], J.M. Gastier-Foster[1,5], K. McBride[2,4], G.E. Herman[2,4].* 1) Department of Pathology and Laboratory Medicine, Nationwide Children's Hospital, Columbus, OH; 2) Center for Molecular and Human Genetics, The Research Institute at Nationwide Children's Hospital, Columbus, OH; 3) Department of Pediatrics, Wright-Patterson Air Force Base, OH; 4) Department of Pediatrics, The Ohio State University, Columbus, OH; 5) Department of Pathology, The Ohio State University, Columbus, OH.

Autism spectrum disorders (ASDs) are a heterogenous and common group of developmental disorders. Affected children demonstrate severe impairments in language and social interaction skills, in addition to stereotypic behaviors. Identifiable genetic causes account for ≤20% of cases. Among these causes, *de novo* and inherited copy number variations (CNVs) have been described in a subset of ASD patients using comparative genomic hybridization (CGH). In recent reports, both structural and sequence variation have been described in the contactin and contactin associated protein (CNTNAP) gene family in association with neurodevelopmental disorders, including ASDs. Contactin proteins are members of the immunoglobulin family of axon-associated cell adhesion molecules that function in the formation and maintenance of neuronal networks. Contactins interact with CNTNAPs to form complexes that promote neuronal migration and axon guidance. Using array CGH, we identified a maternally inherited 1.09 Mb deletion at 3p26.3 encompassing the 5' end of the contactin 4 gene (*CNTN4*) in a patient with severe autism. Based on this finding and previous reports of CNV associated with autism in this region, we sought to further define the role of *CNTN4* as a candidate gene for ASDs. Sequencing of the 22 coding exons of *CNTN4* was performed on 88 patients enrolled in an autism registry at Nationwide Children's Hospital. Inclusion in the registry was based on the clinical diagnosis of an ASD using DSM IV criteria and/or ADOS testing. Four patients were found to have a unique variant within the coding region of *CNTN4*. Each of these variants was inherited from an unaffected parent and altered a highly conserved amino acid residue. Three of the 4 variants were calculated to be damaging on the basis of protein prediction software. To our knowledge, none of these variants has been previously reported. Sequence analysis of *CNTN4* was performed on 107 control samples. One coding variant was detected in our control population and was found to alter a highly conserved amino acid. This variant was not detected in our autism population. In summary, we have found an increased frequency of rare variants in *CNTN4* within an ASD cohort in comparison to normal controls, and we provide additional evidence of CNV in the 3p26 region contributing to an autism phenotype. On the basis of these findings, we believe that *CNTN4* may prove to be an important locus for ASD disease susceptibility.

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## 2028/T/Poster Board #577

**Testing Epilepsy Candidate Genes for Association to Autism in a Genome Wide Association Study Dataset.** *M. Cuccaro[1], D. Ma[1], D. Salyakina[1], R. Tuchman[3], I. Konidari[1], P. Whitehead[1], A. Griswold[1], H.H. Wright[2], R.K. Abramson[2], E.R. Martin[1], J. Jaworski[1], J. Hussman[5], J.R. Gilbert[1], J. Haines[4], M.A. Pericak-Vance[1].* 1) Dept Med, Univ Miami Sch Med, Miami, FL; 2) Neuropsychiatry, USC-SOM, Columia, SC; 3) Dept Neurology, Univ Miami Sch Med, Miami, FL; 4) Cent Hum Gen Res, Vanderbilit Univ, Nashville, TN; 5) Hussman Foundation, Ellicott City, MD.

Autism and epilepsy are common complex disorders which each result in significant behavioral and developmental problems. Conditional prevalence estimates or the rate at which epilepsy is present in autism is conservatively estimated at 25% to 30%. This co-occurrence far exceeds than what would be expected by chance and often results in a more severe phenotype and extremely poor prognosis. The biologic mechanisms that account for this co-occurrence have eluded discovery. Several conceptual models have proposed a common brain pathology in which autism and epilepsy are independent consequences of the same underlying disorder. Given the overlap in these two disorders we propose that epilepsy risk genes are etiologically relevant to autism. In this study we tested the hypothesis that epilepsy related candidate genes may confer risk to autism. Using existing genome wide association study (GWAS) data, we examined 27 candidate genes, selected on the basis of previous reports of association or biological relevance to epilepsy or epilepsy and co-occurring autism. In the initial GWAS, our discovery dataset consisted of 438 Caucasian autism trio families genotyped on the Illumina 1m Beadchip while our validation dataset consisted of 487 autism trio families from the Autism Genetics Resource Exchange (AGRE). In the initial GWAS, all SNPs were tested for association to autism using the Pedigree Disequilibrium Test, a family based test for association. We examined markers in our autism GWAS dataset for each of the 27 candidate genes correcting for the number of available markers in each gene. In addition, only those markers that showed significant association to autism in the MIHG, AGRE, and combined analyses were deemed relevant. Examination of our GWAS results across the 27 genes revealed a significant association to three SNPs (rs11079919, rs2107441, rs9898731) in CACNA1G a calcium channel gene which impacts a variety of functions including neuronal firing patterns. This gene is of greater interest as well given recent evidence from a high density SNP association study which implicated CACNA1G as a candidate gene for autism. We will discuss the implications of examining epilepsy risk genes such as ion channel genes for autism as well as co-occurring autism and epilepsy.

## 2029/T/Poster Board #578

**Neuropeptide S receptor 1, and its ligand neuropeptide S, associate with panic disorder in the Finnish population.** *J. Donner[1,2], S. Pirkola[3,4], J. Suvisaari[3], K. Silander[5], J. Terwilliger[6,7,8], J. Lönnqvist[3,4], L. Peltonen[2,5,9,10], J. Kere[2,11,12], I. Hovatta[1,2,3].* 1) Research Program of Molecular Neurology, University of Helsinki, Helsinki, Finland; 2) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 3) Department of Mental Health and Substance Abuse Services, National Institute for Health and Welfare, Helsinki, Finland; 4) Department of Psychiatry, Helsinki University Central Hospital, Helsinki, Finland; 5) Public Health Genomics Unit, National Institute for Health and Welfare, and FIMM, Institute of Molecular Medicine Finland, Helsinki, Finland; 6) Department of Genetics and Development, Department of Psychiatry, Columbia Genome Center, Columbia University, New York, USA; 7) Division of Medical Genetics, New York State Psychiatric Institute, New York, USA; 8) Faculty of Medicine, University of Helsinki, Helsinki, Finland; 9) Wellcome Trust Sanger Institute, Hinxton, UK; 10) The Broad Institute of MIT and Harvard, Cambridge, USA; 11) Folkhälsan Institute of Genetics, Helsinki, Finland; 12) Department of Biosciences and Nutrition, Karolinska Institutet, and Clinical Research Centre, Karolinska University Hospital, Huddinge, Sweden.

The G protein-coupled receptor NPSR1 (neuropeptide S receptor 1) and its ligand NPS (neuropeptide S) form a signaling system with multidimensional functions. These genes have mainly been implicated in susceptibility to asthma in humans, and regulation of anxiety and arousal in rodents. This is interesting as anxiety disorders and asthma show comorbidity in several epidemiological studies. We investigated a sample collected as part of the Finnish epidemiological Health 2000 Survey, and consisting of a representative sample (n = 6005) of the general adult Finnish population. We first evaluated the epidemiological association between asthma and anxiety disorders. Analysis of specific anxiety disorders (panic disorder, generalized anxiety disorder, social phobia, and agoraphobia) revealed that subjects with agoraphobia had asthma significantly more often than subjects without agoraphobia (14.1% vs. 4.0%, $\chi^2 = 20.1$, $P = 3.0 \times 10^{-4}$). We then addressed the possible role of NPSR1 and NPS as susceptibility genes for human anxiety disorders with a genetic association study in the same anxiety disorder patients (n = 321) and matched controls (n = 653). We genotyped 66 single nucleotide polymorphisms (SNPs) located across NPSR1 and NPS genes Several SNP alleles and haplotypes within both examined genes associated with panic disorder diagnosis specifically. Overall, the strongest support for association in NPSR1 was observed for rs17199659 (panic disorder without agoraphobia; p = 0.001), and in NPS for rs1999635 (panic disorder with and without agoraphobia combined; p =0.007). The discovered findings are particularly intriguing, as panic disorder is the anxiety disorder most frequently co-occurring with asthma, and these diseases share some symptoms, such as a sensation of being smothered, choking, and hyperventilation-induced dyspnea. Our results suggest that NPS-NPSR1 signaling may modulate susceptibility to human anxiety disorders. Therefore, further studies of this recently characterized neuropeptide system in the context of human anxiety are warranted.

## 2030/T/Poster Board #579

**No association between Magel2 and schizophrenia in the Japanese population.** *Y. Fukuo[1], T. Kishi[1], T. Kitajima[1], M. Ikeda[1,4], Y. Yamanouchi[1], Y. Kinoshita[1], K. Kawashima[1], T. Okochi[1], T. Okumura[1], T. Tsunoka[1], T. Inada[3], N. Ozaki[2], N. Iwata[1].* 1) Department of Psychiatry, Fujita health University; 2) Department of Psychiatry, Nagoya University; 3) Neuropsychiatric Research Institute, Seiwa Hospital; 4) Department of Psychological Medicine, School of Medicine, Cardiff University.

Recently the clock genes have been reported to play some roles in neural transmitter systems, including the dopamine system, as well as to regulate circadian rhythms. Abnormalities in both of these mechanisms are thought to be involved in the pathophysiology of such as schizophrenia. Magel2 was reported circadian output gene and association with Prader-Willi syndrome. Recently, Magel2 null mice showed relatively normal motor and cognitive dysfunctions. Therefore, we investigated the association between the three tagging SNPs in Magel2 and the risk of these psychiatric disorders in Japanese patients diagnosed with schizophrenia (733 patients) and 795 Japanese controls. However, no significant association was found with schizophrenia in the allele/genotype-wise and haplotype-wise analysis. In conclusion, we suggest that Magel2 may not play a major role in the pathophysiology of Japanese schizophrenic patients. However, it will be important to replicate and confirm these findings in other independent studies using large samples.

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## 2031/T/Poster Board #580

**Replication of highly associated polymorphisms and candidate genes in an independent dataset for late-onset Alzheimer Disease.** *P.J. Gallins[1], G.W. Beecham[1], A.C. Naj[1], M.A. Slifer[1], P.L. Whitehead[1], G. Cai[2], Y. Kajiwara[2], V. Haroutunian[2], J.R. Gilbert[1], E.R. Martin[1], J.D. Buxbaum[2], J.L. Haines[3], M.A. Pericak-Vance[1].* 1) University of Miami, Miami, FL; 2) Mount Sinai School of Medicine, New York, NY; 3) Vanderbilt University, Nashville, TN.

Alzheimer Disease is the leading cause of dementia in the elderly and has a complex etiology, with strong genetic and environmental determinants. Only *Apolipoprotein E* (*APOE*) polymorphisms have been consistently associated with the risk of late-onset Alzheimer Disease (LOAD), but they contribute only a small portion of the underlying genetic effect. In a previously published genome-wide association study (GWAS) using the Illumina HumanHap 550 beadchip, 32 single-nucleotide polymorphisms (SNPs) in 19 regions outside the vicinity of APOE were highly associated with LOAD (P<5E-5). An additional 29 SNPs in 9 candidate genes (*ADAM12*, *CSF1*, *GBP2*, *KCNMA1*, *NOS2A*, *SORCS2*, *SORCS3*, *SORL1*, *WWC1*) from Alzgene (http://www.alzforum.org/res/com/gen/alzgene/) and four novel regions (1q42, 4q28, 6q14, 19q13) demonstrated nominal association with LOAD in joint analyses of two GWAS datasets incorporating imputation (Beecham et al., AJHG 2009; 84:35-43). To replicate these association signals, genotypes were drawn from the Illumina Human1M beadchip on a novel set of 399 cases and 356 controls, and additional genotyping of 133 SNPs in these candidate genes and novel regions in the combined case-control set was performed using a BioTrove OpenArray assay. In the novel case-control set, SNP associations were replicated in 3 out of 19 highly associated regions (1p33, 6q25, 9p21) from the previous GWAS at P<0.05, with the strongest association of P=0.02 for SNP rs11754661 on 6q25. SNPs within 4 candidate genes (*ADAM12*, *KCNMA1*, *SORCS2*, *WWC1*) demonstrated nominally significant associations with LOAD, with the peak association among these in *KCNMA1* at rs1659789 with P=0.0006. Jointly analyzing the original and novel case-control datasets, SNPs within candidate genes *CSF1* (rs1058885, P=0.04) and *SORL1* (rs624310, P=0.02) and novel region 1q42 (rs17773715, P=0.003) also demonstrated modest associations with LOAD. This study replicates associations of variation in candidate genes with LOAD observed in previous candidate gene association studies and confirmed in meta-analysis (Bertram et al., Nat Genet 2007; 39(1): 17-23), lending additional support to their role in LOAD. Further investigation into the biological roles of these candidate genes in Alzheimer Disease pathology is merited.

## 2032/T/Poster Board #581

**Identification of TRAPPC9 as a Cause of Non-Syndromic Autosomal Recessive Mental Retardation.** *L. Kaufman[1,2], A. Mir[3], A. Noor[1,2], J. Vincent[1].* 1) Neuropsychiatry and Development Lab, Neurogenetics Section, University of Toronto, Toronto, Ontario, Canada; 2) Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada; 3) Dept. of Bioscience, COMSATS Institute of Information Technology, Islamabad, Pakistan.

In this study, we have identified the sixth causal gene for non-syndromic autosomal recessive mental retardation (NS-ARMR). Low IQ and deficits in adaptive behaviours such as social, language and self-help skills characterize MR. It affects 1-3% the population worldwide. Non-syndromic autosomal recessive mental retardation (NS-ARMR) may make up as many as 25% of MR cases. Patients with non-syndromic MR present with mental retardation only, whereas patients with syndromic MR present with additional clinical features affecting multiple systems. Despite the high prevalence of NS-ARMR, little is known about its genetic basis. To date, only five genes have been implicated in this disease: TUSC3, CRBN, GRIK2, PRSS12 and CC2D1A. Understanding the genetic causes for mental retardation is a vital component of elucidating pathological mechanisms, and other potentially pathological genes. In addition, because of the frequent co-occurrence of MR in autism, this may help us identify etiological mechanisms of autism. We have identified a mutation in a large consanguineous NS-ARMR family from Pakistan. Consanguinity, marriage between related individuals, is a powerful tool for identifying rare variants that cause disease. Parents who share DNA through sharing a recent common ancestor will produce offspring with more homozygous regions, which can unmask potentially pathological mutations. Using 5.0 Affymetrix genome-wide SNP chips we analyzed the DNA of affected family members. We used dChip software to perform autozygosity mapping, and identified a 3Mb disease locus. This region, located on chromosome 8q24, contains 12 genes. We used sequencing to elucidate the disease causing gene and mutation. We identified a C>T mutation in exon 7 of the gene TRAPPC9 (trafficking protein particle complex 9), resulting in the nonsense mutation R377X which would prematurely truncate the protein. The mutation was not identified in 290 Pakistani controls. The protein product of TRAPPC9, NIBP, is involved in the activation of the NF-$\kappa$-$\beta$ signaling pathway. It is ubiquitously expressed at low levels, but shows particularly high expression in the muscle, kidney, brain, heart and placenta. It may be involved in neuronal differentiation and neuronal survival. It also likely plays a role in protein trafficking in the golgi apparatus. Future studies on TRAPPC9 will determine its role in MR and may help elucidate a common pathway in NS-ARMR.

## 2033/T/Poster Board #582

**Association analysis of the VWF candidate gene and major depression in the GAIN sample.** *I.P. Kovac[1], J.M. McCaffery[2], N. Frasure-Smith[1,5,8], A. Barhdadi[1,6], Q. Ling Duan[3,4], F. Lespérance[7], P. Théroux[1], G.A. Rouleau[6,8], M.P. Dubé[1,6].* 1) Res Ctr, Montreal Heart Inst, Montreal, PQ, Canada; 2) Weight Control and Diabetes Research Center, Brown Medical School and The Miriam Hospital, Providence, RI; 3) Department of Human Genetics, McGill University, Montreal, QC; 4) Centre for Excellence in Neuromics, University of Montreal and the Centre Hospitalier de l'Université de Montreal, Montreal, QC; 5) Department of Psychiatry and School of Nursing, McGill University; Department of Psychiatry, University of Montreal, Montreal, QC; 6) Department of Medicine, Université de Montréal, Montreal, QC; 7) Department of Psychiatry, Centre Hospitalier de l'Université de Montréal, Montreal, QC; 8) Centre Hospitalier de l'Université de Montréal, Montreal, QC.

In our previous study on depression in coronary artery disease (CAD) patients (McCaffery et al. 2009), we have reported an association between the VWF gene and the Beck Depression Scale measure in 977 cardiac patients. We did not observe this association in subjects without CAD from the same population. In order to further evaluate whether this association is limited to cardiac patients, we aim to test whether VWF is associated with depression in a reference population that is unselected for CAD. Here, we performed an association analysis of the SNPs in the VWF gene region with major depressive disorder using the publicly available data from the GAIN Major Depressive Disorder project. The GAIN data set contained 1754 cases and 1800 controls described previously. As part of quality control, 55 individuals were removed as population outliers. The data contained 42 SNPs in the VWF region on chromosome 12, including our previously associated rs216873 SNP. The 42 SNPs had over 95% genotype call rate, MAF ≥1%, HWE p>10-6. In order to increase coverage for association analyses, we have imputed un-typed SNPs in this region using the MACH software, based on the phased haplotypes of the 60 HapMap CEU individuals at 270 SNPs covering the VWF gene (Hapmap rel21a) as input. After imputation, we excluded SNPs with Rsq<=0.3 (squared correlation between imputed and true genotypes) and monomorphic SNPs (MAF=0), retaining 199/273 SNPs. Further 2 SNPs were excluded due to failing HWE test in controls (SAS 9.1.3), retaining 197 SNPs. We performed association analysis with the case status using an additive genetic model with logistic regression using Proc Logistic (SAS 9.1.3). Analyses were conducted either with sex as a covariate as per our original study (1711 cases and 1787 controls), or with age, sex, smoking and alcohol as covariates (1697 cases and 1653 controls). We used posterior probabilities for the most likely genotype obtained from MACH imputation as weights in these analyses. We corrected for multiple testing by the method of Li and Ji. We did not find significant association between any of the SNPs in the VWF gene region and the case status for major depressive disorder. The p-values for association of rs216873 were 0.69 and 0.59. While the absence of association could be due to various factors, this result is consistent with the hypothesis that the effect of the VWF gene is limited to cardiac patients. Further independent testing will be required.

## 2034/T/Poster Board #583

**Convergent functional genomics: from a BDNF memory model in rat to the identification of genetic variants for cognitive abilities in human.** *S. Le Hellard[1,2], B. Håvik[1,2], T. Espeseth[3], A.J. Lundervold[4], S. Djurovic[5], D.J. Porteous[6], C.R. Bramham[7], I.J. Deary[8], I. Reinvang[3], V.M. Steen[1,2].* 1) Clinical Medicine, University of Bergen, Bergen, Norway; 2) Dr Einar Martens' Research Group for Biological Psychiatry, Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway; 3) Center for the Study of Human Cognition, Department of Psychology, University of Oslo, Norway; 4) Department of Biological and Medical Psychology, University of Bergen, Norway; 5) Department of medical genetics, Oslo University Hospital - Ullevål, 0407 Oslo, Norway; 6) Centre for Cognitive Ageing and Cognitive Epidemiology, Medical Genetics Section, University of Edinburgh Centre for Molecular Medicine, UK; 7) Dept of Biomedicine and Bergen Mental Health Research Center, University of Bergen, Norway; 8) Centre for Cognitive Ageing and Cognitive Epidemiology, Department of Psychology, University of Edinburgh, UK.

Human memory and general cognitive abilities are complex functions with wide variability between individuals. Although empirical evidence for genetic factors underlying cognitive variation is strong, evidence for specific genetic variants has proved challenging. In an effort to identify novel genes involved in cognition we used a convergent functional genomics approach. In a rat model we identified a panel of genes that are strongly up-regulated following BDNF-induced long-term potentiation of synaptic efficacy in the hippocampus. We examined genetic markers tagging these genes for their association with inter-individual differences in performance on cognitive psychometric tests, using three independent samples (N=271, 550 and 1077) of adults individuals. We found significant association, which survives permutation testing, between several variants in *DCLK1* and IQ scores, and verbal memory, consistent in the three samples. Our results show a potential effect of a BDNF-induced gene on cognitive performance in humans.

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## 2035/T/Poster Board #584

**Molecular and clinical characterization of de novo DPYD deletions in unrelated individuals with autism spectrum disorder.** *C.R. Marshall[1], A. Noor[2], S.M. Nikkel[3], A. Lionel[1], C. Noakes[4], C. Fairbrother-Davis[4], W. Roberts[4], J. Vincent[2], S.W. Scherer[1].* 1) The Centre for Applied Genomics and Program in Genetics and Genome Biology, The Hospital for Sick Children and University of Toronto, Toronto, ON, Canada; 2) Centre for Addiction and Mental Health, Clarke Institute and Department of Psychiatry, University of Toronto, Toronto, ON, Canada; 3) Department of Medical Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada; 4) Autism Research Unit, The Hospital for Sick Children, Toronto, ON, Canada.

The dihydropyrimidine dehydrogenese gene (DPYD) spans approximately 850kb of genomic sequence at 1p21.3 and encodes an enzyme that is the initial and rate limiting step in the catabolism of pyrimidine bases. Recessive mutations in this gene cause thymine-uraciluria, which is typically associated with neurological symptoms such as motor and mental delay as well as seizures. A consistent phenotype for dihydropyrimidine dehydrogenese deficiency has yet to emerge and our recent studies have suggested that cases with large hemizygous deletions involving the DPYD locus present with varying complex phenotypes including developmental delay and autistic-like features. As part of a genome scan of 427 unrelated individuals with an autism spectrum disorder (ASD) diagnoses we detected a male proband from a simplex family with a de novo ~1Mb deletion fully encompassing DPYD. In a second unrelated clinical case, a 1.5 Mb de novo deletion encompassing DPYD and Polypyrimidine tract binding protein 2 (PTBP2) was detected in a female patient with an initial diagnosis of Pervasive Developmental Disorder (PDD). A male sibling with a milder phenotype also had the deletion with parent of origin analysis suggesting maternal germline mosaicism. This sibling pair was reassessed and both received a formal ASD diagnosis. Comparison of proband phenotypes did not reveal markedly similar phenotypes suggesting that haploinsufficiency of DPYD presents with a variable phenotype. Exon sequencing of 300 unrelated autistic probands revealed several inherited missense mutations but no nonsense changes. The combined data suggests that, although rare, large deletions involving DPYD may contribute genetic risk to an ASD phenotype in some individuals, perhaps under a multiple-hit model, which we will discuss.

## 2036/T/Poster Board #585

**A rare polymorphism in GABRB3 segregating with childhood absence epilepsy and generalized spike and waves in a French-Canadian family.** *C. Martin[1], P. Lachance-Touchette[1], C. Poulin[1], M. Gravel[1], L. Carmant[2], P. Cossette[1].* 1) Research Center of the CHUM Notre-Dame, Centre d'excellence en neuromique de l'Université de Montréal, Montréal, Qc., Canada; 2) CHU Sainte-Justine, Pediatric Department, Service of Neurology, University of Montreal, Montréal, Qc., Canada.

Mutations in the GABRB3 have been recently associated with Childhood absence epilepsy (CAE) families from Honduras and Mexico. In this study, we aimed to determine the frequency of mutation in this gene in our cohort of families with CAE and other related idiopathic generalized epilepsy (IGE) syndromes. We screened the open reading frame of GABRB3 in 183 French Canadian individuals with IGE including 88 with CAE. A total of 9 SNPs have been identified, 5 of which are novel. The previously described P11S missense mutation was found in three affected and one unaffected individuals from a French-Canadian family. Phenotypes in affected individuals include CAE, asymptomatic generalized spike and wave's discharges and late onset idiopathic generalized tonic clonic seizures. However, the P11S variant was also found in one of our 190 control individuals from French-Canadian origin, suggesting that this variant is rather a rare polymorphism in this population. Our study nonetheless suggests that the P11S mutation in GABRB3 is associated with CAE and related phenotypes. Further screening of other IGE cohorts from various ethnic origins would help confirming the association between this rare functional variant and epilepsy.

## 2037/T/Poster Board #586

**Systematic association analysis and follow-up between genetic variation at the positional and functional candidate gene *SV2A* and schizophrenia in a large combined sample of German descent.** *M. Mattheisen[1,2,3,8], T.W. Mühleisen[1,3,8], S. Herms[1,3], R. Breuer[4], V. Nieratschker[4], I. Nenadic[5], H. Sauer[5], B. Basmanav[1,3], R. Abou Jamra[1,3], J. Schumacher[6], J. Strohmaier[4], S.H. Witt[4], P. Propping[1], M.P. Baur[2], D. Rujescu[7], T.G. Schulze[6], M. Rietschel[4], M.M. Nöthen[1,3], S. Cichon[1,3].* 1) Institute of Human Genetics, University of Bonn, Bonn, Germany; 2) Institute for Medical Biometry, Informatics, and Epidemiology, University of Bonn, Bonn, Germany; 3) Institute of Human Genetics, Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany; 4) Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany; 5) Department of Psychiatry, University of Jena, Jena, Germany; 6) Genetic Basis of Mood and Anxiety Disorders, NIMH/NIH, Bethesda, MD, USA; 7) Division of Molecular and Clinical Neurobiology, Department of Psychiatry, Ludwig-Maximilians University Munich, Munich, Germany; 8) These authors contributed equally.

In the present study, we report the results of the first systematic association study between *SV2A* genetic variation and schizophrenia. There is combined evidence from linkage, functional, animal, and clinical studies, which makes the gene for the synaptic vesicle glycoprotein 2A (SV2A) a promising candidate gene for schizophrenia susceptibility. For Stage I analysis, we genotyped 5 haplotype tagging SNPs selected from HapMap, covering the entire *SV2A* gene and flanking sequences that capture all haplotypes at a frequency > 1% in the CEU population for a large patient-control sample originating from the German population (800 cases and 854 controls). All SNPs with a callrate below 96% (n=1) were excluded. Subsequent additional QC filters did not necessitate to exclude further SNPs. Single marker analysis showed nominal Armitage's Trend Test P values of 0.0061 for SNP rs15931. After inclusion of a follow-up sample of German origin, totalling to 4,218 individuals (40.5% cases), the P value for rs15931 further improved (p = 0.0042). The LD estimates for SNPs in candidate genes for schizophrenia, flanking *SV2A* yielded no r-squared value >0.2 and hence indicate that our findings are independent with respect to previous association findings for the linkage region. In conclusion, our results suggest that *SV2A* may be involved in the development of schizophrenia. However, independent replication studies are warranted to confirm our finding.

## 2038/T/Poster Board #587

**Polymorphism located between *CPT1B* and *CHKB*, and *HLA-DRB1*1501-DQB1*0602* haplotype confer susceptibility to CNS hypersomnias (essential hypersomnia).** *T. Miyagawa[1], M. Honda[2,3], M. Kawashima[1,4], M. Shimada[1], S. Tanaka[2], Y. Honda[3], K. Tokunaga[1].* 1) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; 2) The Sleep Disorders Research Project, Tokyo Institute of Psychiatry, Tokyo, Japan; 3) Japan Somnology Center, Neuropsychiatric Research Institute, Tokyo, Japan; 4) Center for Narcolepsy, Stanford University School of Medicine, Palo Alto, California, United States of America.

We previously reported that SNP rs5770917 located between the carnitine palmitoyltransferase 1B (*CPT1B*) and choline kinase beta (*CHKB*) genes was associated with susceptibility to narcolepsy with cataplexy after performing a genome-wide association study in Japanese and Korean populations ($P = 1.4 \times 10^{-7}$; OR = 1.68). Moreover, it is noteworthy that all narcoleptic patients with cataplexy in Japan carry a human leukocyte antigen (*HLA*)-*DRB1*1501-DQB1*0602* haplotype. This study was conducted in order to investigate whether these genetic markers are associated with Japanese CNS hypersomnias (essential hypersomnia: EHS) other than narcolepsy with cataplexy. Diagnostic criteria for EHS comprised three clinical items: 1) recurrent daytime sleep episodes that occur basically every day over a period of at least 6 months; 2) absence of cataplexy; 3) the condition does not meet the diagnostic criteria of any other disorder causing excessive daytime sleepiness, such as sleep-apnea syndrome. Genotyping for SNP rs5770917 in cases (n = 137) was performed using Taqman genotyping assays. For controls, we used the genotype data (n = 569) from the previous genome-wide association study for narcolepsy with cataplexy. Genotyping for *HLA-DRB1* and *HLA-DQB1* in cases (n = 137) was performed by Luminex Multi-Analyte Profiling system (xMAP) with a WAKFlow HLA typing kit (Wakunaga, Hiroshima, Japan). We did not perform HLA-typing in controls, instead we utilized *HLA-DRB1* and *HLA-DQB1* frequency data obtained from Japanese Society for Histocompatibility and Immunogenetics databank (a total of 516 Japanese general population were genotyped). EHS was significantly associated with SNP rs5770917 ($P = 3.6 \times 10^{-3}$; OR = 1.56) and *HLA-DRB1*1501-DQB1*0602* haplotype ($P = 9.2 \times 10^{-11}$; OR = 3.97). No interaction between the two markers was observed in EHS, thus suggesting that these markers independently affect susceptibility to EHS. CPT1B, CHKB and HLA are candidates for susceptibility to CNS hypersomnias (EHS), as well as narcolepsy with cataplexy.

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## 2039/T/Poster Board #588

**Survey of known candidate genes in a large GWAS study finds support for association of variation in *ACSL6* in schizophrenia.** *T.M. Mühleisen[1,2,8], M. Mattheisen[1,2,3,8], B. Basmanav[1,2], F. Degenhardt[1,2], A. Forstner[1,2], A. Breuer[4], V. Nieratschker[4], S. Witt[1,2], . GROUP Consortium[5], E. Strengman[6], R. Cantor[7], D. Rujescu[8], M.P. Baur[3], M.M. Nöthen[1,2], M. Rietschel[4], R. Ophoff[6,7,9], S. Cichon[1,2,9].* 1) Insitute of Human Genetics, Bonn, Germany; 2) Institute of Human Genetics, Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany; 3) Institute for Medical Biometry, Informatics, and Epidemiology, University of Bonn, Bonn, Germany; 4) Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany; 5) GROUP Consortium: Maastricht University Medical Centre, Academic Medical Centre University of Amsterdam, University Medical Center Groningen, University Medical Center Utrecht, The Netherlands; 6) The Netherlands Department of Medical Genetics and Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands; 7) UCLA Center for Neurobehavioral Genetics, Los Angeles, California, USA; 8) Division of Molecular and Clinical Neurobiology, Department of Psychiatry, Ludwig-Maximilians-University Munich, Munich, Germany; 9) These authors contributed equally to this work.

Schizophrenia is a devastating neuropsychiatric disorder with a life-time prevalence of about 1% in all populations world-wide. Candidate gene and genome-wide association studies (GWAS) have come up with a few very promising genes that are likely to be involved in disease development, but more replication results are still warranted. In the current study, we present the association results of a large GWAS of SCZ for candidate genes. Our GWAS comprised 4883 individuals (1169 cases and 3714 controls) from Germany and the Netherlands. The results are given for a list of SNPs derived from studies that have referred positive and/ or negative association findings and hence cover a collection of candidate genes that have shown evidence for association in at least one study (http://www.schizophreniaforum.org/res/sczgene (accessed 30th of May 2009)). Out of an original list of 7045 SNPs from 786 genes, 1711 SNPs were present in the quality controlled dataset of our GWAS. We identified 95 SNPs with a $P$ value < 0.05 in the Cochran-Mantel-Haenszel test. Five SNPs yielded a $P$ value < 0.001 implicated in 4 genes (*ACSL6*, *RAPGEF6*, *PDE4B* and *DISC1*) and of these 2 SNPs also yielded a $P$ value < 0.0001 (both *ACSL2*, $r^2 = 0.29$). The best result was obtained for rs2240525 in *ACSL6* with a $P$ value of $5.27 * 10^{-6}$. Our results provide additional support for an involvement of these genes in the development of schizophrenia.

## 2040/T/Poster Board #589

**No association between NOS3 and schizophrenia in a Japanese population.** *T. Okochi[1], T. Kishi[1], M. Ikeda[1,3], T. Kitajima[1], Y. Yamnouchi[1], Y. Kinoshita[1], K. Kawashima[1], T. Tsunoka[1], T. Okumura[1], Y. Fukuo[1], T. Inada[4], N. Ozaki[2], N. Iwata[1].* 1) Department of Psychiatry, Fujita Health University; 2) Department of Psychiatry, Nagoya University Graduate School of Medicine; 3) Department of Psychological Medicine, School of Medicine, Cardiff University; 4) Neuropsychiatric Research Institute, Seiwa Hospital.

Nitric oxide (NO) is gaseous neurotransmitter that involved in a variety of mechanisms in the central nervous system. The evidences from pharmacological studies in animal and postmortem studies support an association between NO and psychotic disorders such as schizophrenia and mood disorder. One of the enzymes influencing NO function in the human brain is the endothelial nitric oxide synthase (NOS3). Recent study reported association between haplotype of NOS3 and bipolar disorder. In this study, we conducted a gene-based case-control study between tagging single nucleotide polymorphisms (SNPs) in NOS3 [6 SNPs including 2 functional SNPs (rs2070744, rs1799983)] and schizophrenia in a Japanese population (725 schizophrenia and 699 controls). No significant association was found between tagging SNPs in NOS3 and schizophrenia in the allele/genotype-wise or haplotype-wise analyses. In conclusion, we suggest that NOS3 is unlikely to contribute to susceptibility to schizophrenia in the Japanese population. However, it will be necessary to validate or replicate our association in other, larger population samples.

## 2041/T/Poster Board #590

**Genetic association analysis of functional polymorphisms in neuronal nitric oxide synthase 1 gene (NOS1) and mood disorders and fluvoxamine response in major depressive disorder in the Japanese population.** *T. Okumura[1], T. Kishi[1], T. Okochi[1], M. Ikeda[3], T. Kitajima[1], Y. Yamanouchi[1], Y. Kinoshita[1], K. Kawashima[1], T. Tsunoka[1], T. Inada[4], N. Ozaki[2], N. Iwata[1].* 1) Department of Psychiatry, Fujita Health University, Toyoake, Japan; 2) Department of Psychiatry, Nagoya University Graduate School of Medicine; 3) Department of Psychological Medicine, School of Medicine, Cardiff University, Heath Park, Cardiff; 4) Neuropsychiatric Research Institute, Seiwa Hospital.

[Background]Nitric oxide (NO) has been reported to play roles in neural transmitter release and N-methyl-D-aspartate (NMDA) receptor activation, as well as to be related to oxidative stress. Abnormalities in both of these mechanisms are thought to be involved in the pathophysiology of mood disorders including major depressive disorder (MDD) and bipolar disorder (BP). In addition, several lines of evidence support an association between abnormalities in neuronal nitric oxide synthases (nNOS) and mood disorders. Therefore, we studied the association of nNOS gene (NOS1) with mood disorders and the efficacy of fluvoxamine treatment in Japanese MDD patients. [Materials and methods] Using a SNP (exlc: rs41279104) reported biological functional SNPs, we conducted a genetic association analysis of case-control samples (325 MDD patients, 154 BP patients and 807 controls) in the Japanese population. In addition, we performed association analysis between NOS1 and the efficacy of fluvoxamine treatment in 117 MDD patients. We defined a clinical response as a decrease of more than a 50% in baseline SIGH-D within 8 weeks, and clinical remission as a SIGH-D score of less than 7 at 8 weeks. [Results]We did not detect a significant association of NOS1 with MDD, BP or the fluvoxamine therapeutic response in MDD in allele/genotype-wise analysis. [Discussion] We did not detect an association between only one marker (rs41279104) in NOS1 and Japanese mood disorders patients and fluvoxamine response, but sample sizes were probably too small to allow a meaningful test. Moreover, because we did not perform an association analysis based on Linkage disequilibrium (LD) and a mutation scan of NOS1, a replication in study using a larger sample and based on LD may be required for conclusive results.

## 2042/T/Poster Board #591

**Variants in SLITRK1 are associated with the Obsessive-Compulsive Disorder Spectrum.** *U. Ozomaro[1], R. Moessner[2], M. Cuccaro[1], M. Pericak-Vance[1], M. Wagner[2], G. Feng[3], S. Züchner[1].* 1) Miami Institute for Human Genomics, University of Miami, Miami, USA; 2) Psychiatrische Klinik der Universitaet Bonn, Bonn, Germany; 3) Department of Neurobiology, Duke University Medical Center, Durham, USA.

Obsessive-Compulsive Disorder (OCD) and the spectrum of closely related disorders are characterized by persistent intrusive thoughts (obsessions), repetitive actions (compulsions), and excessive anxiety. Although heritability studies in OCD have shown an increased risk for first degree relatives, and twin studies revealed higher concordance amongst monozygotic twins (compared to dizygotic twins), the identification of the underlying risk-conferring genetic variation by means of classic genetic association studies has proven to be difficult. Recently, the possibility of a larger contribution of rare genetic variants to the risk of psychiatric disorder has been suggested by several successful studies. In accordance with this hypothesis, we applied a rare variant association study design to explore SLITRK1, a gene previously linked to disorders of the OCD spectrum. We directly resequenced SLITRK1 in 279 OCD spectrum cases and in 185 psychiatric controls. We identified two novel nonsynonymous variants in five cases, N400I and T418S. The N400I variant was found in one case, while the T418S change was identified in four cases. Neither variant, nor any other nonsynonymous changes were detected in the controls. In a previous study, we identified two rare variants in SLITRK1, R584K and S593G, whose sequence changes were significantly associated with trichotillomania, an OCD spectrum disorder. Taking into consideration the combined mutation load of our two studies, OCD spectrum cases are significantly associated with variants in SLITRK1 (complete gene sequencing; p=0.043, Fisher's exact test). SLITRK1 codes for a neurite modulating protein and is found in the striatum. Additional support for a role of SLITRK1 in OCD spectrum disorders comes from a recent SLITRK1 knockout mouse characterized by anxiety and by mirroring aspects of the human disorder. We are currently performing functional in-vitro studies to further clarify the molecular consequences of these variants.

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## 2043/T/Poster Board #592

**Association study of the CNV overlapping GSK3β (glycogen synthase kinase-3β) gene with mood disorders.** *E. Saus[1], V. Soria[2], G. Escaramís[3,1], J.M. Crespo[2,4], J. Valero[5], A. Gutiérrez-Zotes[5], L. Martorell[5], E. Vilella[5], J.M. Menchón[2,4], X. Estivill[1,3], M. Urretavizcaya[2,4], M. Gratacòs[3,1].* 1) Genetic Causes of Disease Group, Genes and Disease Program, Center for Genomic Regulation (CRG-UPF), Barcelona, Catalonia, Spain; 2) CIBERSAM (CIBER en Salud Mental), Mood Disorders Clinical and Research Unit, Psychiatry Department, Bellvitge University Hospital, Barcelona, Spain; 3) CIBER en Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain; 4) Department of Clinical Sciences, Bellvitge Campus, Barcelona University, Barcelona, Spain; 5) Grup d'Investigació en Psiquiatria. Hospital Universitari Institut Pere Mata, Rovira i Virgili University, Reus, Spain.

Glycogen synthase kinase-3β (GSK3β), particularly abundant in the CNS (central nervous system), is known to regulate such critical cellular functions as structure, gene expression, mobility and apoptosis. Recent findings support that GSK3β may play a role in the pathophysiology and treatment of mood disorders (MD), in part through its modulatory function in the circadian clock mechanism. Lachman et al. (2006) found a statistically significant increased number of gains in a CNV (copy number variant) partially overlapping with GSK3β in bipolar patients compared with control individuals. Here, we replicated this experiment in a Spanish sample of MD under the hypothesis that this CNV overlapping with GSK3β gene could partially underlie the susceptibility to MD and also could be associated with specific clinical subphenotypes. The clinical sample consisted of 444 patients with MD (256 Unipolar Major Depressive Disorder, 188 Bipolar Disorder) and 428 psychiatrically screened control subjects. All samples were genotyped in triplicate by qPCR (SYBR Green I). All triplicates undergone a quality control measure, and a linear mixed model was generated to normalize all the data allowing a posterior relative quantification of gains and losses. We found a total of 42 samples with gains and 13 samples with losses, accounting for 18 gains and 4 losses in MD patients, and 24 gains and 9 losses in controls. An association study considering the diagnostic (MD vs. controls) adjusting by age and sex was first performed, but no significant result was obtained. Then, we tested 5 different subphenotypes previously related with GSK3β within the MD patients group: polarity, seasonality, chronotype, antidepressant treatment response and age at onset. We only obtained a positive nominal result for seasonality, although statistical significance was lost after Bonferroni correction. In this sense, MD patients carrying changes in GSK3β CNV (especially losses) have a tendency to present a seasonal pattern. While we have not been able to confirm a direct involvement of GSK3β copy number variant in the susceptibility to MD, changes in copy number in GSK3β could partially contribute to the presentation of seasonal pattern in some of these patients.

## 2044/T/Poster Board #593

**Association of language and reading phenotypes with SNPs of KIAA0319 in a population selected for language impairment.** *S. Smith[1], M. Rice[2].* 1) Dept Pediatrics and Munroe Meyer Institute, Univ Nebraska Med Center, Omaha, NE; 2) Dept. Speech-Language and Hearing, Dole Human Devel Center, Univ Kansas, Lawrence, KS.

Language impairment, reading disability, and speech sound disorder are developmental disorders with separate clinical definitions but which often occur together in children, and this comorbidity may be due in part to common genetic influences. Our previous linkage analyses in a population of children selected for language impairment replicated the linkage of reading and language phenotypes to microsatellite markers on chromosome 6p22 in the DYX2 region. The linked markers flanked the two candidate genes for reading disability, DCDC2 and KIAA0319. To confirm this finding and determine if one or both of the candidate genes were involved in language impairment, we followed up with 36 SNPs spanning the genes VMP, DCDC2, KIAA0319, and TTRAP. The subjects were the nuclear families of probands from 86 families totaling 220 children who have been involved in longitudinal language studies by Dr. Rice. The probands were ascertained from speech/language pathologists and were screened for intelligence in the normal range, normal hearing, intelligible speech, and language disability using standardized criteria. Eight phenotypes were evaluated in the children, including measures of language, grammatical morphology, reading, and articulation. Using FBAT analysis (additive hypothesis), only 5 SNPs showed significant association, and all of these were in or near KIAA0319. Three of these SNPs have previously been reported to show association with reading phenotypes in other studies (e.g., Francks et al., 2004; Cope et al., 2005; Luciano et al., 2007), and the same alleles were found to be associated with reading and language problems in our study. These SNPs are in the 5' region and first intron of KIAA0319, suggesting that their association is with elements that regulate the level of expression of the gene. Research supported by NIDCD R01 DC01803 to Dr. Rice.

## 2045/T/Poster Board #594

**Delineation of an overlapping 10q deletion involving PCDH15 with neuropsychiatric disease.** *N. Sobreira[1], N. Feng[1], G. Thomas[3], J. Mulle[4], J. MacGrath[2], S. Warren[4], D. Avramopoulos[1], A. Pulver[2], D. Valle[1].* 1) Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD; 3) Kennedy Krieger Institute, Baltimore, MD; 4) Department of Human Genetics, Emory University, Atlanta, GA.

Schizophrenia (SZ) is a chronic, disabling illness with extensive phenotypic and genetic heterogeneity and an incidence of 1%. The causes are still poorly understood but there is a strong genetic component (80% heritability). Associations between SZ and several candidate genes have been replicated in various samples but other than *DISC1*, causative variants have not been identified. Bipolar disorder (BPD) is a mood disorder characterized by intermittent episodes of mania or hypomania and depression often complicated by frank psychosis. Lifetime risk for BPD has been reported to be as high as 4%. Heritability estimates are similar to those for SZ but even fewer genes have been found with only *CACNA1C* and *ANK3* being identified in well-replicated studies. SZ and BPD have overlapping symptoms and pedigrees with both diagnoses are common. We identified two families segregating SZ and BPD-like phenotypes with overlapping deletions on 10q. The first, JHU2000, includes an affected mother and 2 affected sibs with del(10)(q21.1q21.2) or (q21.2q22.1) identified by standard cytogenetics. The mother has seizures and has been variously diagnosed as BPD or SZ since age 17 yrs. The daughter has attention deficit disorder, and cognitive deficits. The son has ADHD, depression, episodes of mania and the diagnoses of BPD. We utilized quantitative PCR, long-range PCR, and sequencing to define the boundaries of an 8.1 Mb deletion that extends from 55,376,528 bp to 63,475,465 bp at 10q21.1-q21.2. This deletion removes 19 genes including *PCDH15* and *ANK3*. Recessive truncating mutations in *PCDH15* cause Usher syndrome type 1, a deafness-blindness disorder which has been suggested to have an increased incidence of neuropsychiatric disease. The second family, JHU88293, was identified in a genome-wide SNP genotyping screen for CNVs in SZ and has ~4.2 Mb deletion estimated to extend from 53.2 to 57.5 Mb. The female proband has SZ while the father, who also has the deletion, is said to be normal. The minimal overlap in these two families is from 55.4 to 57.5 Mb. *PCDH15* is the only annotated gene in this interval,which also includes a large segment of the gene desert upstream of *ZWINT1*. The observations in these 2 families strongly suggest that a gene or genes in 10q21.1-q21.2, possibly *PCDH15*, contribute risk for a neuropsychiatric disorder with features of SZ and/or BPD.

## 2046/T/Poster Board #595

**Leucine rich repeat gene polymorphisms are associated with autism spectrum disorder susceptibility in populations of European ancestry.** *I. Sousa[1], T.G. Clark[1,2], R. Holt[1], A.T. Pagnamenta[1], N. Sykes[1], E. Mulder[3], A.J. Bailey[4], A. Battaglia[5], S.M. Klauck[6], F. Poustka[7], A.P. Monaco[1], International Molecular Genetic Study of Autism Consortium.* 1) Wellcome Trust Ctr, Univ Oxford, Oxford, United Kingdom; 2) Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK; 3) Department of Psychiatry, Child and Adolescent Psychiatry, University Medical Center Groningen, PO Box 660, 9700 AR Groningen, the Netherlands; 4) University Department of Psychiatry, Warneford Hospital,Oxford, UK; 5) Stella Maris Clinical Research Institute for Child and Adolescent Neuropsychiatry, Calambrone (Pisa), Italy; 6) Division of Molecular Genome Analysis, German Cancer Research Center, Heidelberg, Germany; 7) Department of Child and Adolescent Psychiatry, Johann Wolfgang Goethe-University, Frankfurt/Main, Germany.

Autism is a severe and highly heritable neurodevelopmental disorder characterised by impairments in social interaction, communication, and restricted interests/behaviours. Several cell adhesion transmembrane leucine-rich repeat (LRR) proteins are highly expressed in the nervous system and thought to be key regulators of its development. Here we present an association study analysing the roles of four promising candidate nested genes - *LRRTM1* (2p), *LRRTM3* (10q), *LRRN1* (3p) and *LRRN3* (7q) - in order to identify common genetic risk factors underlying autism spectrum disorders (ASDs). To gain a better understanding of the genetic variation that may underlie these four gene regions and how they may influence susceptibility to ASDs, a family-based association study was undertaken in 735 families of European ancestry selected from four different population cohorts. Significant results were found for *LRRN3* and *LRRTM3* ($P < 0.005$), using both single locus and haplotype approaches. In addition, a case-control analysis was performed supporting the evidence found for *LRRN3* and *LRRTM3* and pointing to new significant results in the latter ($P < 0.005$). Overall, our findings implicate the neuronal leucine rich genes *LRRN3* and *LRRTM3* to have a role in ASD susceptibility.

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## 2047/T/Poster Board #596

**Further investigation of *IMMP2L*, *LRRN3* and *DOCK4* as potential candidate genes for autism susceptibility.** *N.H. Sykes[1], A.T. Pagnamenta[1], G. Lunter[1], H. Kugoh[2], M. Oshimura[2], E. Bacchelli[3], E. Maestrini[3], A.J. Bailey[4], A.P. Monaco[1], IMGSAC (http://www.well.ox.ac.uk/~maestrin/iat.html).* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 2) Department of Molecular and Cell Genetics, Tottori University, Tottori, Japan; 3) Department of Biology, University of Bologna, Bologna, Italy; 4) University Department of Psychiatry, Park Hospital for Children, Oxford.

Autism is a severe neurodevelopmental disorder that usually occurs due to a complex genetic predisposition. The International Molecular Genetic Study of Autism Consortium (IMGSAC) previously identified a susceptibility locus for autism on chromosome 7 (*AUTS1*). A subsequent high-density association analysis of the region tested more than 3000 single nucleotide polymorphisms (SNPs) in all known genes and non-genic highly conserved sequences. SNP genotype data were also used to investigate copy number variation (CNV) within this region. Association and CNV analyses highlighted several genes that warrant further investigation, including *IMMP2L* (which contains a nested gene *LRRN3* in intron 3) and *DOCK4*. An independent replication of association at rs2217262 and the finding of a deletion segregating in a sib-pair family provided support for the involvement of *DOCK4* in autism susceptibility.

To further investigate the genes *IMMP2L*, *LRRN3* and *DOCK4* a number of approaches have since been taken. Firstly, the *DOCK*4 deletion has been characterised at base pair resolution and is being assessed more extensively within the extended family pedigree. Secondly, given the evidence of parent-of-origin effects at the *IMMP2L* locus, the expression of the genes *IMMP2L*, *LRRN3* and *DOCK4* were observed in human/mouse A9 monochromosomal hybrids. The cell lines contained a human maternal or paternal chromosome 7, derived from primary fibroblasts. All three genes were found to have both maternal and paternal expression, which excludes imprinting within these cell lines.

Currently a high-throughput sequencing experiment is being conducted using next generation sequencing technology, to identify rare variants within the genes *IMMP2L*, *LRRN3* and *DOCK4*. Sequencing will be conducted with the Illumina® Genome Analyzer using one affected individual per IMGSAC multiplex family, chosen from a subset that displays linkage to chromosome 7. Initially a pilot study will be performed in which 50 CEU DNAs will be pooled together and sequenced for approximately 75kb of our region of interest. In this way we will be able to verify the ability of the platform to identify rare variants.

## 2048/T/Poster Board #597

**Association between dopamine D4 receptor (*DRD4*) -521 C/T polymorphism and sensation seeking behaviour in female alpine skiers and snowboarders.** *C.T. Thomson[1], C.W. Hanna[2], K.L. Morton[1], P. Wang[1], M.R. Beauchamp[1], J.L. Rupert[1].* 1) Sch Human Kinetics, Univ British Columbia, Vancouver, BC, Canada; 2) Department of Medical Genetics, Univ British Columbia, Vancouver, BC, Canada.

Previous research has shown a large genetic influence over personality traits, especially sensation seeking (SS). Sensation seeking is the desire to seek out new and thrilling experiences. One gene thought to influence this behavioral trait is *DRD4*, in which variants have been associated with SS or novelty in some, but not all studies. The inconsistencies between studies may be due to heterogeneity in both the behaviours and the populations being assessed. Some studies included only males and few studies have *a priori* analyzed males and females separately. SS has been associated with high-risk sports, including skiing; however this is the first study to address the possibility that genetics may play a role in individuals' inclination towards SS in sport. Using the Contextual Sensation Seeking Questionnaire (CSSQ) for Skiing, developed and validated for this study, and the Zuckerman-Kuhlman Personality Questionnaire (ZKPQ), levels of SS in males and females were analyzed in association with *DRD4* -521 C/T. Behavioural analysis of skiers (N = 206) revealed a significant correlation ($r^2$= 0.497, p < 0.001) between skier behaviour (CSSQ) and skier personality scores (ZKPQ). Genotype analysis (N = 109) revealed allele frequencies of 0.54 C and 0.46 T. A significant association was found between the -521 T allele (CT and TT genotypes) and low contextual skiing SS behaviour in the females (N = 54, p = 0.004), however no association was found between ZKPQ SS scores and -521 C/T in females (p = 0.164) nor males (N=55, p = 0.178). This study provides support that the alleles of the DRD4 -521 C/T polymorphism are associated with context-specific SS behaviours in females, but not in males. Whether the lack of association in males is due to variations in the biochemical properties of the dopamine receptor or whether social pressures differentially influence male and female sensation-seeking behaviour are areas that require further investigation.

## 2049/T/Poster Board #598

**A 1q42 Deletion Involving DISC1, DISC2 and TSNAX in an Autism Spectrum Disorder.** *T.F. Beck[1], J.M. Williams[1], D.M. Pearson[1], M.B. Proud[2], S.W. Cheung[1], D.A. Scott[1].* 1) Deparment of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; 2) Department of Pediatrics-Neurology, Baylor College of Medicine, Houston, TX, USA.

Autism spectrum disorders (ASDs) affect approximately 1 in 500 children 3 to 10 years of age, with a significantly higher risk for male children (6.8 times higher) than for females. Individuals with ASDs have impairments in social, communicative, and behavior development that are often accompanied by abnormalities in cognitive functioning, learning, attention, and sensory processing. In this report we describe a three year old male child with an autism spectrum disorder who carries a 2Mb deletion of chromosome 1q42. Array comparative genome hybridization revealed that this deletion involves at least three genesDISC1, DISC2 and TSNAXwhich have been found to be associated with autism and neuropsychiatric disorders in multiple studies and are likely to play key roles in normal central nervous system development. Analysis of this region in the patient's unaffected mother demonstrated the same deletion. To investigate the possibility that our patient's phenotype involved unmasking of paternally inherited deleterious changes, we sequenced the coding regions of DISC1 and TSNAX in our patient. Two nonsynonymous changes were identified in DISC1 but both were judged to be benign using the on-line tool PolyPhen which predicts the impact of an amino acid substitution on protein structure and function using physical and comparative considerations. DISC2 was not screened for changes since it is thought to be a noncoding antisense transcript. These results suggest other genetic and/or environmental factors, some of which may be sex specific, may modify the phenotypic effects of this deletion. While this case provides evidence for the potential role of DISC1, DISC2 and TSNAX in the development of autism spectrum disorders, it is equally clear that caution must be used when providing families with prognostic information and genetic counseling regarding such deletions.

## 2050/T/Poster Board #599

**Mutation analysis of functional candidate genes for Aicardi syndrome.** *X. Wang[1], V.R Sutton[2], T. Eble[2], C. O'neill[1], R. Lewis[2,3], I.B Van den Veyver[1,2].* 1) Dept of Obstetrics and gynecology; 2) Dept of Molecular and human genetics,; 3) Dept of Ophthalmology, Baylor College of Medicine, Houston, TX 77030,USA.

Aicardi syndrome is characterized by developmental abnormalities that primarily affect the eyes and the brain and is associated with early-onset and often intractable seizures.

This severe neurodevelopmental disorder affects almost exclusively females and rarely 47,XXY males, which strongly suggests that Aicardi syndrome is caused by de novo dominant mutations in an X-linked gene. Evidence of excess skewing of X-inactivation in females with Aicardi syndrome further supports this hypothesis. Because genetic linkage approaches to map the gene for Aicardi syndrome cannot be used due to the lack of familial cases, we previously used genome-wide high-resolution array-CGH analysis to screen the DNA of 38 girls with Aicardi syndrome for copy number changes, but no disease-causing changes were detected in these patients. In parallel with these efforts, we are using informed approaches to select X-linked candidate genes for mutation analysis by direct sequencing of PCR-amplified coding exons.

To date, we have analyzed 17 genes, including 5 previously selected genes *ARHGAP6*, *HCCS*, *MID1*, *MSL3L1* and *FLNA*.Twelve additional genes were selected and sequenced based on 1) known or putative function related to Aicardi (*CDKL5, MSN, ARX* conserved sequence blocks in 3'UTR, *IGBP1*); 2) proximally to interesting chromosomal breakpoint in patients with Aicardi syndrome or overlapping phenotypes (*CXORF15, GPM6B, EGFL6*); 3) Interaction with disease-causing genes for similar phenotypes found by network analysis(*CASK, NGFRAP1, NAP1L2, MAGEH1, PCTK1*). So far, no disease-causing mutations or copy number changes by array CGH were detected in these functional candidate genes. Southern analysis with cDNA probes from genes of interest to detect smaller (intragenic) genomic rearrangements, is still ongoing.

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## 2051/T/Poster Board #600

**The effect of chr16p11.2 microdeletions and microduplications on gene expression in Autism Spectrum Disorders and Schizophrenia.** *M. Kusenda[1,2], V. Vacic[1], S. Yoon[1], M. Wigler[1], J. Sebat[1].* 1) Cold Spring Harbor Lab, Cold Spring Harbor, NY 11724; 2) Graduate Program in Genetics, Stony Brook University, NY 11794, USA.

The number of rare variants found to be associated with multiple psychiatric disorders is growing. One such locus is a recurrent ~600kb copy number variant (CNV) at 16p11.2, occurring in approximately 1% of autism and 0.3% of schizophrenia cases, as compared to 0.01% of the general population. We hypothesize that one or more of the 25 genes at this locus contribute to the neurodevelopmental phenotype observed in patients with psychiatric disorders. To determine how gene function is altered by this CNV, we analyzed genome wide expression data from Epstein Barr Virus (EBV) transformed Lymphoblast cell lines (LCL), of patients with autism or schizophrenia who have a de novo or inherited 16p11.2 rearrangement. Using RNA expression profiling by Affymetrix Human Genome U133 Plus 2.0 chip, we examined differential cis and trans gene expression in individuals with 1, 2, or 3 copies of the genomic region. (6, 19, 16 respectively) To avoid skewing of data due to limited sample size we customized the Significance Analysis of Microarrays (SAM) method to utilize all samples while accounting for sources of bias. Our data highlighted 140 genes located both within and outside of the mutation which expression correlates with genotype. Some of these genes play a role in development while others have been associated with psychiatric disorders. We are currently analyzing our list of 140 dysregulated genes to identify pathways and functions relevant to neurodevelopment, and psychiatric disorders. Data generated by this study will give insight into dosage sensitive genes within the risk variant, and may help pinpoint genes which are relevant to pathology of the psychiatric disorders associated with this region.

## 2052/T/Poster Board #601

**Increased Amylase 1 Gene Copy Number in Autism.** *C. Aguado[1], I. Cuscó[1], L.A. Pérez-Jurado[1,2].* 1) Unitat de Genètica, Universitat Pompeu Fabra and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain; 2) Programa de Medicina Molecular i Genètica, Hospital Vall d'Hebrón, Barcelona, Spain.

Autism Spectrum Disorders (ASDs) are a group of conditions with common neurodevelopmental defects affecting socialization, communication skills and behavior, considered to have a multifactorial etiology. Prevalence of ASDs is substantially greater than previously recognised, approaching 1% of the child population, and appears to be on the rise. Whether the increase is due to better ascertainment, increased incidence or both is unclear, and the putative genetic or environmental factors responsible for such an increase remain unknown. One pathogenic hypothesis is that some environmental factors might act on genetically susceptible individuals during early postnatal stages triggering an abnormal neuronal growth or inflammation to alter the normal development of neuronal circuits in ASD. In a search for structural genomic variants using comparative genomic hybridization with BAC microarrays we detected significantly different hybridization signals in the region of the amylase genes at 1p21.1 in ASD patients with respect to controls. We then determined the AMY1 diploid gene copy number using the reported AMY1-specific real-time qPCR assay. We observed that the mean+/-SD number of AMY1 copies in Spaniards is 6.1+/-4.0 (n=89, similar to other Europeans), while it is significantly higher, 9.2+/-5.0, p<0.0001, in the group of idiopathic ASD from the same region (n=85). The analysis of maternal and paternal samples from ASD patients revealed similarly high results in both cases (~8.5+/-4.7 AMY1 copies), suggesting that the increased AMY1 copy number gene in ASD is inherited in most cases without skewed parent of origin. Replication of the results in other ASD cohorts is underway. The AMY1 gene, coding for the salivary alpha-amylase mostly involved in the digestion of starch and other carbohydrates, has been shown to undergo natural selection with increased copy number in farming populations. Much higher AMY1 copy numbers could increase ASD susceptibility by modifying digestion and/or immune response to starch and other foods. Our findings link genetic structural variation to putative dietary factors that could contribute to the pathogenesis of ASD acting either during pregnancy or early in life.

## 2053/T/Poster Board #602

**Inheritance of 15q11-13 Duplications in Autism Pedigrees.** *R.J. Robison[1], N. Matsunami[3], K. Allen-Brady[2], D. Cannon[1], M. Hobbs[4], J. Stevens[3], L. Baird[3], T. Varvil[3], C. Pingree[1], M.F. Leppert[3], W. McMahon[1], H. Coon[1].* 1) Department of Psychiatry, University of Utah, Salt Lake City, UT; 2) Department of Biomedical Informatics, University of Utah, Salt Lake City, UT; 3) Eccles Institute of Human Genetics, University of Utah, Salt Lake City, UT; 4) Division of Infectious Diseases, University of Utah, Salt Lake City, UT.

**Introduction:** Autism Spectrum Disorders (ASDs) are a set of complex, early-onset neurodevelopmental disorders. 15q11-13 contains a cluster of imprinted genes essential for normal neurodevelopment, and is also known as the PWS/AS region. Duplications of 15q11-13 have been associated with ASDs, particularly when maternally inherited, and are thought to occur in up to 5% of autism. These abnormalities often occur as an inverted duplication known as idic(15). **Methods:** 113 subjects with ASDs, along with both parents, were genotyped using the Affymetrix 250K NSP platform. Analysis of copy number state at each SNP probe was performed using the Affymetrix Genotyping Console (GTC), with HapMap CEU samples as controls. 55 of these subjects with ASDs (but not parents) were also genotyped independently on the Affymetrix 6.0 platform. An additional 55 psychiatrically-screened normal control subjects obtained via dbGaP that had been genotyped on the 6.0 platform were also analyzed using GTC software. **Results:** Comparison of Affymetrix 250K and 6.0 platforms for individuals genotyped on both revealed a high degree of qualitative consistency, but a large difference in size of the CNVs detected on each platform. **Discussion:** A high number of maternally-inherited chromosome 15q11-13 duplications was seen in this sample. Common breakpoints predispose 15q to genomic rearrangements and this region is known to be highly polymorphic. While previous studies have found a lower percentage of maternal 15q11-13 duplications, these studies used different methods and diagnostic criteria. Follow-up will include attempting to validate key findings using quantitative PCR, performing additional genotyping using the higher density 6.0 arrays, CNV analysis using multiple software algorithms, and tracing CNVs through extended pedigrees beyond parental transmission. We will also explore the relationship between validated CNVs and particular autism phenotypes. Additional studies are needed to better characterize the role of this important cytogenetic region in ASDs.

## 2054/T/Poster Board #603

**Novel copy number variation in autism.** *S.A. Thomson[1], S.C. Lund[1], B.L. Yaspan[1], D. Pinto[2], E.L. Crawford[1], C.R. Marshall[2], S.W. Scherer[2], E. Kistner[3], N.J. Cox[3], E.H. Cook[4], J.S. Sutcliffe[1].* 1) Centers for Human Genetics Research and Molecular Neuroscience, Vanderbilt Univ, Nashville, TN; 2) Centre for Applied Genomics, Hospital for Sick Children, Toronto, Ontario; 3) Department of Medicine, University of Chicago, Chicago, IL; 4) Institute for Juvenile Research, Department of Psychiatry, University of Illinois-Chicago, Chicago, IL.

Autism is a neurodevelopmental disorder that affects ~1 in 150 individuals and is characterized by deficits in reciprocal social interaction, communication and patterns of repetitive behaviors and restricted interests. Twin and family studies indicate high heritability, but evidence supports a highly complex architecture for the underlying genetic etiology. Recent discoveries point to copy number variation (CNV) as an important class of rare variation that may cause or increase risk for autism. A series of 89 autism probands were screened using either the Affymetrix 6.0 or Illumina 1M SNP platforms. Data were analyzed using multiple algorithms to maximize sensitivity and specificity of CNV detection (Partek, dChip, and AffyConsole for Affy 6.0 and QuantiSNP, PennCNV and Gnosis for Illumina 1M). Variants predicted by 2+ algorithms were prioritized for validation by qPCR and/or genotyping on an independent array platform. Analyses of Affymetrix data from 49 probands identified 3,265 CNV predictions, of which 668 (20%) were called by 2 and 353 (11%) by 3 algorithms. Mean and median sizes were 171kb and 73kb, respectively. Among the 2+ predictions, 482 (47%) were in genic regions and 306 (63%) contained exons. 540 (53%) were predicted to be losses, and 481 (47%) gains. Illumina predictions from 40 probands found 11255 CNV predictions; 1185 (11%) were called by 2 and 399 (4%) by 3 algorithms. Mean and median sizes were 70kb and 21kb, respectively. Among 2+ algorithm predictions, 849 (54%) contained genes; 629 (74%) exons and 220 (26%) intronic regions. The majority were losses (1117; 71%). Prioritized CNVs were confirmed by qPCR and the majority found to be inherited. Most CNVs in autism are inherited, and their interpretation is more difficult. Possible explanations include reduced penetrance in parents or greater overall mutation burden in probands who have multiple variants. CNVs that affect genes implicated previously by de novo variants, or involved in dominantly-inherited MR, are very likely to confer risk. DOCK8 is a gene fitting both scenarios. In conclusion, several of the confirmed CNVs in this study identified loci previously implicated in mental retardation and/or autism, as well as neuronal cell surface molecules with a range of specific functions including cell adhesion, migration, synaptic vesicle release and cellular signaling. These findings further underscore the important role played by CNVs in the genetic etiology of autism.

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## 2055/T/Poster Board #604

**Elucidating the genetic architecture of familial schizophrenia using rare copy number variant and linkage scans.** *B. Xu[1,2], A. Woodroffe[3], L. Rodriguez-Murillo[1], J. Roos[4], E. van Rensburg[5], G. Abecasis[6], J. Gogos[2,7], M. Karayiorgou[1].* 1) Department of Psychiatry, Columbia University, New York, NY; 2) Department of Physiology & Cellular Biophysics, Columbia University, New York, NY; 3) Department of Epidemiology, University of Michigan, Ann Arbor, MI; 4) Weskoppies Hospital, Department of Psychiatry, Pretoria, RSA; 5) University of Pretoria, Department of Genetics, Pretoria, RSA; 6) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 7) Department of Neuroscience, Columbia University, New York, NY.

To elucidate the genetic architecture of familial schizophrenia we combine linkage analysis with studies of fine-level chromosomal variation in families recruited from the Afrikaner population in South Africa. We demonstrate that individually rare inherited copy number variants (CNVs) are more frequent in cases with familial schizophrenia as compared to unaffected controls and affect almost exclusively genic regions. Interestingly, while the prevalence of rare structural variants is similar in familial and sporadic cases, the type of variants is markedly different. In addition, using a high-density linkage scan with a panel of nearly 2000 markers, we identify a region on chromosome 13q34 that shows genome-wide significant linkage to schizophrenia and show that in the families not linked to this locus, there is evidence for linkage to chromosome 1p36. No causative CNVs were identified in either locus. Overall, our results from approaches designed to detect risk variants with relatively low-frequency and high penetrance in a well-defined and relatively homogenous population, provide strong empirical evidence supporting the notion that multiple genetic variants, including individually rare ones, that affect many different genes contribute to the genetic risk of familial schizophrenia. They also highlight differences in the genetic architecture of the familial and sporadic forms of the disease.

## 2056/T/Poster Board #605

**Novel mutations found by resequencing multiple subunits of $GABA_A$ receptor in families with Idiopathic Generalized Epilepsy.** *P. Lachance Touchette[1], C. Meloche[1], P. Brown[3], P. Kinirons[1], J. Poirier[1], L. Lapointe[1], A. Lortie[2], L. Carmant[2], D. Bowie[3], P. Cossette[1].* 1) Center for Excellence in Neuromics of Université de Montréal, CHUM Research Center, Montreal, Canada; 2) CHU Sainte-Justine, Pediatric Department, Service of Neurology, University of Montreal, Montreal, Canada; 3) Department of Pharmacology & Therapeutics of McGill University, Montreal, Canada.

Idiopathic generalized epilepsies (IGE) account for approximately 30 % of all epilepsy syndromes. An increasing number of genes predisposing to the disease have been identified, including mutations in four subunits of the $GABA_A$ receptor genes (GABAR). To investigate the impact of rare variants of GABAR in IGE, we screened the coding regions of 19 genes encoding for all the known subunits of the GABAR in 96 unrelated French-Canadian individuals with the disease, including 46 familial cases. Overall, approximately 15 % of our IGE individuals have novel GABAR mutations, including 13 missense and 1 splice mutations. These mutations were not detected in more than 400 control chromosomes. The impact of each missense mutation was investigated by using several *in silico* methods each of which predict the possible impact of genetic variants on the structure and function of the mature protein. In *GABRA1*, we found an insertion of 25bp close to the splice acceptor site of exon 10 associated with intron retention (P351fsintX), as well as a D219N mutation. In *GABRG2*, we found a P83S mutation. Transfection of these three mutants GABAR in mammalian cells revealed that mutant receptors exhibit reduced expression at the cell surface. In turn, electrophysiological analysis of mutant $GABA_A$ receptors revealed that both P83S and D219N mutations are functional, although the gating properties of these recombinant receptors are significantly altered. Whether the mutations in the other GABAR subunits are associated with similar functional defects remain to be determined.

## 2057/T/Poster Board #606

**Linkage analysis of plasma dopamine β-hydroxylase activity in families segregating schizophrenia.** *J.F. Cubells[1,2], X. Sun[3], R.C. Elston[3], R.W. Bonsall[2], K. Mercer[1], W. Li[1], Y-L. Tang[1], D. Avramopoulos[4], V.K. Lasseter[4], P.S. Wolyniec[4], J.A. McGrath[4], A.E. Pulver[4].* 1) Dept. of Human Genetics, Emory Univ Sch. Med., Atlanta, GA; 2) Dept. of Psychiatry and Behavioral Sciences, Emory Univ Sch. Med., Atlanta, GA; 3) Dept. of Epidemiology and Biostatistics, Case Western Reserve Univ. Sch. Med., Cleveland, OH; 4) Dept. of Psychiatry, Johns Hopkins Sch. Med., Baltimore, MD.

Background: Dopamine β-hydroxylase (DβH) converts dopamine to norepinephrine, and occurs in human plasma, where its activity is under strong genetic control. Prior studies showed linkage between plasma DβH activity and ABO blood type, and subsequent molecular mapping showed that ABO and the structural gene encoding DβH, *DBH*, map closely together, but molecular linkage analysis of plasma DβH activity has not been reported. Methods: A total of 921 individuals from 123 pedigrees were recruited as part of the Maryland Epidemiological Study of Schizophrenia. Enzyme activity, measured using tyramine as a substrate, with product octopamine separated from reactants by HPLC and detected by electrochemical detection, was available for 284 individuals. 208 chromosome-9 SNPs from the Illumina version 4 linkage marker set were determined in 723 individuals. Two additional markers at DBH, previously shown to associate significantly with plasma DβH activity (rs 1611115 and rs 6271, both typed on the Taqman platform), were added to those from the Illumina panel. Segregation analysis was conducted using the SEGREG program, and multipoint model-based linkage analysis with MLOD, in S.A.G.E. Results: Highly significant linkage was observed between markers surrounding *DBH* and square-root transformed plasma DβH activity, with the strongest signal found under low-value dominant inheritance (multipoint LOD score = 6.34). SNPs within the *DBH* genic region were within the 1-unit support interval (LOD = 6.18). Accounting for diagnosis, age, sex, or duration of sample storage did not substantially change the results. Finally, the heritability of plasma DβH was ~0.57, a figure that is about 25% lower than previous estimates. Conclusions: The current results confirm strong linkage between the *DBH* locus and plasma DβH activity, but suggest a different mode of inheritance and somewhat lower total heritability of plasma DβH than prior association studies (which support a co-dominant model). This work sets the stage for examining potential interactions between inherited differences in DβH activity and segregation of symptomatic phenotypes in schizophrenia. Supported by NIH grant R01 MH 077233.

## 2058/T/Poster Board #607

**Genetics of multiple sclerosis.** *A. Bonetti[1,2], K. Koivisto[3], T. Pirttilä[4,5], I. Elovaara[6,15], M. Reunanen[7,8], M. Laaksonen[9,10], J. Ruutiainen[11], L. Peltonen[12,13,14], T. Rantamäki[1,2], P. Tienari[1,2].* 1) Molecular Neuroscience Programme, Biomedicum-Helsinki , University of Helsinki, Finland; 2) Helsinki University Central Hospital, Helsinki, Finland; 3) Central Hospital of Seinajöki, Seinajöki, Finland; 4) Department of Neurology and Neuroscience, University of Kuopio, Finland; 5) Kuopio University Hospital, Kuopio, Finland; 6) Department of Neurology, University of Tampere, Finland; 7) Department of Neurology, University of Oulu, Oulu, Finland; 8) Oulu University Hospital, Oulu, Finland; 9) Turku Immunology Centre, Finland; 10) Departments of Virology and Neurology, University of Turku, Turku, Finland; 11) Masku Neurological Rehabilitation Centre, Finland; 12) National Public Health Institute, University of Helsinki, Finland; 13) FIMM, Institute for Molecular Medicine Finland, Finland; 14) Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 15) Tampere University Hospital, Tampere, Finland.

Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the central nervous system. Multiple genes mediate predisposition to MS, the strongest so far identified is HLA-DR15. Previously, we and others have provided evidence for a role of allelic variation on chromosomes 2q33 and 19q13 in MS susceptiblity. Neither of these loci were, however, detected in a recent genome-wide association study performed in US and UK patients. We have carried out a follow-up study of these regions in a family-based association study in 700 MS families from Finland. In 19q13 we analysed 14 markers, both microsatellites and single nucleotide polymorphisms (SNPs). None of the markers showed statistically significant association with MS, including the previously suggested markers D19S876, APOE, ILT6 and D19S585. In 2q33 a nominally significant trend for association was found distal of CTLA4, with a SNP located in the inducible T-cell co-stimulator (ICOS) gene. This association was, however, non-significant upon correction for multiple comparisons. ICOS is a co-stimulatory molecule that, upon binding to its ligand, functions in parallel with HLA/T-cell receptor in the immunological synapse. The weakly (non-significantly) associated SNP is located in close proximity with an exon that we found in RT-PCR experiments to be alternatively spliced. The stability of the newly-identified transcript variant was analysed in lymphocyte cell lines and transfected cells and its subcellular localisation was analysed in transfected cells. The relative expression of ICOS transcripts was analysed in MS patients' and controls' lymphocytes using real-time PCR. These results demonstrate a novel, possibly regulatory, transcriptional variant of ICOS.

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## 2059/T/Poster Board #608

**Possible association of prokineticin 2 receptor gene (PROKR2) with mood disorders in the Japanese population.** *T. Kishi[1], T. Kitajima[1,2], T. Tsunoka[1], T. Okumura[1], M. Ikeda[1], T. Okochi[1], Y. Kinoshita[1], K. Kawashima[1], Y. Yamanouchi[1], N. Ozaki[3], N. Iwata[1].* 1) Psychiatry, Fujita Health University, Toyoake, Aichi, Japan; 2) Department of Psychological Medicine, School of Medicine, Cardiff University, Heath Park, Cardiff, CF14 4XN, United Kingdom; 3) Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya 466-8850, Japan.

Several investigations have suggested that disruption of circadian rhythms may provide the foundation for the development of mood disorders such as bipolar disorder (BP) and major depressive disorder (MDD). Recent animal studies reported that prokineticin 2 or prokineticin 2 receptor gene deficient mice showed disruptions in circadian and homeostatic regulation of sleep. This evidence indicates that prokineticin 2 gene (PROK2) and prokineticin 2 receptor gene (PROKR2) are good candidate genes for the pathogenesis of mood disorders. To evaluate the association between PROK2, PROKR2, and mood disorders, we conducted a case-control study of Japanese samples (151 bipolar patients, 319 major depressive disorder patients and 340 controls) with four and five tagging SNPs in PROK2 or PROKR2, respectively, selected by HapMap database. We detected a significant association between PROKR2 and major depressive disorder and bipolar disorder in the Japanese population. In conclusion, our findings suggest that PROKR2 may play a role in the pathophysiology of mood disorders in the Japanese population. However, because our samples were small, it will be important to replicate and confirm these findings in other independent studies using larger samples.

## 2060/T/Poster Board #609

**Genomic Characterization of Schizophrenia Candidate Gene Regions.** *A.Q. Nato[1], X. Kong[1,2], F. Chen[1,3], C. He[1,4], D. Chimento[1], B. Byrne[5], J. Naus[6], C. Chiu[1], S. Buyske[1,6], L.M. Brzustowicz[1], T.C. Matise[1].* 1) Dept Gen, Rutgers Univ, Piscataway, NJ; 2) GlaxoSmithKline, King of Prussia, PA; 3) Inst Gen Sci & Policy, Duke Univ, Durham, NC; 4) Lab Stat Gen, Rockefeller Univ, New York, NY; 5) Informatics Inst, UMDNJ, Piscataway, NJ; 6) Dept Stat, Rutgers Univ, Piscataway, NJ.

Schizophrenia (SZ) has been established to have a complex genetic aetiology with a lifetime prevalence commonly given at ~1% but systematic reviews suggested lower values of 0.44% to 0.55%. In this study, we extracted and analyzed data from the 47 published independent genomewide linkage scans for SZ from 1994 to 2009. A smoothing method and a disjoint approach were utilized to determine 19 schizophrenia candidate gene regions (SCRs). We have developed the SCR Browser for identifying the genetic markers implicated in these genome scans. We annotate each SCR by identifying the genes within these regions. We categorize these genes based on whether they are linked to diseases, functions, phenotypes, pathways, or GO terms, and on whether they are linked or associated with SZ in published linkage scans, association studies, meta-analyses, and microarray studies. We also identify copy-number variants, segmental duplications, defined regulatory regions, putative miRNA binding sites, and rearrangement hotspots within each SCR. The total SCR coverage is 427 cM with SCR sizes ranging from 16 cM to 50 cM. We identify subregions within these SCRs using the results from fine-mapping studies coupled with the diverse information used to characterize each of the SCRs. Our approach provides a novel method to determine and prioritize candidate gene regions and genomic elements that may be applied to other complex diseases.

## 2061/T/Poster Board #610

**Resequencing and association study of vesicular glutamate transporter 1 gene (VGLUT1) with schizophrenia.** *Y. Shen[1,2], C. Chen[3,4].* 1) Department of Psychiatry, Tzu-Chi General Hospital and University, Hualien, Taiwan; 2) Institute of Medical Sciences, Tzu-Chi University, Hualien, Taiwan; 3) Division of Mental Health and Addiction Medicine, Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan; 4) Institute of Clinical Medicine, Tzu-Chi University, Hualien, Taiwan.

Dysregulation of glutamate neurotransmission is implicated in the pathphysiology of schizophrenia. Vesicular glutamate transporters (VGLUTs) package glutamate into vesicles in the presynaptic terminal and regulate the release of glutamate. Among these VGLUTs, abnormal VGLUT1 expression has been mostly linked to schizophrenia in postmortem brain studies. The purpose of this study was to investigate the involvement of the gene encoding the human VGLUT1 in the susceptibility to schizophrenia. In this study, we searched for genetic variants in the putative core promoter region, 5`UTR, all the exons, and 3`UTR of the VGLUT1 gene using a direct sequencing strategy in a sample of Han Chinese schizophrenic patients (n = 376) and non-psychotic controls (n = 368) from Taiwan, and conducted a case-control association study. In all regions examined, we identified two common SNPs (g.-248G>C and g.11875C>A) in the VGLUT1 gene. No differences in the allele, genotype, and haplotype frequencies were detected between the patients and control subjects. Besides, we identified eight patient-specific rare mutations in 16 out of 376 patients, including two mutations (g.-296A>G and g.-32C>T) at the core promoter region and 5'UTR, two missense (L516M and P551S) and three synonymous mutations (E24E, L118L, P133P) at exonic regions, and one mutation (g.11252G>A) at the 3'UTR. These rare mutations were not found in 368 control subjects (4.3% versus 0, P = 0.000015). Although the functional significance of these rare mutations remains to be characterized, our study lends support to the multiple rare mutations hypothesis of schizophrenia. Also, our findings provide genetic clues to indicate the involvement of the glutamate transmission pathway in the pathogenesis of schizophrenia.

## 2062/T/Poster Board #611

**Replication of a novel Autism peak region in two ethnically diverse populations.** *J. Jaworski[1], D. Ma[1], D. Salyakina[1], I. Konidari[1], P. Whitehead[1], H. Wright[2], R. Abramsom[2], E. Martin[1], J.P. Hussman[3], J.R. Gilbert[1], M. Cuccaro[1], J.L. Haines[4], M.A. Pericak-Vance[1].* 1) Miami Institute for Human Genomics MIHG, Univ Miami, Miami, FL; 2) School of Medicine, University of South Carolina, Columbia, SC; 3) Hussman Foundation, Ellicott City, Maryland; 4) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN.

Autism is one of the most heritable neuropsychiatric disorders but since no single major gene has been identified, it is now clear that it has a complex genetic etiology. We recently identified common variants in 5p14.1 that carry autism risk in Caucasian families and this was replicated in two other Caucasian datasets derived from the Autism Genetic Resource Exchange (AGRE) and the Children's Hospital of Philadelphia (CHOP). In this study, we tested the generalizability of this result by examining two diverse populations of Hispanics (Hisp) and African Americans (AA). We genotyped the families using the Illumina Human 1M beadchip. The AA dataset included 292 individuals from 85 families with 99 affecteds. The Hisp dataset included 247 individuals from 79 families with 94 affecteds. Both datasets had a mixture of parent-child trios and dyads. To account for missing parents in the dataset, the software program CAPL was used for analysis, which can make efficient use of any mix of case-control and nuclear family data. We examined SNPs within 5p14.1 from 2.58 to 2.60 Mb, the peak region of association in the Caucasian datasets. Within the AA dataset, 11 SNPS had p-values <0.05 with the peak SNP being RS12521157 (p-value= 0.006). Two of the previously reported significant 8 SNPs across this region (p<0.05) were among these 11. The Hispanic dataset had 3 SNPS with p-values <0.05 within the region with the peak being RS6878844 (p=0.03), although none of these 3 was significant in the Caucasian dataset. Despite the significantly smaller sample size in these diverse datasets, our findings indicate that 5p14.1 may indeed harbor an autism susceptibility variation that contributes to the etiology of autism across populations.

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## 2063/T/Poster Board #612

**Combining Co-expression Analysis with Genome-Wide Genetic Data to Identify Novel Candidate Genes in Autism.** *S. Strom, M. Yourshaw, L. Song, B. Merriman, H. Lee, S.F. Nelson.* UCLA, Los Angeles, CA.

Genome-wide association (GWAS) and copy number variant (CNV) analysis have identified several new genes likely contributing to Autism Spectrum Disorder (ASD) risk. However, these few genes explain only a small fraction of the estimated heritability of ASD. As yet unknown genes conferring ASD risk may generate non-significant association signals due to heterogeneity or low effect size. Analysis of the co-expression of a given gene with published ASD-related genes provides a path to highlighting the most interesting genes from amongst a multitude of false positive signals present due to chance. Co-expression correlation scores between a seed list of probe-sets from 13 genes likely to contribute to autism taken from a recent review and the over 18,000 unique genes assayed by the Affymetrix U133_2.0 microarray were obtained via the UCLA Gene Expression Tool (UGET). As a proof-of-principle, we demonstrate that mean gene-correlation scores were substantially higher for genes known to be mutated in ASD relative to a brain expression control group. We further use this tool to explore known positional candidate regions, and identify that there is significantly increased gene-gene correlation of known autism gene seed list with all genes adjacent to nominal association signals (p<0.001) in GWAS datasets. Additionally, several genes with mutations/variants linked to ASD are detected in a joint analysis incorporating co-expression with known ASD genes, SNP association, and CNV data. A small list of novel candidates are proposed, having a high likelihood of contributing to autism based on these combined analyses. Sequencing data on several novel candidates in autistic patients is presented.

## 2064/T/Poster Board #613

**In Down syndrome, synaptojanin1 over-expression is responsible for the enlargement of early endosomes, the first hallmark of Alzheimer disease pathology.** *JC. Cossec[1], S. Stora[2], C. Ripoll[3], A. Hoischen[4], S. Antonarakis[5], M. Lecourtois[6], Y. Grattau[2], J. Veltman[4], C. Duyckaerts[1], J. Delabar[3], MC. Potier[1].* 1) CRICM CNRS UMR7225, INSERM UMR975, UPMC, Pitié-Salpêtrière Hospital, 47, Bd de l'Hôpital 75013 Paris, France; 2) Institut Jérôme Lejeune, France; 3) BFA, CNRS EAC7059, Paris, France; 4) Dept. of Human Genet., Nijmegen, Netherlands; 5) Univ. of Geneva Med. Sch., Geneva, Switzerland; 6) INSERM U614, Rouen, France.

Enlarged endosomes are believed to be the first morphological change observed in Alzheimer's disease brains (AD). They are found in sporadic cases with no pathological evidence of AD yet, in Down syndrome (DS) as early as 28 weeks of gestation. It is believed that high levels of amyloid peptides (Aβ) could be the cause of these morphological changes. However in familial AD with mutations in the presenilin genes that over produce Aβ, the endosomal compartment appeared to be unchanged. We thus searched for other factors that could modify the endosome morphology in DS. In order to identify the genes from human chromosome 21 (Hsa21) responsible for this cellular phenotype, we searched for the presence of enlarged endosomes in blood cells and in transformed lymphocytes from DS patients. Blood mononuclear cells were isolated on a ficoll gradient, cultured for a few hours at 37°C to let them recover. Immunofluorescence labelling of the early endosomes was performed using an antibody against the early endosome antigen protein EEA1. Using confocal microscopy, we showed that the mean size of the endosomes was significantly increased (35%;p<0.001; n=10 for DS and controls) in cells from DS patients as compared to age matched controls. We then measured the size of early endosomes in lymphoblastoid cell lines (LCL) generated by transformation of lymphocytes with the Epstein Bar virus. As in mononuclear blood cells, LCLs from DS patients contained large endosomes. The mean size as measured by immunofluorescence was 30% higher (p<0.001;n=11 for DS and n=8 for controls). Enlarged endosomes were absent in the 3 LCLs carrying APP microduplications. This result suggested that APP is not involved in the enlargement of early endosomes in this cell type. In four out of seven LCLs from DS patients carrying partial trisomy 21 we could very clearly identify enlarged endosomes. By correlating the presence of enlarged endosomes to the triplicated genomic segment from Hsa21 we identified SYNJ1 as a candidate gene. SYNJ1 gene codes for the phosphoinositide phosphatase synaptojanin 1 protein, a key regulator of the signalling phospholipids phosphatidylinositol-4,5-biphosphate [PtdIns(4,5)P2]. We observed abnormally large endosomes in SHSY5Y cells transfected with SYNJ1 plasmid. In conclusion we have shown that synapyojanin 1 over-expression pertubing the PtdIns(4,5)P2 homeostasy of the cell, leads to defects in endocytosis associated with morphological changes of the endosomes.

## 2065/T/Poster Board #614

**SNP rs16969968 in CHRNA5 Associated with Heroin Dependence.** *E.O. Johnson[1], L.J. Bierut[2], J.C. Wang[2], A.M. Goate[2], A.H. Kral[1].* 1) Behavioral Hlth Epidemiology, RTI International, Res. Triangle Pk, NC; 2) Washington University School of Medicine, St. Louis, MO.

Neuronal nicotinic acetylcholine receptors modulate the dopamine reward pathway. In prior studies we identified an association between nicotine dependence and the non-synonymous single nucleotide polymorphism (SNP) rs16969968 in the nicotinic acetylcholine receptor α5 subunit (CHRNA5) gene (Bierut et al., 2007; Saccone et al. 2007), which has been replicated in independent samples (Bierut et al., 2008; Berrettini et al., 2008; Thorgeirsson et al. 2008; Stevens et al., 2008; Weiss et al., 2008). The minor (A) allele increases the risk of nicotine dependence (approximately 2-fold in a recessive model) and results in a aspartic acid to asparagine change at position 398 (D398N) which appears to alter receptor response to nicotine in vitro (Bierut et al., 2008). Recently, our group found the major (G) allele, relative to the minor (A) allele, increased the risk for cocaine dependence, suggesting that the genetic associations are reversed for cocaine versus nicotine dependence (Grucza et al., 2008). Here we test the hypothesis that the G allele in rs16969968 is associated with increased risk of heroin dependence. METHODS: Cases of heroin dependence came from the Urban Health Study of injection drug users (n = 267) and the non-drug abusing controls from the Family Study of Cocaine Dependence (n = 244), all were of European decent. RESULTS: The primary multiplicative model showed a significant association with heroin dependence (p = 0.05; G allele frequency 69% vs. 63% among case and controls). However, a recessive model of inheritance was found to be the best model indicating a 45% increased risk of heroin dependence associated with a G/G genotype relative to A/G or A/A genotypes (OR = 1.45, 95% CI 1.01-2.08, p = 0.04). CONCLUSION: The association between rs16969968 and heroin dependence is in the same direction as that found for cocaine dependence and the reverse of its association with nicotine dependence further supporting the potential importance of this SNP in the development of multiple substance use disorders.

## 2066/T/Poster Board #615

**Saitohin and APOE variants influence clinical features in institutionalized persons with Alzheimer Disease.** *D.L. Schutte[1], D.A. Reed[2].* 1) Col Nursing, Michigan State Univ, East Lansing, MI; 2) Aging, Disability, and Long-Term Care Program, Cecil G. Sheps Center for Health Services Research, University of North Carolina-Chapel Hill.

Alzheimer disease (AD) is a prevalent neurodegenerative disorder, characterized by dementia. AD exhibits variability in age at onset and specific cognitive, functional, and behavioral features even into the advanced stages of disease. Little is known about factors, including genetic variation, that influence clinical variability in this population. The purpose of this study was to examine the extent to which genetic variability influences phenotypic variability in institutionalized persons with advanced AD. Thirty-seven subjects diagnosed with probable AD were recruited from five long-term care facilities. Whole blood or cheek cells were collected for DNA extraction and genotyping. Subjects were genotyped for sequence variants in several candidate genes, including Apolipoprotein E (APOE) and Saitohin (STH). Measures of cognitive status (Global Deterioration Scale, Severe Impairment Battery), functional ability (Functional Abilities Checklist), and agitation (Cohen-Mansfield Agitation Inventory) were collected at four-month intervals over one year. Subjects exhibited a mean age at onset of 76.8 years. All subjects exhibited moderate to severe levels of cognitive impairment. A multilevel model approach to repeated measures revealed a genotype effect on cognition and functional ability. Thirty-five percent of the variation in cognitive status between subjects was accounted for by the presence of the APOE-ε4 allele and STH-7R allele (p < .001). APOE-ε4 (p=.088) and STH-7R (p=.043) alleles explained 22% of the inter-individual variation in functional ability. Statistical modeling of behavior was also completed using GEE. Modeling of physical aggression did not yield a significant genotype effect at the STH locus. However, for the APOE locus, a time X APOE-ε4 allele interaction was identified (p=.030), resulting from a convergence of physical aggression scores over time. The modeling of verbal agitation yielded no significant effects of time, gender, or genotype at either locus. Genotyping of additional tagging SNPS across the STH and surrounding MAPT gene are underway to enable a haplotype approach to this analysis. These results from this pilot study will guide the design of a larger study to examine both resident and facility characteristics as potential predictors of clinical subgroups in institutionalized persons with Alzheimer disease towards the goal of developing and testing more targeted interventions.

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## 2067/T/Poster Board #616

**Association of *ALDH5A1* (*SSADH*) with human capital formation and cognitive function.** *A.V. Smith[1], D.J. Benjamin[2], C.F. Chabris[3], S.M. Purcell[4], E.L. Glaeser[5], L.J. Launer[6], T.B. Harris[6], V. Gudnason[1,7], D.I. Laibson[4].* 1) Icelandic Heart Association, Kopavogur, Iceland; 2) Cornell University, Ithaca, NY, USA; 3) Union College, Schenectady, NY, USA; 4) Massachusetts General Hospital, Boston, MA, USA; 5) Harvard University, Boston, MA, USA; 6) National Institute on Aging, National Institutes of Health, Bethesda, MD, USA; 7) University of Iceland, Reykjavik, Iceland.

In recent years, there has been increasing interest in identifying genetic influences on economic behavior. Twin studies have documented heritability for income, education, and other economic phenotypes. In order to identify particular genetic variants that influence economic outcomes, we searched for associations between 8 phenotypes of economic interest--occupation, housing wealth, labor supply, human capital, time discounting, social capital, happiness, and overall health--and a set of candidate genes that may be involved in cognition and decision making. In an ethnically homogeneous sample of 2,289 participants in the Reykjavik Study-Age Gene/Environment Susceptibility Study (RS-AGES), we genotyped 415 single nucleotide polymorphisms (SNPs) across 68 genes that have published associations with cognition-related phenotypes and/or are part of the dopamine or serotonin systems. After correcting for multiple testing, we find statistically significant associations between a composite measure of discounting-related behaviors (smoking, drinking, and BMI) and the cognition-related gene *BDNF*; between a composite of social capital accumulation and the dopamine-system gene *DRD2*; and between human capital (years of schooling and number of languages spoken) and the cognition-related gene *ALDH5A1* (*SSADH*). We tested for replication in a non-overlapping sample of approximately 1700 RS-AGES participants. While the discounting and social capital results did not replicate, we found additional evidence supporting an association between human capital and *ALDH5A1* (*SSADH*). This result is consistent with a possible influence of this gene, which encodes an enzyme that metabolizes GABA, on general cognitive ability.

## 2068/T/Poster Board #617

**Association of a Functional Haplotype of the Discs, Large Homolog 4 (Drosophila) Gene with Schizophrenia in Han Chinese.** *M. Cheng[1], D.L. Liao[2], H.M. Tsai[2], J.Y. Chen[3], Y.C. Wang[3], I.C. Lai[3], C.H. Chen[1,2].* 1) Institute of Medical Sciences, Tzu-Chi University, Hualien, Taiwan; 2) Division of Mental Health and Addition Medicine, Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan; 3) Department of Psychiatry, Yuli Veterans Hospital, Hualien, Taiwan.

Compelling evidence support that hypofunction of N-methyl-D-aspartate (NMDA) receptor is associated with the pathophysiology of schizophrenia. Postsynaptic density protein 95 (PSD95) interacts with NMDA receptors and plays an important role in modulating of NMDA receptor activity. Altered expression of the PSD95 in the brain has been linked to schizophrenia in several postmortem studies. The purpose of this study was to investigate whether variants of the human discs, large homolog 4 (Drosophila) (DLG4) gene that encodes PSD95 conferring susceptibility to schizophrenia. We re-sequenced the 5'UTR, all the exons, and the 3'UTR of the DLG4 gene in a sample of Han Chinese schizophrenic patients (n=434) and control subjects (n=438) from Taiwan and conducted a genetic association study. We also assessed the function of the SNPs and haplotyes of the DLG4 gene identified in this study using a reporter gene assay. We did not detect any mutations at the exonic regions of the DLG4 gene associated with schizophrenia in this sample. Nevertheless, we identified a haplotype at the 5'UTR of the DLG4 gene that was nominally associated with schizophrenia (odd ratio: 1.24, 95% confidence interval: 1.005-1.522). Reporter gene assay showed that this haplotype has significantly increased activity as compared to other haplotypes. Thus, our study indicates that DLG4 gene is associated with schizophrenia.

## 2069/T/Poster Board #618

**Positional Cloning of a Gene for Distal Motor Neuron Disease on 7q34-q36.** *A.P. Drew[1,2], I.P. Blair[1,2], K.L. Williams[1], G.A. Nicholson[1,2,3].* 1) Northcott Neurosciences, ANZAC Research Institute, Concord, New South Wales, Australia; 2) Faculty of Medicine, University of Sydney, Sydney, New South Wales, Australia; 3) Molecular Medicine Laboratory, Concord Hospital, Concord, New South Wales, Australia.

The motor neuron disorders are a group of neurodegenerative diseases that cause the selective progressive death of motor neurons, with an absence of sensory involvement. Motor neuron disease (MND) is genetically and clinically heterogeneous with the biological basis of the disease is poorly understood. MND ranges from the rapidly progressive fatal form amyotrophic lateral sclerosis (ALS), to slowly progressive forms including hereditary motor neuropathy (HMN). Approximately 10-15% of MND is familial, with the remainder being sporadic or complex genetic traits. The significant genetic and clinical overlap between types of MND imply shared pathogenic pathways. Distal HMN is a MND which affects only the lower motor neurons, causing muscle weakness and wasting, resulting in a lifelong disability. Through genetic linkage studies of a large dHMN family, a novel motor neuron disease locus was mapped to chr. 7q34-q36 (1). Haplotype analysis has defined a 12.9Mb interval flanked by the markers D7S2513 and D7S637. Meiotic recombination events in unaffected individuals suggest the candidate interval is a 6.9Mb region flanked by markers D7S615 and D7S2546. The disease interval encompasses over 100 annotated genes. To identify a pathogenic mutation we selected candidate genes prioritised by association with neurological disorders, interaction with known MND genes and expression in neuronal tissues. Exons and flanking intronic regions were sequenced in 34 genes. Following this, we sequenced one affected individual for the 7q34-q36 disease interval using NimbleGen sequence capture arrays. we achieved 80% coverage, with 18 novel nonsynonymous changes. 7 have been excluded as part of chromosomal translocations with 11 remaining to be screened. Twenty-four HMN families have been genotyped for the 7q34-q36 locus. Ten of these families carry haplotypes that segregate with the disease locus on 7q34-q36 and cannot be excluded. These families were too small to achieve significant linkage scores. The largest family with 5 affected individuals achieved a LOD score of 1.72, suggestive of linkage. Comparison of these haplotypes identified 3 smaller haplotypes associated with the disease group compared to a control group (n=55). The overlap between the families suggests common ancestry and therefore a smaller disease interval. Identification of this novel MND gene will give insights into the biological basis of both familial and sporadic motor neuron degeneration.

## 2070/T/Poster Board #619

**Variants within a 150 kb region on chromosome 15q25.1 that includes CHRNB4 and ADAMTS7 are associated with age at onset of habitual smoking.** *J. Wang[1], S. Bertelsen[1], L. Fox[1], J. Budde[1], A. Hinrichs[1], J. Rice[1], J. Nurnberger Jr.[2], V. Hesselbrock[3], H. Edenberg[4], L. Bierut[1], A. Goate[1], COGA Collaborators.* 1) Psychiatry, Washington Univ Sch Med, St Louis, MO; 2) Institute of Psychiatric Research, Indiana Univ Sch Med, Indianapolis, IN; 3) Department of Psychiatry, Univ Connecticut Sch Med, Farmington, CT; 4) Department of Biochemistry and Molecular Biology, Indiana University, Indianapolis, IN.

Several genome-wide association and candidate gene studies have linked chromosome 15q24-q25.1 (a region including the CHRNA5-CHRNA3-CHRNB4 gene cluster) with alcohol dependence, nicotine dependence and smoking-related illnesses such as lung cancer and chronic obstructive pulmonary disease. To further examine the impact of this gene cluster region on the development of substance use disorders, we tested whether variants within and flanking the CHRNA5/A3/B4 gene cluster affect age at onset of habitual smoking (20 cigarettes/day for at least six months) and nicotine dependence in a cross sectional sample of adolescents and young adults from the COGA (Collaborative Study of the Genetics of Alcoholism) families. Subjects were recruited from families affected with alcoholism (either as a first or second degree relative) and the comparison families. Participants are those who have had at least one assessment between the years 1989-2008 when they were between the ages of 12 and 25 years. The assessment used the SSAGA-IV interview, a comprehensive assessment of alcohol and other substance use and related behaviors. Using Cox proportional hazards regression analysis, we observed that variants located upstream of CHRNB4 and within an adjacent gene, ADAMTS7 significantly predict age at onset of habitual smoking. These variants are not highly correlated ($0.28<r2<0.56$) with variants that we have previously reported to affect risk for nicotine dependence and smoking related diseases. This suggests that a novel mechanism underlies the association with age at onset of habitual smoking. This work may provide genetic information that will lead to better prevention and intervention for substance use disorders among adolescents and young adults.

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## 2071/T/Poster Board #620

**New generation sequencing for the identification of novel variant late-infantile neuronal ceroid lipofuscinosis (vLINCL) genes.** *M. Kousi[1], A.J. Coffey[2], E. Jakkula[3], M. Topcu[4], S. Gokben[5], D. Yuksel[6], A. Palotie[2,7,8], A.E. Lehesjoki[1].* 1) Folkhälsan Institute of Genetics, Department of Medical Genetics and Neuroscience Center, University of Helsinki, Helsinki, Finland; 2) The Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 3) Institute of Molecular Medicine Finland and The National Institute for Health and Welfare, Helsinki, Finland; 4) Department of Pediatrics, Hacettepe University Faculty of Medicine, Section of Child Neurology, Ankara, Turkey; 5) Department of Pediatrics, Ege University Medical Faculty, Izmir, Turkey; 6) Dr. Sami Ulus Children's Hospital, Ankara, Turkey; 7) The Broad Institute of MIT and Harvard, Cambridge, MA, USA; 8) Department of Clinical Chemistry, University of Helsinki.

The neuronal ceroid lipofuscinoses (NCLs) comprise the most common neurodegenerative disorders encountered in childhood. The clinical symptoms involve epileptic seizures, myoclonus, progressive mental and motor deterioration, visual failure and premature death. Ten subtypes of human NCLs are recognized today. Despite the progress made in identifying human NCL genes, eight of which are known to date, the knowledge of the genetic spectrum underlying this group of disorders remains still elusive. We aim to identify the underlying defected genes in 28 Turkish patients with a putative variant late-infantile NCL (vLINCL) diagnosis, in whom we excluded all known human NCL loci by sequence analysis. Towards this goal, we performed a genome-wide SNP scan in the 28 patients and four unaffected family members from 23 mostly consanguineous families using the Illumina's human610-Quad SNP chip. Homozygosity analysis was done with the PLINK program and the Illumina's Beadstudio-suite. Several regions with overlapping homozygosity in a subset of the patients were identified. Twelve candidate loci were determined by overlapping homozygosity between the siblings of three nuclear families. Two distinct loci map on each of chromosomes 16, 11, 3, and 1 and one locus on each of chromosomes 22, 17, 8 and 2. The loci harbor a total of 1,007 genes. In order to identify the genes involved with NCL we are resequencing the coding regions of all of the genes in the candidate intervals using an array-based sequence capture protocol and Illumina sequencing. When successful, our approach will lead to the identification of three novel vLINCL genes given that the three families share no common homozygous regions across the genome. Our findings suggest that the molecular genetic basis of vLINCL is more heterogeneous than previously anticipated. Completion of the genetic spectrum underlying NCLs will provide tools for their improved diagnosis and classification, as well as for understanding the underlying disease mechanisms.

## 2072/T/Poster Board #621

**Variants of the Matrix Metalloproteinase-2 (*MMP-2*) but not the Matrix Metalloproteinase-9 (*MMP-9*) genes significantly influence functional outcome after stroke.** *H. Manso[1,2], T. Krug[2,3], J. Sobral[1,2], I. Albergaria[1], G. Gaspar[1], J.M. Ferro[4], S.A. Oliveira[2,3], A.M. Vicente[1,2].* 1) Instituto Nacional de Saude Dr.Ricardo Jorge, Lisboa, Portugal; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 3) Instituto de Medicina Molecular, Lisboa, Portugal; 4) Serviço de Neurologia, Hospital de Santa Maria, Lisboa, Portugal.

Background and Purpose - Multiple lines of evidence suggest that genetic factors contribute to stroke recovery. The matrix metalloproteinases -2 (MMP-2) and -9 (MMP-9) are modulators of extracellular matrix components, with important regulatory functions in the Central Nervous System (CNS). Shortly after stroke, MMP-2 and MMP-9 have mainly damaging effects for brain tissue. However, MMPs also have a beneficial activity in angiogenesis and neurovascular remodelling during the delayed neuroinflammatory response phase, thus possibly contributing to stroke functional recovery. Methods - In the present study, the role of *MMP-2* and *MMP-9* genetic variants in stroke recovery was investigated in 546 stroke patients. Functional outcome was assessed three months after a stroke episode using the modified Rankin Scale (mRS), and patients were classified in two groups: good recovery (mRS≤1) or poor recovery (mRS>1). Haplotype tagging single nucleotide polymorphisms (SNPs) in the *MMP-2* (N=21) and *MMP-9* (N=4) genes were genotyped and tested for association with stroke outcome, adjusting for significant non-genetic clinical variables. Results - Six SNPs in the *MMP-2* gene were significantly associated with stroke outcome ($0.0018<P<0.0415$), two of which survived the Bonferroni correction for multiple testing. In the subset of ischemic stroke patients, association of five of these SNPs remained positive ($0.0042<P<0.0306$). No significant associations were found for the *MMP-9* gene. Conclusions - The results presented strongly indicate that *MMP-2* genetic variants are an important mediator of functional outcome after stroke.

## 2073/T/Poster Board #622

**Sequencing of *NGN2* in autism cohort identifies rare *de novo* coding mutations.** *D.S. Rudd[1], L.K. Davis[1], G.H. Kim[2], A. Jones[3], T.H. Wassink[3].* 1) Interdisciplinary Genetics Program, University of Iowa, Iowa City, IA; 2) Department of Pediatrics, University of Iowa, Iowa City, IA; 3) Department of Psychiatry, University of Iowa, Iowa City, IA.

*NGN2* (Neurogenin2) is a basic helix-loop-helix transcription factor that is known to be involved in early brain development where it enhances synaptic specificity. *NGN2* is comprised of a single exon (~800 bp) with only one known SNP and there are various mouse models available for multiple alleles of *NGN2*. Mutant mice display defects in their central nervous systems, a decrease in Cajal-Retizus cells and mis-localization of synapses in the dorsal telencephalon and the forebrain. *PAX6* and *PAX3* are transcription factors that regulate expression of *NGN2* in the dorsal and ventral sides of the neural tube, respectively. Combined with previous results that have implicated *PAX6* mutations with autism susceptibility and the required functionality of *NGN2* in early brain development, it is proposed that *NGN2* is a candidate gene for autism. 210 children with autism spectrum diagnoses from simplex and multiplex families have been sequenced to date. 200 controls are currently being sequenced with no novel base-pair changes identified to date. Preliminary sequencing results from the proband panel have identified rare mutations in *NGN2*. Among the previously unannotated sequence changes that have been identified there exists two missense mutations and one synonymous mutation. All three mutations appear to be *de novo*. [This still needs to be confirmed with micro-satellite markers.] Additional variants are being characterized.

## 2074/T/Poster Board #623

**A susceptibility locus for bipolar affective disorder identified on chromosome 3p22.3.** *R. Secolin[1], C. Banzato[2], M.C.M. Oliveira[2], M.F.R. Bittar[1], P. Dalgalarrondo[2], I. Lopes-Cendes[1].* 1) Dept of Medical Genetics, University of Campinas - UNICAMP, Campinas, SP, Brazil; 2) Dept of Medical Psychology and Psychiatry, University of Campinas - UNICAMP, Campinas, SP, Brazil.

**Rationale:** Bipolar affective disorder (BPAD) is a common psychiatric illness, with a prevalence of 0.8-2.6 % in the general population. Clinical features include episodes of mania or hypomania, interspersed with periods of depression. Genetic factors are known to contribute to the etiology of BPAD. A large genome-wide association study performed in the British population identified 21 candidate loci across the genome. However, in order to confirm and strength these findings, further replication studies in different populations are needed. In this study we aimed to evaluate these 21 candidate loci previously reported using a family-based association approach. **Methods:** We evaluated 74 pedigrees with BPAD, with a total of 411 individuals, including 96 patients who fulfilled clinical criteria for BPAD according to DSM-IV classification. We genotyped SNPs using real-time PCR (TaqMan® system, Applied Biosystems). We used the TDT POWER CALCULATOR program to verify statistical power of our sample and genotyped data was processed by the LINKGEN program, which also estimates minor allele frequency (MAF). Mendelian inconsistencies and Hardy-Weinberg Equilibrium (HWE) were evaluated by PEDCHECK and HAPLOVIEW softwares, respectively. Family-based association analysis was performed by the UNPHASED software. **Results:** We found that our sample has statistical power higher than 80% to detect association. We found significant association signals for four SNPs: rs2989476 ($p = 0.0031$), rs7570682 ($p = 0.0407$), rs9834970 ($p = 0.00013$) and rs11622475 ($p = 0.0153$). However, only SNP rs9834970 maintained statistically significance for association after Bonferroni correction ($p_{corrected} = 0.0025$), with an OR = 2.64 (95% CI= 1.30 - 5.35). **Conclusion:** We demonstrated that SNP rs9834970, located on chromosome 3p22.3, is associated with the disease phenotype in our BPAD pedigrees. This SNP maps to an intergenic region and is not known to be associated to regulatory genomic sequences. Additional SNPs are being genotyped in the candidate region in order to estimate haplotype association and to identify the putative causal variant associated with BPAD in the group of patients included in our study. Supported by FAPESP.

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## 2075/T/Poster Board #624

**Study of regional candidate genes for endophenotypes and clinical features of schizophrenia in a population isolate.** *J. Wedenoja[1,2], A. Tuulio-Henriksson[3,4], J. Suvisaari[3], A. Loukola[1], I. Surakka[1], T. Varilo[1,2,5], S. Mottaqui-Tabar[1], P. Lahermo[6], E. Serkkola[7], S. Ripatti[1,8], M. Ranki-Pesonen[7], J. Lönnqvist[3,9], L. Peltonen[1,2,5,10], T. Paunio[1,3,9].* 1) Institute for Molecular Medicine Finland FIMM, University of Helsinki and National Institute for Health and Welfare, Helsinki, Finland; 2) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 3) Department of Mental Health and Substance Abuse Services, National Institute for Health and Welfare, Helsinki, Finland; 4) Department of Psychology, University of Helsinki, Helsinki, Finland; 5) Program in Medical and Population Genetics, Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, MA, USA; 6) Genome and Technology Center, Institute for Molecular Medicine Finland FIMM, University of Helsinki, Helsinki, Finland; 7) Research and Development, Orion Pharma, Espoo, Finland; 8) Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden; 9) Department of Psychiatry, Helsinki University Central Hospital, Helsinki, Finland; 10) Human Genetics, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK.

Challenges in identification of predisposing genes for schizophrenia favor the use of detailed disorder features and endophenotypes which potentially are more closely related to the underlying biology. In our Finnish sample of familial schizophrenia, we have identified schizophrenia loci on chromosomes 2q, 4q, and 5q. Of these, a recent meta-analysis of 32 genome-wide linkage studies also highlighted the 2q and 5q regions. Additionally, we have identified loci for schizophrenia endophenotypes on chromosomes 2q for visual working memory, and on 4q for verbal learning and memory, both overlapping the schizophrenia loci. Here, we selected 104 regional, functionally relevant candidate genes from the linked 2q33.1-2q37.3, 4q13.1-4q26, and 5q31.1-5q33.3 regions, and genotyped altogether 1511 tagging SNPs in a nationwide Finnish sample of 293 neuropsychologically tested schizophrenia families (1111 individuals), as well as 375 independent population controls. We performed association analysis of clinical diagnostic categories, clinical disorder features, and several endophenotypic traits representing the central cognitive functions impaired in schizophrenia. Our approach allowed identification of several promising associations, of which especially interesting is *ERBB4*, showing association with the severity of positive symptoms (P=0.0003) and impairments in traits related to verbal abilities (P= 0.0001). Interestingly, *ERBB4* has been previously associated with schizophrenia and verbal working memory. Similarly, *GRIA1* showed association with the severity of positive symptoms (P=0.00008), and has been previously associated with schizophrenia. Of the others, *EPHA4* showed association with schizophrenia spectrum disorders (P=0.0003) and several clinical disorder features (P=0.00006), and *EPHA5* with several verbal learning and memory traits (P=0.0001). Our findings support the view that the genetic risk of schizophrenia is at least partially mediated via the effects of the candidate genes on intermediate endophenotypes and support the use of these factors in search of specific risk variants.

## 2076/T/Poster Board #625

**A family-based association study of DNA sequence variants in GRM7 and CHL1 with schizophrenia in an Indonesian population.** *D.B. Wildenauer[1,2], C. Ganda[1,4], N. Amir[3], R. Heriani[3], I. Irmansyah[3], A. Kusumawardhani[3], M. Nasrun[3], I. Widyawati[3], S.G. Schwab[1,4].* 1) School of Psychiatry and clinical , University of Western Australia, Perth, Australia; 2) Centre of Clinical Research in Neuropsychiatry (CCRN), Mt Claremont, Western Australia; 3) Department of Psychiatry, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia; 4) Western Australian Institute for Medical Research, Perth, Western Australia.

Differences in clinical presentation and outcome of schizophrenia in patients from developing countries as opposed to developed countries may suggest the presence of specific environmental and/or genetic factors. In order to address this question, we collected in Indonesia a sample of 124 families with two or more affected siblings together with their parents or unaffected siblings in cases where one parent was missing. Using this sample for linkage analysis, we reported genome-wide significant linkage to chromosome 3p in a sib-pair sample from 124 families from Indonesia. Two promising candidate genes within the linked region are the metabotropic glutamate receptor subtype 7 (GRM7), involved in glutamatergic neurotransmission, and the close homolog of L1 (CHL1), which plays a critical role in neurodevelopment. We genotyped 18 SNPs in GRM7 and one SNP in CHL1 in the sample of 124 sib-pair families, in which linkage with schizophrenia had been detected. Transmission disequilibrium analysis revealed nominally significant transmission distortion of rs17031835 in intron 1 of GRM7 (P = 0.004, not corrected for multiple testing), along with a number of haplotypes containing rs17031835. No other single marker was found to be significantly associated with schizophrenia in our sample. The results from our study provide support for the idea that glutamatergic neurotransmission and specifically the GRM7 gene might be relevant to the development of schizophrenia in the Indonesian population.

## 2077/T/Poster Board #626

**Rare variant discovery using pooled DNA for next generation sequencing; Multiple rare alleles of the ATP-binding cassette transporter A1 (ABCA1) gene as risk factors for Alzheimer's disease.** *M.K. Lupton[1], M. Daniilidou[2], M. Tsolaki[2], R. Wroe[1], S. Lovestone[1], J.F. Powell[1].* 1) Neuroscience, Institute of Psychiatry, Kings college London, London, United Kingdom; 2) Third Neurological Clinic of Aristotle University, Papanikolaou Hospital, Thessaloniki, Macedonia, Greece.

Hypercholesterolemia and the ε4 allele of the cholesterol-carrying apolipoprotein E gene are major risk factors for Alzheimer's disease (AD). Intracellular cholesterol has been shown to influence the generation of amyloid-beta peptides and the cholesterol efflux pump, ABCA1 regulates cellular cholesterol levels. Rare alleles in this gene have been shown to contribute significantly to low plasma HDL cholesterol levels. While, there has been a failure to find consistent evidence of a genetic contribution by common variants (SNPs with MAF>0.05) of this gene to AD, we hypothesized that rare variants may increase risk of AD.

We pooled DNA from 311 AD cases and 355 controls. All coding regions and splice sites were amplified by PCR which was sequenced using the next generation Illumina Genome Analyzer. Data was analysed using the alignment program MAQ. SNP identification methods were designed using Perl scripts and STATA. The impact of novel amino acid allelic variants was predicted using multiple sequence alignments and protein 3D structures. The method has been validated by individually genotyping a common SNP, and one exon has been Sanger sequenced in individuals to compare allele frequencies and assess error rate.

From the common SNPs identified one was found to have a highly significantly different frequency in case compared to controls which was confirmed in a larger sample set. There was found to be significantly more potentially pathogenic variants in cases compared to controls for rare variants (MAF<0.01). This is one of the first studies to utilise the next generation technology to carry out deep re-sequencing of pooled DNA samples rather than individuals. We have shown that it is possible to use pooled DNA samples to obtain reliable allele frequency data even for rare SNPs.

Our study confirms the hypothesis that individually rare sequence variants are sufficiently pathogenic and common in aggregate to contribute to variation in AD risk. This promising technique will make screening for all sequence variants in candidate regions more affordable.

## 2078/T/Poster Board #627

**Two independent mutations in the ZNF526 gene are associated with non-syndromic autosomal recessive mental retardation.** *L. Abbasi Moheb[1], L. Riff jensen[2], M. Garshasbi[1], K. Kahrizi[2], S. Ghadami[1], K. Wrogemann[1], H. Hu[1], R. Kariminejad[3], F. Behjati[2], M. Mohseni[2], M. Falah[4], H. Khodai[5], M. Kasiri[6], H. Darvish[2], A. Tzschach[1], H.H. Ropers[1], H. Najmabadi[2,3], A.W. Kuss[1].* 1) Human Molecular Genetics, Max Planck Institute for Molecular Genetics, Berlin, Germany; 2) University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; 3) Kariminejad-Najmabadi Pathology and Genetics Center, Tehran, Iran; 4) Tehran Welfare Institution, Tehran, Iran; 5) Yazd Welfare Institution, Yazd, Iran; 6) Shahre Kord Welfare Institution, Shahre Kord, Iran.

Mental retardation (MR) is with a prevalence of about two percent one of the most common forms of genetic handicaps. So far, however, very little is known about the genetic basis of this disorder. Especially the contribution of autosomal recessive hereditary defects is largely unresolved and to date, only five genes have been found to be directly associated with non-syndromic autosomal recessive MR (NS-ARMR). We have previously identified numerous novel loci for NS-ARMR, including MRT4-11 (Najmabadi et al., 2007, Hum. Genet. 121(1):43-8). Here we report on two different single nucleotide changes in the *ZNF526* gene, which maps to MRT11, localized in a 10 Mbp linkage interval on chromosome 19 (19q13.2-13.32). These nucleotide changes were found to co-segregate with moderate to severe NS-ARMR in three large, apparently unrelated Iranian families and were absent in 572 control chromosomes. *ZNF526* encodes a C2H2 zinc finger protein with multiple functional domains. It is expressed in brain, has DNA binding properties and is thought to be involved in gene regulation. Both mutations affect functional domains of the gene product and impair its function, as suggested by in silico protein modelling and confirmed by the presence of specific changes in the gene expression patterns of lymphoblastoid cells. Several zinc finger proteins have been shown to be involved in the pathogenesis of X-linked MR, but *ZNF526* is the first to be implicated in ARMR. Moreover, *ZNF526* is only the second gene known to carry MR-associated mutations in independent families. This may suggest that *ZNF526* defects are among the more common causes of NS-ARMR, at least in the Iranian population. CHIP-seq experiments are in progress to identify regulatory targets of the ZNF526 protein.

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## 2079/T/Poster Board #628

**Partial duplication of CSMD1 (CUB and sushi multiple domains 1) (arr cgh 8p23.2((2,756,521->3,188,217))x3)associated with myoclonic seizures in a one year old female.** *J. Bartley[1], E. Friedrich[2].* 1) Miller Children's Hospital, Long Beach, CA; 2) UC Irvine Medical Center, Orange, CA.

A one-year old female with myoclonic seizures was found to have a partial duplication of CSMD1 (Signature Genomic Laboratories, LLC). Her brain MRI was normal and her seizures were poorly controlled with medications and a ketogenic diet. Her mother also had a duplication of the same intronic oligomers; but she does not seizures. The extent of the duplication is approximately 431.7 Kb in size with a single copy gain of 46 oligonucleotide probes in the short arm of chromosome 8 at 8p23.2. CUB and sushi exon number have not determined to be the same in the patient and her mother. The numbers and positions of CUB and sushi domains and transmembrane domain are completely conserved among the human CSMD (CSMD1, CSMD2, CSMD3) gene family. CSMD3 at 8q23.3-q24.1 is a candidate gene for mutations causing benign adult familial myoclonic epilepsy (BBRC 309 (2003) 143-154). The rat analog of CSMD1 mRNA is expressed in developing CNS and epithelial tissues with enrichment of CSMD1 in the nerve growth cone, the amoeboid-leading edge of the growing neuron (J Immunology, (2006) 4419-4430). A duplication of 8p23.1-8p23.2 (maternal origin) with the distal breakpoint interrupting the CSMD1 gene between introns 7 and 11 was associated with speech delay, autism and learning disorders (Eur J Hum Gen 17(2009)37-43). CSMD1 mutations appear to be a candidate for causing myoclonic seizures or developmental delay and autism in children.

## 2080/T/Poster Board #629

**The influence of sleep deprivation on gene expression in humans.** *R. Pellegrino[1], C.S Guindalini[1], D.Y Sunaga[2], M.L Andersen[1], R. Martins[1], S. Tufik[1].* 1) Laboratório de Biologia Molecular do Sono - Departamento de Psicobiologia - Universidade Federal de São Paulo - Unifesp; 2) Instituto de Biociências da USP - Centro de Estudos do Genoma Humano - Universidade de São Paulo.

Chronic sleep loss is increasingly common in industrialized societies. Sleep deprivation (SD) induces several behavioral, neurochemical, cellular and metabolic alterations. We performed genome-wide mRNA expression profiles in total blood RNA using a high-density oligonucleotide array (U133 Plus 2.0 Affymetrix, Santa Clara, California) in sleep deprived men. Healthy volunteers (18-25 years old) were subjected to 48 hours of SD, followed by 24 hours of sleep recovery (SR). All participants were carefully screened with a detailed medical history, physical, and neurological examination, routine blood tests, and urine toxicology for psychotropic drugs. All assays were performed by the MolecularCore Microarray Core (Affymetrix) following the manufacturer's standard protocol. We identified 34 DEGs (differentiated expressed genes) after 48 hours of SD and 76 DEGs after 24 hours of SR, when compared to basal levels. Moreover, 92 DEGs were detected between SD versus SR groups. The DEGs included genes related to circadian rhythms, inflammatory response and cell division control. We carried out pathway analysis on the identified DEGS using the Ingenuity Pathway Analysis (IPA) tool. Pathways involving cellular growth and proliferation, hematological system development, cancer and immunological disease, as well as cell death were significantly altered after 48 hours of SD and the subsequent recovery. Our results show that long periods of sleep loss activates genes related to a number of biological processes that might have great impact on health. These data should also motivate further investigations to define the molecular effects of sleep deprivation and its implications for diverse diseases.

## 2081/T/Poster Board #630

**Genetics of non-coding RNA in alcoholism.** *A. Pietrzykowski.* Rutgers University, Cook Campus, School of Environmental and Biological Sciences, Department of Animal Sciences, 67 Poultry Farm Lane, New Brunswick, 08901.

Alcoholism is a chronic, debilitating disease and a major cause of morbidity and mortality worldwide. There is substantial evidence for genetic linkage of specific locations on some chromosomes including chromosome 1 and 15 to alcohol-related phenotypes. As our understanding of the role of protein coding genes in the development of alcoholism is well advanced, contribution of non protein coding genes, including microRNA, just started to be investigated. microRNAs encode regulatory, small RNA molecules, which typically control expression of hundreds of targets. This powerful ability places microRNAs in a unique position of master regulators of gene expression. Recently, others and we have defined a subset of microRNA species particularly important for alcohol actions in the developing and mature neurons. Specifically, miR-9 stands out as a key target of alcohol, contributing e.g. to the development of alcohol tolerance. Moreover, miR-9 expression is acutely and chronically modulated by alcohol. Here we show that at least two out of three miR-9 genes are located in high alcohol susceptibility loci located on chromosomes 1 and 15. Moreover, promoters of miR-9 genes have very little homology and SNPs profile varies among miR-9 genes and their regulatory regions, suggesting differential expression of each miR-9 gene. Further studies will test the genetic association and functional implications of SNPs related to miR-9 genes in non-alcoholic and alcoholic populations. Our studies provide new concepts of the development and therapy of alcoholism.

## 2082/T/Poster Board #631

**The Role of the Mitochondria in Autism.** *J. Conroy[1], AT. Pagnamenta[2], N. Shah[1], R. Segurado[3], J. Casey[1], R. Regan[1], T. Magelhaes[4], K. Tansey[3], D. Pinto[5], JB. Cazier[2], A. Vicente[4], A. Green[1,6], M. Gill[3], L. Gallagher[3], AP. Monaco[2], S. Ennis[1,6], Autism Genome Project (www.autismgenome.org).* 1) School of Medicine, Univeristy College Dublin, Dublin, Ireland; 2) The Wellcome Trust Centre for Human Genetics, University of Oxford, UK; 3) Department of Psychiatry, Trinity College Dublin, Ireland; 4) Instituto Gulbenkian de Ciencia, Oeiras, Portugal; 5) The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Canada; 6) The National Centre for Medical Genetics, Ireland.

Autism Spectrum Disorders (ASD) are neurodevelopmental disorders characterised by impairments in communication, social interaction, and repetitive/restrictive behaviour. Deletions in the mitochondrial genome, biochemical markers of mitochondrial dysfunction and mitochondrial disorders have been reported in individual patients with autism, for example Leigh syndrome and various oxidative phosphorylation (OXPHOS) complex deficiencies. Furthermore, two studies showed evidence that 20% and 28% of autism cases respectively, exhibited hyperlactacidemia, a known marker of mitochondrial dysfunction (Oliveira et al. 2005 and Correia et al. 2006). In larger studies, associations have also been reported with markers in the *SLC25A12* gene. The international AGP consortium has carried out whole-genome SNP and CNV analysis on a cohort of ~1500 ASD families using the Illumina 1M SNP array. To complement these studies we set out to investigate the potential role of the mitochondria in the development of autism. Nuclear genes that contribute to the structure, function and maintenance of the organelle have been identified from various online mitochondrial databases. Only those genes that showed expression in the central nervous system were tested for association using TDT set based test in "plink". Following 10,000 permutations, a number of genes that contain significantly associated SNPs have been identified. Forty-nine gene sets have an mperm p value less than 0.05, eleven of which had p values less than 0.01. These genes (n = 49) range in function including active transmembrane transport, NAD binding, protein transport and ligase activity. The Illumina 1M SNP array also contains probes for 163 polymorphisms in the mitochondrial genome. Association analysis was undertaken using the paternal genotypes as a control dataset. The most significant result was a rare Ile-Val change in the *MT-ND6* that was 6 times more frequent in probands compared to controls ($p = 0.001$). However this did not withstand correction for multiple testing. The analysis should be extended to include additional autism spectrum disorder cohorts for sufficient power to detect effects of these rare mtDNA variants. A number of other lines of investigation are taking place. These include the analysis of deletions and duplications within the mitochondrial and nuclear genome in probands and their parents. Gene-gene interactions in the OXPHOS complexes are also being tested in this larger autism cohort.

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## 2083/T/Poster Board #632

**Exploring Mitochondrial Variation in Autism.** *J.R. Gilbert[1], J.L. McCauley[1], D.J. Hedges[1], M.A. Schmidt[1], H.H. Wright[2], R.K. Abramson[2], M.L. Cuccaro[1], E.R. Martin[1], J.L. Haines[3], M.A. Pericak-Vance[1].* 1) Miami Institute for Human Genomics, Miller School of Medicine, University of Miami, Miami, FL; 2) University of South Carolina, School of Medicine, Columbia, SC; 3) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN.

There has been increasing speculation that oxidative stress and abnormal energy metabolism may play a role in Autism Spectrum Disorders (ASD). There has been a profound lack of investigation into the role of mitochondrial variation in autism, despite numerous clinical reports describing patients with mitochondrial disorders or mutations who have symptoms consistent with ASD. Our laboratory sought to comprehensively examine the role of mitochondrial variation and nuclear-encoded mitochondrial gene variation with regard to ASD risk. We sequenced the mtDNA from a dataset of ~400 Caucasian proband-father pairs (379 probands/372 fathers) using the Affymetrix Human Mito2.0 chip. We examined haplogroups, characterizing both common and rare mitochondrial variations, and tested for association of both haplogroups and mitochondrial variation with ASDs. Additionally, we utilized genome-wide single nucleotide polymorphism data generated from the Illumina 1-M beadchip on all individuals to test for gene x gene interactions between mitochondrial variations and nuclear-encoded mitochondrial gene variations. Our initial examination of European-Caucasian haplogroups and haplogroup defining single-nucleotide polymorphisms (SNPs) to assess the odds of carrying each mitochondrial haplogroup/ or haplogroup defining SNP in autism probands compared to unaffected fathers indicated that there is no significant difference in haplogroup frequencies between these two groups. The examination of variation across the mtDNA genome via single marker tests of association also failed to show convincing evidence for association to autism, although it should be noted there is limited power in this current dataset to detect smaller effect sizes. Furthermore, we examined our dataset for evidence of greater total mtDNA variation in autism probands compared to their fathers including the counts of both synonymous and non-synonymous changes between cases and controls with no significant difference. As there appears to be no main effects in our current dataset, we have begun to examine the interplay between nuclear-encoded and mitochondrial variation. These data provide insight into mitochondrial variation on a level never before performed in autism. Our data as a whole indicate an absence of main effects, suggesting that the role of mitochondrial variation in ASD risk may be subtle or interactive in nature.

## 2084/T/Poster Board #633

**A novel missense mutation associated with GEFS+ in a Chinese family.** *X. Cui[1], Y. Liu[1], F. Zeng[2], T. Zhan[1], Z. Tang[1], Q. Wang[1], M. Liu[1].* 1) Huazhong University of Science and Technology, Wuhan, Hubei, China; 2) Centre Hospital of Xuan Cheng, An Hui Province, China.

Generalized epilepsy with febrile seizures plus (GEFS+) is a common familial epilepsy syndrome, which generally develops in patients' childhood. Several genes have been reported to be associated with GEFS+, including sodium-channel $\beta$1-subunit(SCN1B) , sodium-channel $\alpha$1-subunit(SCN1A), sodium-channel $\alpha$2-subunit(SCN2A), gamma-aminobutyric acid receptor-delta(GABRD) , ,and gamma-2 subunit of the gamma-aminobutyric acid receptor(GABRG2) as well. In this report, we investigated a Chinese family with GEFS+, which was autosomal dominantly inherited. Linkage analysis localized the disease-causing gene to Chromosome 2q24, with a maximum lod score of 3.01($\theta$=0.00) at D2S2330, where SCN1A harbored nearby. DNA sequence for whole coding region of SCN1A revealed a nucleotide substitution of C577 with T, causing a missense mutation L193F of SCN1A. The mutation was found to segregate with GEFS+ in this Chinese family and not present in 200 normal controls. Interestingly, the GEFS+ symptoms in the family displayed significant intra-family heterogeneity. The mutation located at the s3 segment of domain I, which might alter the $\alpha$-helix motif of this transmembrane peptide. Further studies for the L193F mutation might offer a better understanding to the function of SCN1A, and the correlation between the genotype of SCN1A and clinical expression of GEFS+.

## 2085/T/Poster Board #634

**A mutation in lysosomal integral membrane protein type 2 (*LIMP2*) associated with myoclonic epilepsy in a patient with type 3 Gaucher disease.** *J. DePaolo, O. Goker-Alpan, N. Tayebi, B. Stubblefield, N. Gupta, E. Goldin, E. Sidransky.* nhgri/nih, bethesda, md.

Lysosomal integral membrane protein type 2 (LIMP-2) is responsible for the proper sorting of glucocerebrosidase to the lysosome. Mutations in the *LIMP2/SCARB2* gene have been implicated in inherited forms of myoclonic epilepsy. Vast clinical heterogeneity is observed among patients with Gaucher disease (GD) even among individuals with the same genotype. We investigated whether a mutation in the *LIMP2* gene could impact phenotype, possibly interfering with the trafficking of glucocerebrosidase to the lysosome. Specifically, we examined the case of two siblings with congruent mutations in glucocerebrosidase (GBA), but with extremely different phenotypes. One developed progressive myoclonic epilepsy in adolescence, and subsequently dementia, while the second was relatively asymptomatic. We sequenced *LIMP2* in both sibs, and found that only the one with more severe GD and myoclonic epilepsy carried a heterozygous point mutation in *LIMP2* on amino acid residue 471, changing it from Glu to Gly. The acidic residue E471, located in the carboxyl domain is reported to be important in movement of protein within the endocytic pathway. Western blots performed on fibroblast cell lysates from both patients demonstrated that the sib with the *LIMP2* mutation had markedly decreased levels of LIMP-2. This was confirmed by immunofluorescence studies demonstrating a dramatic decrease in cellular LIMP-2 in the sib with the mutation. These results appear to shed light on the drastically different phenotypes encountered in these siblings and may have implications regarding the role of *LIMP2* in myoclonic epilepsy.

## 2086/T/Poster Board #635

**Confirmation of the association between Calpastatin and Parkinson's disease.** *A.S. Allen[1], G.A. Satten[2].* 1) Biostatistics & Bioinformatics, DukeUniversity, Durham, NC; 2) Centers for Disease Control and Prevention, Atlanta, GA.

**Background:** We recently used haplotype sharing to analyze the NINDS Parkinson's disease (PD) genome-wide association study (GWAS). Our analysis identified a genomic region containing the calpastatin (CAST) gene as associated with risk of PD (Allen and Satten, Genetic Epi. published online April 13, 2009). Calpastatin inhibits calpains which have been implicated in several neurodegenerative disorders including PD.

**Methods:** We investigated the CAST region in an independent dataset comprised of 926 Parkinson's patients and 832 neurologically normal controls taken from the Center for Inherited Disease research GWAS in Familial PD study. Fifty SNPs where chosen from the region containing CAST and were tested for PD association using two haplotype sharing tests as well as the single-SNP Mantel-Haenszel (MH) test. Adjustment for population stratification was carried out using the stratification score approach of Epstein, Allen, and Satten (AJHG 2007 ;80(5):921-30). Permutation was used to adjust for the 150 tests performed (3 tests at each of 50 loci).

**Results:** After adjusting for multiple comparisons, we found the single SNP Mantel-Haenszel test to be significant (adjusted p=0.0199) with maximum signal at rs1559085. The haplotype sharing tests did not identify any significant loci after adjustment for multiple testing. SNP rs1559085 is also part of the signal identified in the NINDS dataset and effect measures for this SNP are comparable in the two studies (allelic odds ratios: 1.45 - CIDR; 1.56 - NINDS ).

**Conclusions:** Loci in the calpastatin gene are associated with risk for PD. This, along with a plausible role for calpastatin in PD etiology, suggest Calpastatin as a priority for further study.

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## 2087/T/Poster Board #636

***GBA* mutations and clinical features of patients evaluated at a referral clinic for Parkinson disease.** *J. Choi[1], G. Lopez[2], N. Gupta[1], B. Stubblefield[1], O. Goker-Alpan[1], N. Tayebi[1], E. Sidransky[1].* 1) Section on Molecular Neurogenetics, Medical Genetics Branch, NHGRI, National Institutes of Health, Bethesda, Maryland; 2) Office of Clinical Director, Medical Neurology Branch, NINDS, National Institutes of Health, Bethesda, Maryland.

Recent findings demonstrate an increased frequency of mutations in glucocerebrosidase (*GBA*), the enzyme deficient in the autosomal recessive lysosomal glycolipid storage disorder Gaucher disease (GD), among patients with Parkinson disease (PD). Parkinson disease is a common, complex motor disorder, resulting from the loss of dopamine-producing neurons. In this study, all patients referred to a new Parkinson disease clinic at the National Institutes of Health Clinical Center were prospectively evaluated in a uniform fashion by a single neurologist, using a standardized Parkinson examination. DNA from 214 PD patients and healthy controls were screened for mutations in *GBA* by sequencing all 11 exons of the gene. Seven mutant *GBA* alleles were identified among 135 patients with PD including mutations D409H, L444P, N370S, R463C, and R496H. Each was a heterozygote. No *GBA* mutations were found among 79 healthy controls. The frequency of *GBA* mutations among the PD cohort was statistically significant (p-value of 0.0484 from Fisher's exact test). Despite the limited sample size, we explored the association of *GBA* mutations with specific clinical features. On average, PD subjects carrying *GBA* mutations presented with symptoms on 3 years earlier, and showed a trend towards an increase in tremor, bradykinesia, balancing problems, shuffling gait, cognitive involvement, and dystonia. This overall increase in motor and cognitive features among patients with *GBA* mutations was reflected in their standardized scores. In patients with PD without *GBA* mutation the mean Hoehn and Yahr score was 2.2, mean UPDRS (III) score 25.4, mean MMSE score 28.8, and mean MoCA score 25.2; whereas for patients with *GBA* mutations the mean Hoehn and Yahr score was 2.6, mean UPDRS (III) score 33.4, mean MMSE score 29.1, and mean MoCA score 27.0. The lower age of onset trend supports other studies in the literature suggesting that carriers of *GBA* mutations have earlier disease onset. Further studies of the association between GD and PD will enhance our understanding of pathophysiology of the two disorders, will impact genetic counseling of patients and families and may lead to new diagnostic and therapeutic strategies for Parkinson disease.

## 2088/T/Poster Board #637

**FUS mutations are a rare cause of sporadic amyotrophic lateral sclerosis.** *S. Lai[1,2,3], Y. Abramzony[1], J.C. Schymick[1,4], R. Guerreiro[5], D.A. Stephan[6], G. Mora[7], G. Restagno[8], A. Chiò[9], B.J. Traynor[1] on behalf of The ITALSGEN Consortium.* 1) Neuromuscular Diseases Research Group, Laboratory of Neurogenetics, NIA, Bethesda, MD, USA; 2) Department of Neurology, Chang Gung Memorial Hospital and College of Medicine, Taiwan, Republic of China; 3) Department of Molecular Neuroscience and Reta Lila Weston Laboratories, Institute of Neurology, University College London, London, UK; 4) Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK; 5) Molecular Genetics Unit, Laboratory of Neurogenetics, NIA, Bethesda, MD, USA; 6) Navigenics, Inc, Foster City, CA, USA; 7) Salvatore Maugeri Foundation, Lissone, Italy; 8) Molecular Genetics Unit, Department of Clinical Pathology, A.S.O. O.I.R.M.-S.Anna, Turin, Italy; 9) Department of Neuroscience, University of Turin, Turin, Italy.

**Background** Recently, the *fused in sarcoma/translated in liposarcoma (FUS)* gene, located on chromosome 16p11.2, has been identified as a disease gene in familial amyotrophic lateral sclerosis. However the importance of this gene in the commoner sporadic form of the disease is not known. **Aim** The aim of this study was to further define the spectrum of *FUS* gene mutations in a large series of sporadic ALS (SALS) patients. **Methods** DNA samples were collected from 1,749 SALS of American and Italian origin. The entire coding region was sequenced in 276 SALS. As previous publications have implicated exon 15 as a hotspot for mutations within this gene, this exon was sequenced in an additional 1,473 SALS. Detected variants were evaluated in a control cohort consisting of 280 neurologically normal Italian subjects and 460 neurologically normal US subjects. **Results** Among the 1,749 SALS patients we found a total of 8 cases with missense mutations (0.4%). Of these, c.1561C>T (leading to a p.R521C change in the amino acid sequence, n = 1 case), c.1562G>A (p.R521H, n = 1) and c.1566G>A (p.R521R, n = 4) have been previously been described, whereas c.1552A>G (p.R518G, n = 1) and c.1575G>T (p.P525P, n = 1) were novel. None of these variants were present in controls. **Discussion** *FUS* gene mutations have been found in less than 0.5% of apparently SALS patients. Two novel mutations of FUS that appear to underlie motor neuron degeneration were identified.

## 2089/T/Poster Board #638

**Testing the genes and pathways behind fetal motoneuron disease as candidate genes for ALS.** *H.O. Nousiainen[1], H. Laaksovirta[2], K. Silander[1], K. Rehnström[1], H. Kääriäinen[1], M. Kestilä[1], L. Peltonen[1,3,4,5].* 1) National Institute for Health and Welfare, Public Health Genomics Unit and FIMM, Institute for Molecular Medicine Finland, Helsinki, Finland; 2) Department of Clinical Neurosciences, Helsinki University Central Hospital, Helsinki, Finland; 3) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 4) The Broad Institute, MIT, Boston, MA, USA; 5) The Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK.

Amyotrophic Lateral Sclerosis (ALS) is a late-onset, rapidly progressing neurodegenerative disease affecting motor neurons. The etiology of most ALS cases remains unknown, but mutations in Cu/Zn superoxide dismutase *SOD1*, and also *TARDBP* and *FUS*, encoding the RNA binding proteins TDP-43 and fused in liposarcoma have been implicated in a small fraction of cases of both familial and sporadic ALS. Deleterious variants in the phosphoinositide phosphatase *FIG4*, which is mutated in the juvenile form of the hereditary motor and sensory neuropathy Charcot-Marie-Tooth disease CMT4J (MIM 611228), have also been found in ALS patients. Here we have taken a pathway based approach in searching for ALS genes triggered by our recent findings in two fetal motoneuron diseases: Lethal Congenital Contracture Syndrome (LCCS1, MIM 253310) and Lethal Arthrogryposis with Anterior Horn cell Disease (LAAHD, MIM 611890). Both diseases are caused by mutations in the *GLE1* gene, which encodes for a protein that is involved in mRNA export and protein synthesis. GLE1 function is dependent of a small organic molecule, inositol hexakisphosphate ($IP_6$). Interestingly, the causative mutations of two additional lethal motoneuron diseases (LCCS2 and LCCS3) have been recently attributed to defects in enzymes that modulate the phosphatidylinositol-3-kinase/Akt signaling cascade, a pathway intimately connected with the production of $IP_6$. This suggests a role for mRNA processing in these disorders and a relationship between inositol signaling, mRNA processing, and neuronal survival. We analyzed a nationwide cohort of 250 Finnish ALS patients, both familial and sporadic, and 450 Finnish controls, excluding patients with mutations in *SOD1* (n= 18). We genotyped the remaining patient samples for 93 SNPs in six genes involved in mRNA transport and phosphoinositide signaling. We performed association and haplotype analysis using PLINK. Preliminary results revealed suggestive association between haplotypes in *ITPR2* and ALS. We observed enrichment of a rare haplotype in cases, with frequencies 0.090 and 0.060 in cases and controls, respectively. *ITPR2* has also been previously associated with ALS in European populations. Further characterization of the associated region will potentially provide additional information on the molecular mechanisms behind ALS and increase our understanding of the pathogenesis of this devastating disease.

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## 2090/T/Poster Board #639

**Allelic spectrum of rare mutations within CNTNAP family members in autism spectrum disorders and related phenotypes.** *S. Szelinger[1], J.D. Gerber[1], C. Smith[2], A. Sekar[1], S. Brautigam[2], J.O. Long[1], T. Pawlowski[1], J. Pruzin[1], M. Redman[1], A.A. Kurdoglu[1], M.J. Huentelman[1], D.W. Craig[1].* 1) Neurogenomics, Translational Genomics Research Institute, Phoenix, AZ; 2) Southwest Autism Research & Resource Center, Phoenix, AZ.

The genetic basis of common complex neurobehavioral diseases such as autism, schizophrenia, and bipolar disorder are largely unknown. Multiple genome-wide association studies suggest that 'common-variants' alone do not explain a substantial portion of the variance for this highly heritable disorder. As an initial investigation into how multiple 'rare-variants' may lead to a spectrum of sub-phenotypes within and across neurobehavioral disorders, we have investigated the spectrum of rare variants within the gene *contactin associated protein 2* (CNTNAP2), along with several neurexin family members for individuals with and without autism. CNTNAP2 has been previously associated with epilepsy, Tourette's syndrome, and schizophrenia. The genes CNTNAP1, CNTNAP2, and CNTNAP4 were sequenced using both capillary Sanger-based sequencing and multiplexed sequencing on the Illumina Genome Analyzer II. Genetic variants identified from analysis of sequence traces were validated by TaqMan assays and/or further sequencing and bioinformatically evaluated for pathogenicity. Within CNTNAP2 mutations predicted to be deleterious we further examined sub-phenotypic comorbidities, such as seizures, the appearance of repetitive behaviors, and related phenotypes. A total of 300 different autistic individuals were sequenced for one or more of these genes using individuals collected from the Autism Genetic Research Exchange (AGRE) and the Southwest Autism Research and Resource Center (SARRC). Controls were obtained from lymphoblastic cell-lines provided by the National Institute of Mental Health (NIMH) and the Coriell Cell Repository. Additional controls for the same region were examined using sequence traces aligned from the International 1,000 Genomes Project. For genetic variants not found in controls, ABI TaqMan assays were designed and these variants were screened across additional controls cleared of neurological defects. Cumulative analysis indicates that the allelic spectrum of deleterious CNTNAP2 mutations can lead to substantially different comorbidities, even across a single multiplex family, suggesting that rare disease-causing mutations within CNTNAP2 require a 'second hit' that remains unknown. Overall, the diversity of sub-phenotypes observed within CNTNAP2 mutations associated with autism underscores the forthcoming challenges for elucidating how rare-variants lead to common complex neurological disorders.

## 2091/T/Poster Board #640

**A new locus for pure Spinocerebellar Ataxia associated with Erythrokeratodermia maps to chromosome 6p12.3-q16.1.** *M. Turcotte Gauthier[1], D.K. Nguyen[1], C. Meloche[1], J. Poirier[1], S.L. Girard[1], S. Forlani[2], E. Di Gregorio[4], B. Borroni[4], G. De Michele[5], A. Filla[5], D. Verbeek[6], B.P.C. Van de Warrenburg[6], C.A. Drouin[3], A. Durr[2], A. Brice[2], G. Stevanin[2], A. Brusco[4], P. Cossette[1].* 1) Centre of Excellence in Neuromics of Université de Montréal, CHUM Research Center, Montreal, Quebec, Canada; 2) INSERM/UPMC U975, Centre de Recherche Institut du Cerveau et de la Moelle Epinière, GHU Pitie-Salpetriere, Paris, France; 3) Department of Dermatology, Centre Hospitalier Régional Grand-Portage, Rivière-du-Loup, Québec, Canada; 4) Department of Neurology, University of Brescia, Italy; 5) Federico II University, Naples, Italy; 6) University Nijmegen Medical Centre, the Netherlands.

Giroux and Barbeau previously described a unique French-Canadian family segregating a combination of ataxia and hyperkeratotic erythematous plaques in an autosomal dominant mode. These skin lesions were compatible with erythrokeratodermia (EK). The family has been partially collected and the disease was potentially linked to chromosome 1p35. However, the identification of the mutated gene is pending. The objective of this study is to revisit the clinical phenotype and genetic mapping of this peculiar family. We recently reassessed the family with detailed examinations, brain MRI and EMG studies.We recollected the family and performed a whole genome scan analysis by using microsatellites markers. We also analyzed four additional families from European ancestry with autosomal dominant SCA (from the EuroSCA consortium), for which a genome scan have shown positive LOD score to the same chromosomal region. The neurological phenotype in the French-Canadian family is compatible with a pure autosomal dominant cerebellar ataxia (ADCA type III in Harding's classification) associated with cerebellar atrophy starting in late 40s. We mapped the locus for the disease on chromosome 6p12.3-q16.1 with a maximum LOD score of 5.33 for marker *D6S452*. Haplotype analysis of this family allowed us to refine the candidate gene region between markers *D6S459* and *D6S417* (46.8Mb). Linkage to this locus was confirmed in the four additional families with a maximum compiled LOD score of 9.9. So far, the sequencing of 17 candidate genes, search for CAG/CTG expansion and copy number analysis did not allow the identification of the causative gene. We have mapped a new locus for a pure autosomal dominant cerebellar ataxia (SCA31) in a total of five families from European ancestry, including a large French-Canadian family associated with EK.

## 2092/T/Poster Board #641

**Lingos in ET and PD.** *C. Vilarino-Guell.* Dept Neuroscience, Mayo Clinic, Jacksonville, FL.

A variant in LINGO1 (rs9652490) has recently been found associated with increased risk of essential tremor (ET). We set out to replicate this association in an independent case-control series of 356 essential tremor patients from North America. In addition, given the clinical and pathological overlap between essential tremor and Parkinson disease (PD), we also evaluate the effect of LINGO1 rs9652490 in two case-control series of Parkinson disease from the US and Norway. The analysis of our ET patient-control series indicated a significant association between the LINGO1 rs9652490 A allele and risk of disease (P=0.014; OR=2.2). Similarly, LINGO1 was also found significantly associated with an increased risk of PD in the US (P= 0.012; OR=2.1) and Norway series (P=0.008; OR=1.7). Combining both PD series this analysis resulted in a highly significant association (P=3.4x10-4) and a 1.8-fold increased disease risk. Combining PD and ET cases we obtained a highly significant 1.9-fold increased risk of disease (P=1.3x10-4). The genetic association of the LINGO1 locus with both ET and PD susceptibility is supported by the functional role of its encoded protein. LINGO1 is a central nervous system-specific protein component of the Nogo-66 receptor (NgR1)/p75/LINGO1 signaling complex implicated in inhibition of oligodendrocyte differentiation, axonal myelination and regeneration, and neuronal survival. The expression of LINGO1 is elevated in the substantia nigra of patients with PD compared to age-matched controls, and in ventral midbrain neurons in animal models of PD after neurotoxic lesions. Furthermore, reduction of LINGO1 activity has been shown to improve survival, growth and function of dopaminergic neurons both in primary cell cultures and in vivo experimental models of parkinsonism in rodents. Genetic association of both ET and PD with LINGO1 provides the first compelling evidence that the pathophysiology of these common movement disorders is connected.

## 2093/T/Poster Board #642

**Functioal studies of MLC1 gene novel variants in four Chinese Patients with Megalencephalic Leukoencephalopathy with Subcortical Cysts(MLC).** *J. Wang[1], A. Dhauchak[2], H. Wei[1,3], H. Xiong[1], Y. Wu[1], D. Colman[2], X. Wu[1], Y. Jiang[1].* 1) Peking University First Hospital, Beijing, China; 2) Montreal Neurological Institute, Montreal,Quebec,Canada; 3) Shanxi Medical University, Taiyuan, Shanxi, P. R. China.

Megalencephalic leukoencephalopathy with subcortical cysts (MLC, MIM# 604004) is an autosomal recessively inherited disease resulting from a deficiency of MLC1 protein. No previous data about this gene mutation in China have been reported.The objective was to identify and analyze MLC1 mutations in four Chinese patients with (MLC). Methods Direct DNA sequencing, Immunoblots, and Immunocytochemistry. Results We detected four mutations (p. G73E, p.Y198X, p. A275T, c.772-1G change to C splicing mutation in IVS9-1, and c.*48A change to T) of which four were novel. We immunostained transfected arstrocyte cells for HA-MLC1 and calnexin, an ER marker.WT MLC1 was at arstrocyte borders, whereas MLC1 mutants G73E and Y198X are mainly intracellularly localized , except for A275T, which mainly at cell borders. Western blot analyses in arstrocyte cells transfected with WT MLC1 and with the amino acid changes G73E, Y198X and A275T, in comparison with the WT MLC1 in MLC1 expression, G73E mutation showed a reduced protein expression in transfected arstrocytes, Y198X reduced at 36kDa and showed a band at about 25kDa in artrocyte cells, and no changes were observed for the A275T. The c.772-1G change to C splicing site mutation resulted in the splice acceptor AG sequence of intron nine mutate to AC. This mutation may disrupt the open reading frame (ORF) of the MLC1 gene and cause the aberrant splicing of the following exons. Conclusion MLC1 mutants associated with MLC likely disrupt MLC1 protein expression and locolization. This would be the first report about MLC1 mutations in patients in China (Supported by National Key Research Project "11-5" (No: 2006BAI05A07), Beijing Municipal Natural Science Key Project (No: 08G1469), "973" Project of the Science and Technology Ministry of China (No. 2007CB5119004) and the International Collaborative Genetic Research Training Grant (NIH/FIC, No. D43 TW06176)).

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## 2094/T/Poster Board #643

**Genetic analysis and cellular localization of glucocerebrosidase in Parkinson's disease.** *M. Westerlund[1], A. Anvret[1], C. Ran[1], A. Carmine Belin[1], A. Zettergren[2], H. Nissbrandt[2], P. Söderkvist[3], T. Willows[4], C. Lind[5], O. Sydow[5], D. Galter[1], L. Olson[1].* 1) Department of Neuroscience, Karolinska Institutet, 171 77 Stockholm; 2) Department of Pharmacology, Sahlgrenska Academy at Göteborg University, 405 30 Göteborg; 3) Department of Biomedicine and Surgery, Faculty of Health Sciences, Linköping University, 581 85 Linköping; 4) Department of Neurology, Karolinska University Hospital, Huddinge, 141 86 Stockholm; 5) Department of Neurology, Karolinska University Hospital Stockholm, Solna, 171 76 Stockholm Sweden.

Recent studies have demonstrated an association between mutations in glucocerebrosidase (*GBA*), originally implicated in Gaucher's disease, and an increased risk of both familial and sporadic Parkinson's disease. The glucocerebrosidase gene is located on chromosome 1q21 and encodes a lysosomic protein which cleaves the beta-glucosidic linkage of glycosylceramide, an intermediate formed during glycolipid metabolism. To investigate the possible involvement of *GBA* in Parkinson's disease, we used pyrosequencing and real time PCR to study the non-synonymous variants T369M, N370S and L444P in a material consisting of 456 confirmed Swedish Parkinson cases and 732 unrelated control individuals. Differences in allele and genotype frequencies were compared using a Chi square test with two-sided p-values. Characterization of gene expression patterns is a prerequisite for understanding the involvement of the gene in disease. We therefore characterized the cellular localization of *GBA* mRNA in human and mouse tissues using in situ hybridization. We hypothesize that pathogenic variants in the *GBA* gene may confer risk for Parkinson's disease, and that the risk may vary between populations. Further genetic and biochemical studies on *GBA* will possibly bring new insights into the pathophysiology of Parkinson disease, which in turn can result in earlier diagnosis and design of relevant therapeutic strategies.

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## 2095/T/Poster Board #644

**Investigating the genetic basis of amyotrophic lateral sclerosis: analysis of known genes and search for new loci.** *K.L. Williams[1], J. Durnall[1], A. Thoeng[1,2], J.A. Solski[1], V. Thomas[1,2], J. Crawford[1,3], S. Gopinath[1], S. Warraich[1,4], G.A. Nicholson[1,5], I.P. Blair[1,4].* 1) Northcott Neuroscience, ANZAC Res Inst, Sydney, NSW, Australia; 2) Department of Physiology, University of Sydney, NSW, Australia; 3) School of Biological Sciences, Macquarie University, NSW, Australia; 4) Faculty of Medicine, University of Sydney, NSW, Australia; 5) Molecular Medicine Laboratory, Concord Hospital, NSW, Australia.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that causes the progressive degeneration of motor neurons. Familial ALS (FALS) accounts for approximately 10% of ALS cases with the remainder being sporadic (SALS). ALS is a genetically heterogenous disorder. To date, known genes only account for a small proportion of cases. We sought to investigate known ALS genes in a large cohort of ALS families, to firstly establish whether there is wider support for the proposed pathological role of these genes, and secondly, to determine the proportion of disease that can be attributed to mutations in these genes. The phenotypic expressivity of each mutation was also investigated. A genome scan is also underway among this ALS cohort to identify new disease loci. We analysed 147 Australian ALS families by direct DNA sequencing for mutations in *SOD1*, *DCTN1*, *ANG*, *CHMP2B*, *VEGF*, *TARDBP*, *FUS*, and *FIG4*. Haplotype analysis was also performed to identify any potential founder mutations. We determined that mutations in known ALS genes account for 20% of Australian ALS families, and comprise *SOD1* (15%), *FUS* (2.7%), *ANG* (2.0%), and *TARDBP* (1.4%) mutations. No mutations were identified in *DCTN1*, *CHMP2B*, *VEGF* and *FIG4*. None of the identified mutations in *SOD1* and *ANG* were present in a screen of 492 control chromosomes. We identified a novel missense mutation in *TARDBP* that was absent in 708 control chromosomes. This mutation (p.G294V) substitutes a highly conserved residue and is predicted to disrupt the glycine-rich domain in the C-terminus of TDP-43. We also identified a large *FUS* family in which the mutation segregated with the disease and provided a maximum LOD score of 4.54. Haplotype analysis suggested that no founder effect was present among *FUS* families with the same mutation thereby implicating the presence of a mutation hotspot. Among families with identified mutations, variable phenotypic expressivity was observed with gene and mutation specific effects for disease onset and duration. The genetic defects among the remaining 117 ALS families (80%) within our cohort are yet to be identified. A whole genome scan for linkage is currently underway in a subset of these families in order to identify new candidate loci for familial ALS.

## 2096/T/Poster Board #645

**Mitochondrial Rho GTPase, MIRO1, and Parkinson's disease.** *A. Anvret[1], M. Westerlund[1], C. Ran[1], D. Galter[1], C. Lind[2], T. Willows[3], O. Sydow[2], L. Olson[1], A. Carmine Belin[1].* 1) Department of Neuroscience, Karolinska Institutet, Retzius väg 8, 171 77 Stockholm, Sweden; 2) Department of Neurology, Karolinska University Hospital Solna, 171 76 Stockholm, Sweden; 3) Department of Neurology, Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden.

Mitochondria are essential in all eukaryotic cells to generate ATP and they also play a critical role in cell death mechanisms and calcium buffering. In neurons the mitochondrial network is highly dynamic, undergoing changes in morphology and motility. Mitochondria are actively recruited to specific neuronal compartments, e.g. synaptic terminals, soma, hillock and nodes of Ranvier, reflecting needs for localized energy production and calcium storage. Mitochondrial dysfunction has been implicated in Parkinson's disease. MIRO1/RHOT1 (Ras homolog gene family member T1) belongs to the mitochondrial Rho GTPase family and is involved in neuronal transport of mitochondria. The protein contains two GTPase domains, two calcium binding EF-hands and a transmembrane domain in the C-terminal, locating MIRO1 to the mitochondrial outer membrane. As a mitochondrial Rho GTPase, MIRO1 is suggested to be involved in both anterograde and retrograde transport of mitochondria and their subcellular distribution. We hypothesize that mutations in MIRO1 may lead to disturbed mitochondrial trafficking in neurons and increased risk of neurodegenerative events, such as those observed in Parkinson's disease. This is the first genetic study of MIRO1 in relation to Parkinson's disease. We investigated three different single nucleotide polymorphisms (SNP) in MIRO1; a missense, a frameshift and a synonymous SNP, in an aged-matched Swedish Parkinson case-control material using real time PCR (TaqMan). Two of the SNPs are located in each of the EF-hands and one is located in the GTPase domain. However our data does not suggest any significant association of the investigated SNPs in MIRO1 with Parkinson's disease. A related mitochondrial Rho GTPase protein, MIRO2, is also of interest due to its similar function to MIRO1 and should be equally investigated. In a further step we will study the localization and expression level of these genes at the mRNA level in rodent and human tissue using in situ hybridization and quantitative real time PCR.

## 2097/T/Poster Board #646

**Association studies of 12 candidate SNPs with late-onset Alzheimer's disease.** *L.C. Burns, R.L. Minster, F.Y.K. Demirci, S.T. DeKosky, M.I. Kamboh.* Human Genetics, University of Pittsburgh, Pittsburgh, PA.

Late-onset Alzheimer's disease (LOAD) is a multifactorial disease with the potential involvement of multiple genes. There is a known association between *APOE* gene variants and LOAD. No additional genes have been consistently demonstrated to be associated with risk of LOAD. Multiple recent genome-wide association studies (GWAS) (*Neuron* 2007, 54:713; *Am J Hum Genet* 2008, 83:623; *Am J Hum Genet* 2009, 84:35; *Nat Genet* 2009, 41:192) have found variants showing significant association with LOAD on chromosomes 11, 12 and 14 and on the X chromosome. We examined seven single nucleotide polymorphisms (SNPs) that surpassed genome-wide levels of significance and also selected five additional significant SNPs that lie within reported linkage regions. We genotyped these twelve new significant SNPs based on GWAS as well the E2/E3/E4 *APOE* polymorphism using fluorogenic 5' nuclease assays in up to 1,009 Caucasian Americans with LOAD and up to 1,010 age-matched healthy Caucasian Americans. Our data show no statistically significant associations between the twelve new SNPs examined and the risk of AD. Stratification by *APOE*4* carrier status also failed to reveal statistically significant associations. We have been unable to replicate the published associations from GWAS with our large case-control sample. Further study may be necessary to definitively rule out associations between these variants and LOAD.

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## 2098/T/Poster Board #647

**IQ discrepancy as a quantitative autism endophenotype: strong evidence for loci on chromosomes 10 and 16.** *N.H. Chapman[1], A. Estes[2], J. Munson[2], R. Bernier[2], S.J. Webb[2], J. Rothstein[1], G.D. Schellenberg[6], G. Dawson[5,7], E.M. Wijsman[1,3,4].* 1) Department of Medicine, University of Washington, Seattle, WA; 2) Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA; 3) Department of Genome Sciences, University of Washington, Seattle, WA; 4) Department of Biostatistics, University of Washington, Seattle, WA; 5) Department of Psychology, University of Washington, Seattle, WA; 6) Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia PA; 7) Autism Speaks.

In studies of cognitive abilities of autistic individuals, there is a tendency for performance IQ (PIQ) to exceed verbal IQ (VIQ). This may reflect deficits in language skills that are a key part of the autism diagnosis. The difference between PIQ and VIQ (IQ discrepancy) thus has potential as a quantitative endophenotype in genetic studies of autism. One advantage of IQ discrepancy is that it can be measured on adults, thus providing more reliable parental data than other endophenotypes such as age at first word or phrase. We present the first genetic analyses of IQ discrepancy as an autism endophenotype, in 310 multiplex and mostly Caucasian families, collected as part of the NIH CPEA. IQ discrepancy scores, as measured by short forms of WPPSI-R , WISC-III or WAIS-III, are available on 76% of individuals in these families. Genetic data available includes a genome scan of 387 STRs (85% of individuals), and 10k SNP data (provided by the AGP) in a subset of 187 families. We performed clustering analyses of a discrete IQ discrepancy phenotype "high IQD" (PIQ - VIQ ≥ 15) and demonstrated significant evidence (p=0.0067) that individuals with high-IQD cluster in families. MCMC oligogenic segregation analysis demonstrates that an average of 3.9 QTLs contribute to IQ discrepancy, and identifies three clear models, with heritabilities of 33%, 18% and 20%. Genome-scan analyses utilizing MCMC joint segregation and linkage analysis of the STRs identified 5 chromosomal regions with substantial evidence of linkage. Empirical p-values were calculated for each of the 5 regions, requiring >5 months of CPU time. The regions and empirical p-values are 10p12 (p=0.001), 16q23 (p=0.015), 2p21(0.03), 6q25(p=0.047) and 15q23-25 (p=0.053). The genetic models for the QTLs on chr 10 and 16 clearly correspond to two of the models observed in the segregation analysis. QTDT analysis using 10k SNP data was performed in the regions of the strongest signals on chr 10 and 16. On each chromosome, one SNP achieved significance: on chr 10, 5 cM away from the marker (p=0.009); and on chr 16 immediately under the peak linkage signal (p=0.014). The location of the chr 10 signal coincides with a region noted in the first analysis of the IMGSAC data. The chromosome 16 signal coincides exactly with a linkage signal for non-word repetition in specific language impairment, and involves a region that is home to several cadherin genes.

## 2099/T/Poster Board #648

**Gene expression provides evidence that cell adhesion is involved in the etiology of schizophrenia.** *O. Evgrafov[1], B. Wrobel[2], X. Kang[1], G. Simpson[1], D. Malaspina[3], J. Knowles[1].* 1) Dept Psychiatry, Univ Southern California, Los Angeles, CA; 2) Otolaryngology, Univ Southern California, Los Angeles, CA; 3) Psychiatry, New York University, New York, NY.

We investigated the expression of poly-A RNA isolated from cultured neuronal cells derived from olfactory neuroepithelium (CNON) of 8 patients with schizophrenia (SZ) and 8 control individuals. Human neuroepithelium constantly produces olfactory neurons from neural stem cells and this process is simulated in CNON cultures, which are primarily composed of neuronal progenitors. The tight environmental control provided by cell culture, in comparison to using post-mortem brain samples, provided a greater signal-to-noise ratio and yielded a clear separation of SZ and control samples, in a principle component analysis. In contrast to neurons from adult brain, CNON cells express genes that are specific for development of neural system. Thus, this cellular model may be useful for the identification of genes for neurodevelopmental disorders, including most of the psychiatric disorders. We measured gene expression using Human Exon 1.0 ST Arrays (Affymetrix), preprocessed the data with RMA or GC-RMA followed by Tukey gene summarization. The data were then analyzed by ANOVA (with correction for two batches) using Partek Genomics Suite (Partek). We have also performed RNA-Seq using an Illumina Genome Analyzer II, on a portion of these samples. The ten of the top fifteen most differentially expressed genes with an uncorrected p<0.05 were validated by qPCR, as was the gene with the overall best p-value (CDH11). Genes showed low expression level (<0.001 of ACTB) detected by qPCR or RNA-Seq have been excluded from the further analysis. Most of the differentially expressed genes were expressed at a lower level in the SZ samples, than the controls. The three most differentially expressed genes were CDH6, VCAM1 and S1PR1 (EDG1). Another cadherin, CDH11, was differentially expressed (p<0.05, one-tail t-test), when validated by qPCR. Three of these are cell adhesion molecules, and the fourth, S1PR1, directly regulates cadherin expression and is involved in regulation of VCAM1. Cell adhesion molecules are known to play an important role in brain development and the observation of lower levels of these molecules in the SZ group may point to a possible mechanism for the altered neurodevelopment observed in individuals with schizophrenia. This maybe a general phenomenon of some of the psychiatric disorders as two recent GWASs of autism, another disorder with impaired social functioning, have found association with SNPs located between CDH9 and CDH10.

## 2100/T/Poster Board #649

**Hereditary spastic paraplegia with thin corpus callosum (HSP-TCC) is a frequent phenotype in Brazilian patients with hereditary spastic paraplegia.** *M.C. França Jr, C.M. Lopes, C.V. MAURER-MORELLI, M. Rosa, A. D'Abreu, I. Lopes-Cendes.* Medical Genetics, University of Campinas, Campinas, São Paulo, Brazil.

Objectives: To estimate the frequency of HSP-TCC among Brazilian patients with autosomal recessive HSP (ARHSP) and to investigate causative mutations in SPG11. Background: HSP-TCC has been mapped to chromosome (ch) 15q13-15 in Japanese families and considered to be rare in the west. Design/Methods: 52 ARHSP patients from 23 different families were clinically and radiologically evaluated in order to estimate the prevalence of HSP-TCC. MRI scans were performed in a 2T scanner and sagittal T1 weighted images used to evaluate CC volumes. In addition, we sequenced all exons of the SPACTASIN gene in patients with the HSP-TCC phenotype. Results: Fifteen individuals from 9 unrelated families had HSP-TCC as determined by MRI scans. Their mean age at first examination was 34 years and duration of disease 17.2 years. Gait abnormalities were the initial symptom, followed by slow cognitive decline. Six patients had sensory-motor axonal neuropathy and 6 individuals presented cerebellar atrophy on MRI. We found at least one mutation in exon 4 of the SPACTASIN gene. Conclusion: HSP-TCC was not rare among HSP patients seen at our neurogenetics clinic, representing 39% of all HSP families included in the present study (7/20), to date only one mutation in the SPACTASIN gene was found confirming the presence of genetic heterogeneity associated with HSP-TCC phenotype. Support: FAPESP.

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## 2101/T/Poster Board #650

**Clinical value of *SCN1A* mutation screening in patients with generalized epilepsy with febrile seizures.** *M.C. Gonsales[1], P.M. Preto[2], M.M. Guerreiro[2], I. Lopes-Cendes[1].* 1) Department of Medical Genetics, University of Campinas, Campinas, Brazil; 2) Department of Neurology, University of Campinas, Campinas, Brazil.

Rationale: Mutations in the gene that codes for the $\alpha$-subunit of a neuronal voltage-gated sodium channel (*SCN1A*) have been identified in families with generalized epilepsy with febrile seizures plus (GEFS+). The phenotypic expression of GEFS+ comprises a spectrum of clinical phenotypes including severe syndromes such as myoclonic astatic epilepsy (MAE) and severe myoclonic epilepsy of infancy (SMEI). Molecular studies suggest a common genetic basis for these epilepsies and mutations in the *SCN1A* gene have also been found in SMEI patients. However, the prognostic value of these mutations and a possible correlation with the different clinical subtypes remain controversial. In addition, studies of mutations in candidate genes are important as they provide a better understanding of the physiopathologic mechanisms responsible for epileptic activity. The aim of this study was to search for mutations in the *SCN1A* gene in a group of patients with the GEFS+ spectrum and establish a genotype-phenotype correlation. Methods: Mutation screening was performed in 24 probands, eight of them with SMEI, fifteen with MAE and one with GEFS+. DNA for molecular analysis was obtained from peripheral blood, through the phenol-chloroform extraction method. All 26 exons of the *SCN1A* gene were amplified by polymerase chain reaction (PCR) and analyzed by denaturing high-performance liquid chromatography (DHPLC). Exons for which abnormal chromatographic patterns were observed have been sequenced. Results: To date, 20 *SCN1A* variants were identified, from which five are novel mutations. Three missense mutations (c.829T→C, c.971A→C and c.5434T→C) that lead to amino acid residue substitutions (C277R, H324P and W1812R) were found in patients with the most malignant phenotype of the spectrum, SMEI. According to computational analyses, these substitutions affect the function of the protein. We also found two variants that possibly alter splice donor sites (IVS4+1G→A and IVS8+3G→T) in two patients with SMEI. Both variants may change the structure of the protein and therefore be related with the severity of the phenotype. Conclusions: Potentially deleterious *SCN1A* variants were found only in patients with SMEI. Therefore, our results indicate that in patients with no familial aggregation of epilepsy (sporadic cases) molecular testing for clinical purposes seems to yield best results in patients with the most severe phenotype of the spectrum.

## 2102/T/Poster Board #651

**Association analysis of estrogen receptor (ER) binding polymorphisms in women with major depression.** *L. Graae[1], R. Karlsson[1], C. Zhao[2], S. Paddock[1].* 1) Karolinska Institutet, Neuroscience, Stockholm, Sweden; 2) Karolinska Institutet, Bioscience and Nutrition, Stockholm, Sweden.

Major depression is nearly twice as common in women compared to men with a lifetime prevalence of 21% in women (12.7% in men; Payne, Int Rev Psychiatry, 2003). Several observations suggest a possible involvement of estrogen in the pathobiology of mood disorders in women. For example, periods of hormonal fluctuations or estrogen instability have been associated with increased vulnerability to depression among susceptible women. Intracellular estrogen receptors (ERs) function as transcription factors by binding to specific DNA sequences and regulating gene transcription. Changes in the DNA sequence where the ER-complex binds could possibly affect the binding and lead to abnormal function. This might alter transcription levels of nearby genes and could be part of the disease mechanism. The aim of this study was to investigate if differences exist in ER DNA-binding sequences in depressed vs. non-depressed women. Genome-wide genotype data for 2 276 women (1200 cases and 1076 controls) were obtained from the Genetic Association Information Network (GAIN). Out of 438129 SNPs that survived the quality control analyses, 217 SNPs, found to be placed in ER DNA-binding regions, were selected for the association analysis. ER DNA-binding regions were obtained from the literature (Carroll et al. 2006, Lin et al. 2007 and unpublished data from Zhao et al. at the Department of Bioscience and Nutrition, KI, Sweden). If a SNP placed in these regions was not directly genotyped by GAIN, a SNP in high LD (r2>0.89) with the SNP of interest was chosen instead, where available. No single SNP was significant after Bonferroni correction. The p-value for the top SNP was 0.00318. This SNP is located in PITPNC1, a gene involved in regulation of the the phosphatidyl inositol pathway, which has earlier been implicated in the pathogenesis of affective disorders. Lithium and other mood stabilizing drugs, for example, are suggested to work regulatory on this pathway. One explanation for not finding a significant association in this study is that variation in ER binding sequences lacks relevance for the disease mechanism. Another reason could be that the sample size was too small. Finally, since for some interesting SNPs, neither the SNP itself nor any SNP in high LD was genotyped in the GAIN study, we may have missed true associations. We are now carrying out imputation of those missing SNPs in order to obtain more information from the available sample.

## 2103/T/Poster Board #652

**Association Studies of Autism Spectrum Disorders in Populations of European Ancestry.** *R. Holt[1], I. Sousa[1], E. Bacchelli[2], D. Brocklebank[1], E. Maestrini[2], G. Barnby[1], A.J. Bailey[3], A.P. Monaco[1], EU Autism MOLGEN Consortium.* 1) WTCHG, Univ Oxford, Oxford, United Kingdom; 2) Dipartimento di Biologia, Università di Bologna, Bologna, Italy; 3) University Department of Psychiatry, Park Hospital for Children, Oxford, United Kingdom.

Over the past decade, research on the genetic variants underlying autism and autism spectrum disorder (ASD) susceptibility has focused on two main lines of research; linkage and candidate gene studies. The vast amount of effort expended has resulted in a variety of chromosomal loci and genes being implicated in susceptibility to these disorders. Candidate gene studies have proved particularly intractable, with many studies failing to replicate previously reported associations. In a previous study of the genetics of ASD, we used four sets of samples of European ancestry; the International Molecular Genetics Study of Autism Consortium (IMGSAC) and Paris Autism Research International Study (PARIS) cohorts, and populations from Finland and the north of the Netherlands. Genotyping was performed using two 384 SNP Illumina GoldenGate® arrays to examine linkage regions and candidate genes implicated in ASD. Our results showed suggestive evidence of linkage on chromosome 2 in a subset of populations, and evidence of association to *RELN* and *GRIK2* across the entire sample. Here, we use the SNPs from the linkage array to perform association analysis in two relatively isolated European cohorts from Finland and the north of the Netherlands. Association analysis of these SNPs revealed significant associations in *MKL2* to autism and ASD (rs756472, autism $P = 2.46 \times 10^{-5}$, ASD $P = 4.31 \times 10^{-5}$) and *SND1* to autism (rs1881084, $P = 7.76 \times 10^{-5}$) in the Finnish and North Dutch cohorts, respectively. In addition, we have taken the five most strongly associated SNPs from our previous candidate gene study and genotyped them in an additional 470 trios from the IMGSAC and Northern Dutch cohorts. No significant association was identified in this replication cohort. However, when the replication data was combined with our original results to produce a total cohort of 848 trios an increased association to *RELN* (rs362780, $P = 0.001$) and marginally reduced association to *GRIK2* (rs2518261, $P = 0.008$) was identified. Therefore, our results both continue to highlight the difficulties in studying the genetics of autism, strengthen the case for further study of the role of *RELN* and *GRIK2* in ASDs, and identify two new potential candidate genes, *MKL2* and *SND1*.

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## 2104/T/Poster Board #653

**Variants of the transcription factor gene *GATA2* and the GATA-2 target region in α-synuclein (*SNCA*) are associated with Parkinson's disease (PD).** *D.M. Kay[1], J. Montimurro[1], M.G. Schlossmacher[2], E.H. Bresnick[3], C. Stevens[1], C.P. Zabetian[4,5], S.A. Factor[6], D.S. Higgins[7], C.R. Scherzer[8,9], H. Payami[1,9].* 1) Division of Genetics, Wadsworth Center, New York State Department of Health, 150 New Scotland Ave, Albany, NY, US; 2) Division of Neurosciences - OHRI, University of Ottawa, 451 Smyth Road #1462, Ottawa, Ontario, Canada; 3) University of Wisconsin School of Medicine, 383 Medical Sciences Center, 1300 University Avenue, Madison, WI, US; 4) Department of Neurology, School of Medicine, University of Washington, Seattle, WA, US; 5) Geriatric Research Education and Clinical Center, VA Puget Sound Health Care System, Seattle, WA, US; 6) Department of Neurology, Emory University School of Medicine, Atlanta, GA, US; 7) Parkinson's Disease and Movement Disorder Center, Albany Medical Center, Albany, NY, US; 8) Laboratory for Neurogenomics, Center for Neurologic Diseases, Harvard Medical School and Brigham & Women's Hospital, 65 Landsdowne Street, Suite 307A, Cambridge, MA, US; 9) equal contributors.

**Background** Increased *SNCA* dosage due to locus multiplication causes autosomal dominant PD and variation in *SNCA* expression may be critical in common, genetically complex PD. By combining gene expression analysis in humans with genetic complementation and gene silencing in cells, we discovered that GATA transcription factors regulate *SNCA* expression. Endogenous GATA-2 is highly expressed in PD-vulnerable substantia nigra, occupies a conserved region within *SNCA* intron 1, and modulates *SNCA* expression in dopaminergic cells. Here we identify variants in *GATA2* and the GATA-2 binding site region in *SNCA* that are associated with PD. **Methods** Subjects included 1971 cases and 2111 controls from NeuroGenetics Research Consortium. Six *GATA2* tagSNPs were genotyped in 602 cases and 631 controls, tested for association, and two promising SNPs were genotyped in the remaining subjects. *SNCA* intron 1 had not been studied in Caucasians; we therefore sequenced 602 cases and 583 controls, tested the identified SNPs and haplotypes for association with PD, and genotyped the remainder of samples for the promising SNPs. **Results** Two *GATA2* SNPs were associated with early onset and familial PD (all $p<0.01$). The two SNPs were not in strong linkage disequilibrium (LD); their effects on PD seemed independent. In *SNCA*, we identified 18 variants. The 11 rare variants were found in cases and controls. Among the 7 polymorphisms, 3 were significantly associated with PD. Haplotype analysis revealed that one SNP was the primary PD-associated variant, and the other two were hitchhiking via LD. The association of this SNP with PD was robust and highly significant in all PD ($p<10^{-5}$), and categories by age at onset (early $p=0.05$, late $p<10^{-4}$) and family history (positive $p=0.002$, negative $p=10^{-5}$). Risk conferred by intron 1 is not the same as risk associated with REP1. There was no evidence for statistical interaction (synergy) between *SNCA* and *GATA2*; their effects on PD risk were additive. In early-onset PD, where *GATA2* has notable effect, the largest odds ratio (OR) for either locus was ~1.4. Considered jointly, OR was 2.0 to 2.5 (p=0.001-0.005). **Conclusion** In a case-control study of 4082 subjects we find a substantial and novel association between polymorphisms in *GATA2* as well as the GATA-2 binding site region in *SNCA* and PD. Consistent with these results, variation in *SNCA* expression regulated by transcription factor GATA-2 may confer susceptibility to common, genetically complex PD.

## 2105/T/Poster Board #654

**Pleiotropic effects of the 11p13 locus on speech dyspraxia and susceptibility to Rolandic epilepsy.** *D.K. Pal[1], L.J. Strug[2], T. Clarke[1], P. Lieberman[3].* 1) Columbia University Medical Center, New York, NY, USA; 2) University of Toronto and Hospital for Sick Children, Toronto, Canada; 3) Department of Cognitive and Linguistic Sciences, Brown University, Providence, RI, USA.

Introduction. Speech sound disorder (SSD) is a common developmental comorbidity in children with Rolandic epilepsy, which is a developmental epilepsy syndrome with speech arrest and electroencephalographic (EEG) discharges emanating predominantly from the perisylvian cortex. The EEG discharges were recently mapped to variants in Elongator Complex Protein 4 (ELP4). We aimed to determine what mechanism of SSD operates in RE patients, and whether this SSD mapped to the same 11p13 locus as the EEG. Methods. We used computerized digital analysis of recorded speech to evaluate vowel duration, voice-onset time and other speech motor parameters. We performed parametric linkage analysis on 38 families, genotyped at 48 STR markers spaced at 4cM across chromosome 11 using the combined affectedness status of EEG/SSD. Results. Acoustic analysis revealed clear evidence of speech dyspraxia in all SSD affected individuals. In two-point linkage analysis, the maximum LOD score for EEG/SSD was 4.61 at D11S4102, the same marker at which we had found the maximum LOD score for EEG alone (EEG alone, LOD=3.4; SSD alone, LOD=0.83); in multipoint analysis, the highest multipoint LOD score was 7.54 at D11S914, again at the same marker where we found the maximum multipoint LOD score for the EEG (EEG alone, LOD=4.3), and with no heterogeneity. Discussion. We present evidence for speech dyspraxia underlying SSD in RE families, implicating impairment of cortico-striato-cortical tracts. Moreover, the linkage results suggest the hypothesis that ELP4 variants may have pleiotropic effects on speech development and susceptibility to epilepsy in children.

## 2106/T/Poster Board #655

**Clinical characterization and mapping of a late-onset painful recessive sensory neuropathy.** *M.L. Putorti[1,2], M. Srour[1,2], J. Mathieu[3], B. Bernard[1,2,3].* 1) Laboratoire de neurogénétique et motricité, CRCHUM Hôpital Notre-Dame, Montréal, QC, Canada; 2) Neuromics Center for Excellence of Université de Montréal, Université de Montréal, CRCHUM Hôpital Notre-Dame, Montréal, QC, Canada; 3) Carrefour de la Santé de Jonquière, Saguenay, QC, Canada.

Late-onset sensory neuropathies are usually believed to be acquired or sporadic. We have identified 16 cases belonging to 9 French-Canadian families that present on average at age 48 (27-64) with a painful sensory polyneuropathy that evolves into a sensory ataxia. Initial electrophysiological studies are not always significantly abnormal but progress to a severe axonal sensory, and to a lesser extend motor, peripheral neuropathy. The extent of the sensory loss can be quite profound in elderly patients with complete loss of position and vibration senses in extremities. The presence of one case with consanguineous parents, the equal male to female ratio (7 females/ 9 males), the absence of history of affected parents, and the 16/45 ratio of affected to unaffected cases supported a recessive mode of transmission. Considering that many of our families originated form the Saguenay-Lac Saint-Jean region of Quebec well known for its recessive founder effects, we completed a SNP genome wide scan using the Illumina Human610-Quad BeadChip. We uncovered one region of 1,1Mb shared by many of our families. Linkage using STS markers established that our largest families were mapped to this region with a maximum LOD score of 5,2. This study is the first to uncover a recessive locus responsible for a late-onset recessive painful neuropathy that evolves into a sensory ataxia.

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## 2107/T/Poster Board #656

**LRRK2 variation and risk of Parkinson's disease.** *O. Ross, Genetic Epidemiology of Parkinson's Disease Consortium.* Dept Neuroscience, Mayo Clinic, Jacksonville, FL.

Leucine-rich repeat kinase 2 (LRRK2) mutations were first identified to cause familial late-onset parkinsonism, and showed idiopathic Parkinson disease (PD) may have a major genetic component. The identification of the Lrrk2 G2019S subsequently ignited the field due to its relatively high frequency in familial and seemingly sporadic PD. Overall Lrrk2 p.G2019S is found in 10 to 30% of PD patients of Ashkenazi Jewish or North African Berber ethnicity and increases risk by approximately 22 fold, the age-associated incidence of PD in carriers being over 80% at 70 years. Recently we and others identified the first two common ancestral variants acting as a 'risk-factors' for PD; LRRK2 pG2385R and pR1628P, that appear specific to Asian populations and combined may contribute to risk in over 10% of patients. To date over 75 non-synonymous LRRK2 variants have been identified through out the coding sequence of the gene. In addition there are over 30 synonymous LRRK2 coding SNPs that may influence LRRK2 transcription regulation or affect the rate of Lrrk2 protein translation, subsequent folding and protein-protein interactions. However pathogenicity, prevalence and penetrance of many remain to be determined and their given frequencies combined also indicate the potential for compound inheritance. We are genotyping over 100 exonic LRRK2 variants through the combined GEO-PD consortium patient-controls series (8,000 subjects). We have observed a number of variants that are common between patients and controls, and variants that appear to only be present in patients suggestive of pathogenicity. We will present our results which will help elucidate the role of rare LRRK2 variants in PD risk, inform diagnostic testing and future therapeutic strategies.

## 2108/T/Poster Board #657

**Association of SNPs in the CHRNA5/CHRNA3/CHRNB4 Gene Cluster with Conduct Disorder-Related Phenotypes.** *S.H. Stephens, M.B. McQueen, S.E. Young, J.K. Hewitt, M.A. Ehringer.* Institute for Behavioral Genetics, Integrative Physiology Dept, Univ Colorado, Boulder, CO.

Work from several groups provides convincing evidence for an association between several of the nAChR genes and alcohol and nicotine behaviors in humans, including lung cancer (Bierut et al, 2007; Schlaepfer et al, 2008; Wang et al, 2008; Amos et al, 2008). This work has been based on evidence that common genetic factors contribute to alcohol, nicotine, and substance use disorders, particularly in adolescence. Some of this drug use co-morbidity may be a manifestation of a more general underlying trait, characterized by a range of disinhibitory behaviors such as conduct disorder (CD). Recently, we have shown evidence for association between CD-related phenotypes and SNPs in the CHRNA5/A3/B4 gene cluster in two separate samples, as well as evidence that two SNPs in the region may lead to differences in gene expression (Schlaepfer et al, under revision). In the current study, we have examined the same SNPs in the CHRNA5/A3/B4 genes with CD-related phenotypes in a third sample, the National Longitudinal Study of Adolescent Health (Add Health). The genetic pairs subsample of the Add Health project includes approximately 2500 subjects which have been assessed at three waves during adolescence and young adulthood. The software package PBAT (Laird and Lange, 2006) was used to carry out family-based association tests on six CD-related measures across Waves I, II and III. Results of genetic association analyses in the Caucasian sample revealed evidence for association between CD-related phenotypes and SNPs rs16969968 ($P=0.038$) and rs8040868 ($P=0.009$). SNP rs16969968, located in exon 5 of CHRNA5, has been associated with nicotine dependence, lung cancer, and cocaine dependence. SNP rs8040868, located in exon 2 of CHRNA3 has shown suggestive evidence for association with CD-related phenotypes in a separate sample population (Schlaepfer et al, under review). Ongoing work using gene expression assays to further characterize the functional importance of additional SNPs in this region are in progress, including examination in lung cancer cell lines. Our results support the hypothesis that allelic variants in the CHRNA5/CHRNA3/CHRNB4 contribute to an underlying genetic predisposition to disinhibitory behaviors such as conduct disorder. These phenotypes are important precursors to substance use disorders, thereby providing additional insight into the possible role of these genes in the developmental trajectory of these disorders.

## 2109/T/Poster Board #658

**Polymorphism detection in gene GRIN 1 of ionotropic glutamate receptor activated by NMDA of a population from Bogota.** *I. Zarante[1], L. Lareo[2], P. Ayala[1], D. Ojeda[1].* 1) Instituto de Genetica Humana, Facultad de Medicina,Pontificia Universidad Javeriana, Bogota, Colombia; 2) Departamento de Nutricion y Bioquimica, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogota, Colombia.

The ionotropic glutamate receptor activated by N-methyl-D-aspartate is composed by three different kinds of subunits NR1, NR2A to D and NR3A and B, which are codified by GRIN1, GRIN2 and GRIN3 genes. Since the GRIN`s genomic variability is closely related to the genetic history of the studied population, it was necessary to develop a detailed study of the frequency of the polymorphisms of the gene GRIN-1 in Colombian population. The main goal of this research was identifying polymorphisms present in 5'-UTR region and Exon 6 of gene GRIN-1, among 101 samples of umbilical cord taken on filter paper of healthy newborns at the University Hospital San Ignacio in Bogota. It was found that polymorphism A1970G with minor allele frequencies of 28.21%, doesn't differ significantly from the frequencies in Caucasian and Native American populations. Polymorphism G1140A with minor allele frequencies of 1.49% did not show any significant statistic difference with the Taiwan population. Polymorphism A1160G just showed one allelic form, the A allele.

## 2110/T/Poster Board #659

**Single nucleotide polymorphisms within D1 and D2 dopamine receptor genes are associated with heroin abuse and striatal gene expression.** *M.M. Jacobs[1], E. Keller[2], Y.L. Hurd[1].* 1) Departments of Psychiatry and Pharmacology & Systems Therapeutics, Mount Sinai School of Medicine, New York, NY, USA; 2) Department of Forensic Medicine, Semmelweis University, HU 1091, Budapest, Hungary.

Drug addiction is a complex disorder with genetic factors accounting for approximately 40-60% of overall vulnerability. Vulnerability to heroin abuse appears to have one of the strongest genetic loads compared to abuse of other addictive substances. Converging lines of evidence obtained from rodent and human brain studies have identified discrete components of cortico-striato-thalamo-cortical circuits as linked to discrete behavioral traits implicated in addiction. The corticostriatonigral circuit constitutes the "Go" pathway that facilities behavioral responses and reward sensitivity, whereas the corticostriatopallidal circuit constitutes the "No-Go" pathway involved in suppressing inappropriate responses in normal adults. The striatonigrial and striatopallidal pathways are distinct in their gene expression pattern with preferential expression of dopamine D1 (DRD1) receptors in the striatonigral pathway and dopamine D2 (DRD2) receptors in the striatopallidal pathway. Using a homogenous European Caucasian population consisting of 81 heroin abusers and 46 age-matched controls, we examined 4 single nucleotide polymorphisms (SNPs) of DRD1 and DRD2. Alleles of 1 SNP within DRD1 and 3 SNPs within DRD2 are significantly associated with heroin abuse ($P<0.05$). Additionally, expanding these analyses to haplotypes containing associated alleles improves association; a 3-marker haplotype of DRD1 and a 4-marker haplotype of DRD2 are significantly associated with heroin abuse ($P<0.01$ and 0.04, respectively). To examine DRD1 and DRD2 gene expression, we purified RNA from the putamen in a subset of these individuals and performed quantitative PCR analysis. We observed that these associated polymorphisms of DRD1 and DRD2 are significantly associated with altered gene expression in heroin abusers. Future studies will examine gene expression and genetic polymorphisms of additional genes specific to striatonigral and striatopallidal circuitry to determine their contribution to addiction vulnerability.

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## 2111/T/Poster Board #660

**De novo mutations in autism affected subjects from a large cohort screening of polymorphisms in genes IL1RAPL1 and IL1RAPL2 by pooling and next generation sequencing.** *J.J. Pruzin, J.J. Corneveux, H.L. Benson, J.L. Dinh, J.S. Beckstrom, S.M. Beckstrom-Sternberg, S.D. Mastrian, J.V. Pearson, M.J. Huentelman.* Neurogenomics, Translational Genomics Research Institute, Phoenix, AZ.

Though a strong genetic component is proven to predispose a person to Autism Spectrum Disorders (ASD), the search for specific risk factors has been largely unsuccessful. This possibly results from the heterogeneity of the disorder both phenotypically and genetically. Perhaps an accumulation of a menu of rare and common variants can result in ASD. We devised a method to sequence large numbers of people with the capability of detecting both rare and common polymorphisms and chose the previously ASD linked gene Interleukin-1 Receptor Accessory Protein-Like 1 (IL1RAPL1), involved in calcium-regulated vesicle release and dendrite differentiation, on which to try the method. In this study 250 individuals were screened for exonic changes in both IL1RAPL1, and related gene IL1RAPL2, by pooled sequencing on the Illumina Genome Analyzer II. Five equimolar pools of 50 people each were created and enriched for the two genes of interest by PCR. The PCR products were recombined in equimolar ratios and sequenced. Coverage averaged from 856X to 1538X depending on pool. A minimum of 500X coverage with a deviation from the reference at a prevalence of 5% or greater was used to determine possible polymorphisms. A total of 12 polymorphisms in IL1RAPL1, found at a prevalence of 6% to 25%, were identified for verification. The two most functionally relevant changes are at positions chrX:29,882,707 and chrX:29,882,709 (UCSC version hg18) causing changes of Asp to Val and Arg to Gly respectively. Both polymorphisms are located in the Toll-interleukin 1 Receptor (TIR) domain, implicated in protein-protein interactions and thought to be important to the signal transduction pathway. IL1RAPL2 yielded twenty polymorphisms at prevalences of 6% to 43%, nine of which are located in the untranslated region. One mutation present in 2 or 3 people, located at chrX:104,848,086 (UCSC version hg18), results in a premature stop codon on exon 7 and the loss of half of the Ig-like C2-type 3 portion of the topological domain and entire TIR, and transmembrane regions of the protein.

## 2112/T/Poster Board #661

**Exploratory sequence analysis of candidate genes for repetitive and restricted behaviors in autism.** *E.L. Crawford[1], E. Kistner[2], N.J. Cox[2], E.H. Cook[3], J.S. Sutcliffe[1].* 1) Centers for Molecular Neuroscience and Human Genetics Research, Dept of Mol Phys & Biophys, Vanderbilt University, Nashville, TN; 2) Department of Medicine, University of Chicago, Chicago, IL; 3) Institute for Juvenile Research, Department of Psychiatry, University of Illinois-Chicago, Chicago, IL.

Autism is a neurodevelopmental disorder that affects approximately 1 in 150 individuals and is characterized by deficits in reciprocal social interaction, communication and patterns of repetitive behaviors and restricted interests. Twin and family studies indicate high heritability, but evidence supports a highly complex architecture for the underlying genetic etiology. Serotonin dysregulation has long been implicated in autism, and rare autism-associated variants in the serotonin transporter (SERT; gene symbol: SLC6A4) lead to gains of function involving increased activity and abnormal regulation. In autism families, these SERT variants result in more severe rigid-compulsive behaviors, but one has also been observed in multiple pedigrees with obsessive compulsive disorder (OCD). Selective serotonin reuptake inhibitors (SSRIs) that selectively target SERT are a key front-line therapy for OCD. Thus, SERT variants and SSRI treatment efficacy highlight a shared etiology between autism and OCD involving serotonin regulation via SERT. We are pursuing related hypotheses that genes and/or pathways related to SERT regulation and SSRI-responsive OCD behaviors harbor autism susceptibility alleles. In this study, we begin testing these hypotheses through a sequencing-based variant discovery paradigm in unrelated autism probands and controls for two loci, DLGAP3 and ADAMTS6. DLGAP3 is also known as SAPAP3 (SAP90/PSD95-associated protein 3), and mutations have been described in cases of OCD and trichotillomania, and a mouse knock-out shows analogous behaviors that are responsive to treatment with SSRIs. ADAMTS6 encodes a disintegrin and metalloproteinase with thrombospondin motifs 6, and this locus was identified based on its containing SNPs that significantly predict variation in gene expression of SLC6A4. Exonic sequences for these two genes have been sequenced in a pilot sample of 200 cases and controls. Multiple synonymous and nonsynonymous variants have been detected in both loci. While data at this time do not permit a conclusion that either of these genes shows a greater mutation burden in autism vs. controls, the current study is under-powered to detect particularly rare variants and modest differences in numbers of apparently damaging sequence variants in cases vs. controls. We conclude that further study may be warranted for both of these genes.

## 2113/T/Poster Board #662

**Genetic and cellular localization studies of the Protein Kinase AKT1 in Parkinson's disease.** *C. Ran[1], M. Westerlund[1], A. Anvret[1], C. Lind[2], T. Willows[3], O. Sydow[2], D. Galter[1], A. Carmine Belin[1].* 1) Department of Neuroscience, Karolinska Institutet, Retzius väg 8, 171 77 Stockholm, Sweden; 2) Department of Neurology, Karolinska University Hospital Solna, 171 76 Stockholm, Sweden; 3) Department of Neurology, Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden.

AKT1 (V-akt murine thymoma viral oncogene homolog 1) is one of three genes in the Akt/PKB protein kinase family. This family has a broad spectrum of substrates implicated in multiple signaling pathways. They are known to regulate cellular growth, proliferation, metabolism, transcriptional activity, and in particular, cell survival. AKT1 promotes survival by regulating several important mediators of apoptosis and cell survival, as well as expression of pro-survival and pro-apoptotic genes. AKT1 is fully activated when phosphorylated and once activated, it dissociates from the membrane and is translocated to the cytoplasm or nucleus to phosphorylate its substrates. As a potent mediator of cell survival, AKT1 has been associated with several forms of human cancers, but can also be linked to other pathologies. Notably, AKT1 seems to be associated with neurological disorders of the dopamine system. AKT1 is localized to chromosome 14q32.32 and this locus has been linked to bipolar disorder. Furthermore, several single nucleotide polymorphisms (SNP), low expression levels as well as reduced enzyme activity of AKT1 has further been found to associate with schizophrenia. Recently, the AKT1 pathway was suggested to play a protective role in Parkinson's disease (PD). In 2008, Xiromerisou et al. discovered a haplotype more frequently occurring in healthy controls than in PD patients in a Greek case-control material. AKT1 has also been found to show lower levels of phosphorylation in dopamine neurons of PD patients. We are investigating the protective haplotype reported by Xiromerisou et al. in a Swedish PD case-control material using pyrosequencing. 315 controls and 243 PD patients were genotyped for four SNPs: rs1130214 in the 5'UTR and rs2494743, rs2498788, rs2494746 in intron 3. We did not find a significant association for any of the investigated SNPs with PD. A haplotype analysis did not reveal any haplotypes associated with an altered risk of developing PD. To study the cellular expression pattern of AKT1 in rodent and postmortem human tissue, we used radioactive in situ hybridization and oligonucleotide probes designed to hybridize with AKT1 mRNA. Moreover, we compared the expression levels of AKT1 mRNA in postmortem tissue from PD patients and controls in order to study the possibility of Akt1/PKB$\alpha$ exerting a protective effect on neurodegenerative diseases.

## 2114/T/Poster Board #663

**Copy number variation at the spinal muscular atrophy locus and susceptibility to autism.** *I. Cusco[1], C. Aguado[1], LA. Pérez-Jurado[1,2].* 1) Unitat de Genètica, Univ Pompeu Fabra & Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain; 2) Programa de Medicina Molecular i Genètica, Hospital Vall d'Hebron, Barcelona.

Autism spectrum disorders (ASD) are a group of neurodevelopmental disorders characterized by impairments in reciprocal social interaction and communication skills, restricted interests and stereotyped behaviors, with a recently established prevalence as high as 1 in 150 newborns. There is strong evidence for a genetic etiology of ASD given the 90% concordance rates in monozygotic twins and the ~50 fold increased risk for siblings. ASD is found in association with comorbid genetic conditions in 10% of cases and can be caused by several rare monogenic mutations and submicroscopic rearrangements, but it is mainly considered a complex multifactorial disorder involving multiple genes. However, the etiology of ASD in the majority of patients remains unknown and common susceptibility factors have not been identified yet. Here we report a novel genetic susceptibility factor at the highly variable 5q13.2 Spinal Muscular Atrophy locus showing strong association with the disorder. We detected a significantly different pattern of deletions and duplications at two genes (GTF2H2 and SMN2) in Spanish ASD patients (n=215) compared with controls (n=453). Data were replicated using a small subset of the AGRE consortium cohort (85 cases). Overall, individuals with gain of GTF2H2 copies and deletion of the SMN2 gene showed an increased risk for ASD with odds ratio of 14.77 (range 5.89 - 44.75, p= 8.93e-13). The analysis of 24 trios revealed that most SMN2 losses were inherited from either parent, while the GTF2H2 gains could be de novo in ~2/3 ASD probands. Copy number changes showed a strong correlation with gene expression levels by quantitative RTPCR and western blotting in lymphocytes. GTF2H2 coding for the TFIIH2 subunit of the RNA polymerase transcription initiation factor IIH involved in basal transcription and nucleotide excision repair, and SMN2 coding for the survival of motor neuron protein (SMN) involved in splicing regulation and SMN$\Delta$7 with unknown function, are therefore novel ASD candidates. Our findings open new directions to investigate the pathogenesis of the ASD phenotype and suggest that other genetic susceptibility factors for complex disorders may locate in highly instable genomic regions poorly tagged by current genotyping methods.

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## 2115/T/Poster Board #664

**Variants in and around *DPYSL2* confer sex-specific risks for schizophrenia.** *Y. Liu[1], P. Chen[1], V. Lasseter[2], D. Fallin[3], J. McGrath[2], P. Wolyniec[2], G. Nestadt[2], K. Liang[4], A. Pulver[2], D. Avramopoulos[1], D. Valle[1].* 1) Institute of Genetic Medicine,Johns Hopkins University School of Medicine Baltimore, MD; 2) Department of Psychiatry, Johns Hopkins University School of Medicine Baltimore, MD; 3) 3Department of Epidemiology,Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 4) 4Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

Schizophrenia (SZ) is a common disabling disorder typically with onset in young adults and multiple sex-specific phenotypic differences: females make up only ~30% of the cases; have later age of onset; higher familiarity; and, a somewhat different presentation (more affective symptoms). Association studies from multiple groups including our own have implicated *DPYSL2* (8p21, 26.5Mb), which encodes a cytosolic protein that regulates axonal growth, as a candidate gene for SZ. Recently, in a SNP fine-mapping study covering 4Mb on 8p21, we identified significant association with SNPs in intron 3 (rs5029306, p<0.0005) and 5Kb 3' (rs12155555, p<0.0001) of *DPYSL2* in an Ashkenazi Jewish (AJ) population. To find additional potential causative variants on the risk haplotype specified by these variants, we sequenced all 14 exons and 21 conserved non-coding sequences (cNCSs) in and around *DPYSL2* in 41 AJ SZ probands and 6 controls. We identified 86 SNPs in total and based on frequency, location and conservation, selected 5 non-coding SNPs and one 11-bp indel for follow-up genotyping in 729 AJ SZ (465 males, 264 females) and 1542 AJ controls. We also included one SNP (rs17088251) ~7kb 3' of *DPYSL2*, on the basis of its association in a genome wide study, ranking in the top of 0.06% for AJ women published by Shifman, et.al, 2008. Of the 7 variants, 4 showed no difference between cases and controls. In agreement with Shifman et.al., rs17088251 showed a case-control difference in females only, (p<0.05). Another SNP in the region, rs5029306, showed a stronger female specific association alone (p= 0.0017) and in a haplotype with rs17088251 (p=0.0013). More strikingly, 3 variants in the gene-free region 3' of *DPYSL2* showed a significant association specific to males both individually ($p<10^{-4}$-$10^{-5}$) and as a three SNP haplotype ($p<10^{-5}$). These variants and their distance beyond the 3' end of *DPYSL2* are: rs57045236, ~1kb; rs12155555, ~5kb and an unannotated SNP (un26633562), ~62Kb. In summary, our results: 1) replicate a female-specific risk of rs17088251 in AJ; 2) identify a SZ association with two haplotypes, one in the body of *DPYSL2*, which is female-specific and the other in 3' of *DPYSL2*, which is male-specific. These results suggest that variants in a single SZ candidate gene confer risk for SZ in multiple sex-specific ways, perhaps by cis-acting estrogen/androgen response elements in and around *DPYSL2*.

## 2116/T/Poster Board #665

**Fine mapping of AHI1 on 6q23 as a susceptibility gene to schizophrenia.** *F. Torri[1], A. Akelai[2], S. Lupoli[3], M. Sironi[4], D. Amann-Zalcenstein[5], M. Fumagalli[4], R. Cagliani[4], C. Dal Fiume[1], E. Ben-Asher[5], K. Kanyas[2], D. Lancet[5], P. Cozzi[6], E. Osimo[1], L. Strik Lievers[1], E. Salvi[1], A. Orro[6,7], J. Beckmann[8], B. Lerer[9], F. Macciardi[1,10].* 1) University of Milan, Genomics and Bionformatics Platform, Fondazione Filarete, Milan, Italy; 2) Hadassah-Hebrew University Medical Center, Jerusalem, Israel; 3) INSPE, Scientific Institute San Raffaele, Milan, Italy; 4) Scientific Institute IRCCS E. Medea, Bosisio Parini (LC), Italy; 5) Weizmann Institute of Science, Rehovot, Israel; 6) ITB, CNR, Milan, Italy; 7) CILEA Consortium, Segrate, Milan, Italy; 8) University of Lausanne, Lausanne, Switzerland; 9) Miller School of Medicine, University of Miami, Miami, FL, United States; 10) Dept of Psychiatry and Human Behavior, UCI, Irvine, CA, USA.

Schizophrenia (SCZ) is a multifactorial disorder where probably multiple genes of small to moderate effect act in combination also with environmental factors to increase the risk of illness. In previous studies, using a set of Arab-Israeli families, we identified by linkage and association mapping a susceptibility region to SCZ on the long arm of chromosome 6 (6q23.3)(1,2,3); this association was also replicated in an independent sample. The peak region includes the Abelson Helper Integration Site 1 (AHI1) and a putative gene for a human-specific hypothalamic mRNA BC040979. Here we densely map the most probable candidates in this area, performing a finer mapping of the originally identified linkage peak. The strongest single SNP and haplotype association lies within a 500 kb genomic region (135.6-136.1 Mb) encompassing the AHI1 gene and the BC040979 locus, supporting the role of AHI1 as a susceptibility gene to SCZ. Interestingly the second highest significant subregion is immediately downstream and includes PDE7B and MAP7 genes. Resequencing of a 10kb region within AHI1 in ethnically defined populations suggested that the gene has undergone a selective sweep in Europeans, in agreement with previous studies (4,5). Network analysis indicated the presence of two haplotype clades, with SCZ-susceptibility haplotypes clustering within the major clade. REFERENCES: 1)Lerer B. et al.,Mol Psychiatry. 2003 May;8:488-98. 2)Levi A. et al., Eur J Hum Genet. 2005 Jun;13:763-71. 3)Amann-Zalcenstein D. et al., Eur J Hum Genet. 2006 Oct;14:1111-9. 4) Ferland RJ et al., Nat Genet. 2004 Sep;36:1008-13. 5)Barreiro LB et al., Nat Genet. 2008 Mar;40:340-5.

## 2117/T/Poster Board #666

**Overexpressed Cyclophilin B Protectes beta Amyloid(25-35)-Induced Oxidative Stress in PC12 cell death via Reative Oxygen Species.** *J. Kwon, E. Kim, W. Choe.* Department of Biochemistry and Molecular Biology, Medical Science and Engineering Research Center for Bioreaction to Reactive Oxygen Species, Biomedical Science Institute, School of Medicine, Kyunghee University, Seoul 130-701, Korea.

Alzheimer's disease (AD) is characterized by neuronal loss and extracellular senile plaque, whose major constituent is β-amyloid protein (Aβ), a 3943 amino acid peptide derived from amyloid precursor protein. Aβ-induced neurotoxicity has been attributed in various studies to Ca2+ influx and generation of ROS. The previous studies confirmed that Aβ (2535) caused elevation of [Ca2+]c, glutamate release, ROS generation and then apoptotic neuronal cell death.

Neuronal apoptosis is one of the pathologies of Alzheimer's disease, and it has been hypothesized to be induced, at least in some degree, by beta amyloid (Aβ) protein. The mechanism by which Aβ induces cell death is not clear yet. However, it has been suggested one of the mechanisms is oxidative stress. Beta-amyloid (Aβ)-induced neurotoxicity is mediated through the generation of reactive oxygen species (ROS) and elevation of intracellular calcium. Cyclophilin B belongs to protein groups that have peptidyl-prolyl cis-trans isomerase activity (PPIase). Recent studies have shown diverse functions of cyclophilin family, and we suggest that cyclophilin B might play an antioxidant. The purpose of this study is whether overexpressed

cyclophilin B reduces Aβ -induced oxidative stress or not. To determine the effect, we used rat pheochromovytoma (pc12) cells and performed MTT assay to check the cell viability. Exposure of PC12 cells to the Aβ25-35 fragment resulted in apoptotic cell, while PC12 cells which are overexpressed cyclophilin B reduced Aβ -induced cell death. Our results suggest that overexpressed cyclophilin B has a protective effect against the Aβ-induced oxidative damage and eventually inhibited apoptotic cell death.

## 2118/T/Poster Board #667

**Late-Life Depression Is Heritable Among Old Order Amish.** *M. Slifer[1], W. Scott[1], P. Gallins[1], M. Pericak-Vance[1], J. Haines[2].* 1) MIHG, Univ Miami, Miami, FL; 2) CHGR, Vanderbilt University, Nashville, TN.

Little is known about the heritability of late-life depression. The environment plays a significant role in the development of depressive symptoms at all ages. However, the magnitude of effects of specific environmental stressors varies widely among published reports. Environmental exposures may be less heterogeneous among Amish populations. As a result, the Amish may be informative for discovery of genetic effects on traits like depression that exhibit important but difficult to capture and characterize environmental interactions. In this study, we use complete pedigree information from a set of old-order Amish participants to determine the heritability of late-life depressive symptoms. Since cognitive impairment is confounded with depressive symptoms in elderly populations, we restricted our sample to cognitively intact Amish elders. We examined 380 cognitively intact participants (mean age 80y) using the Geriatric Depression Scale (GDS). Heritability was calculated with the SOLAR© statistical software package. Overall heritability of GDS score is 0.44 (p-value=8.8X10-6). Heritability of GDS score among the 159 elderly Amish men is 0.23 and among 221 elderly Amish women is 0.76. The heritability of depressive symptoms among the elderly Amish women is about twice that reported in other Caucasian populations and is statistically significant (p-value= 3.1X10-4). These data suggest that depressive symptoms among Amish elders is heritable, particularly among women. Historically, discovery and replication of specific genetic variants associated with depression has been frustrating. The Amish may represent an informative population in which to discover genomic variation associated with late-life depression.

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## 2119/T/Poster Board #668

**A latent variable approach to optimizing genetic phenotypes for substance use disorders: Behavioral disinhibition.** *S.E. Young[1], M.C. Stallings[1,2], J.K. Hewitt[1,2]*. 1) Institute for Behavioral Genetics, University of Colorado, Boulder, CO; 2) Department of Psychology, University of Colorado, Boulder, CO.

The NIDA Genetics Consortium has recently begun an active investigation of drug-related behavioral traits that will afford more statistical power in molecular genetic studies than categorical, diagnostic methods traditionally used. In order to contribute to this effort, our Colorado NIDA Center studies are building on previous work demonstrating that substance use behavior is part of a spectrum of externalizing problems (e.g., conduct disorder, impulsivity) that reflect, in part, behavioral disinhibition (BD). We characterize BD as a lifelong disposition toward the excessive pursuit of exciting appetitive stimuli, impulsivity, risk-taking, and the unusual disregard of aversive consequences of deviant behavior. Previous research has shown that these behavioral problems tend to cluster because of a shared array of genetic risk factors. As we follow our Center participants from adolescence into early adulthood, we focus on more developmentally salient indices of BD including adult antisocial behavior, symptoms of substance abuse and dependence, and precocious/promiscuous sexual behavior. We present a latent variable approach to developing a maximally heritable and highly reliable phenotype for application to genetic linkage and association studies of substance use disorders. This method captures the common variance among highly intercorrelated, quantitative behavioral measures, while avoiding the noise associated with the impurity and measurement error in individual assessments. We examined the genetic and environmental architecture of BD as defined by the common variance among adolescent conduct disorder symptoms, adult antisocial personality symptoms, substance abuse and dependence symptoms, precocious sexual behavior, and novelty seeking. A significant portion of the variance in each of the five observed phenotypes contributed to BD. Results showed that 69% of the variance in BD was due to additive genetic influences ($p < .01$), while shared environmental influences accounted for only 13% of the variance ($p > .05$). Only precocious sexual behavior showed significant residual genetic effects (33%). These results provide compelling evidence that BD is a genetically driven propensity for a spectrum of deviant externalizing behaviors, which are known to be central to the development of substance use disorders. BD is a highly heritable, reliable phenotype that may be useful for future linkage and association studies.

## 2120/T/Poster Board #669

**Focal Neuropathological and Laminar Gene Expression Abnormalities in Dorsalateral Prefrontal Cortex in Autism.** *M. Chow[1], M. Boyle[2], S. Roy[1], T. Wynshaw-Boris[4], E. Lein[2], S. Colomarino[3], E. Courchesne[1]*. 1) UC San Diego, La Jolla, CA; 2) Allen Institute for Brain Science, Seattle, WA; 3) Autism Speaks Inc, Los Angeles, CA; 4) UC San Francisco, San Francisco, CA.

Although it is accepted that autism is a heritable neurobiological disorder, many questions remain including etiology as well as onset of underlying neurobiological defects. We hypothesize that autism begins prenatally due to abnormal cell proliferation, migration and/or laminar organization. Such possibilities have never been systematically examined in the young developing autistic cerebral cortex, but their elucidation would shed light on controversies (putative postnatal neuroinflammation, speculations about vaccines). To qualitatively and quantitatively examine the latter two possibilities, we performed in situ hybridization(ISH) as well as Nissl staining of 20 micron thick frozen sections of dorsolateral prefrontal cortex in 8 autistic male cases aged 2-14 years and 8 age-matched control males. ISH data was systematically generated using high throughput semiautomated procedures for a panel of 25 robust lamina, neuronal, and/or glial cell type-specific markers in order to search for detailed cellular cytoarchitectural abnormalities in autistic samples, as well as for a set of genes implicated in autism through gene association studies. Ten uniformly spaced sections were hybridized to digoxigenin-labeled riboprobes for each gene, with interleaved Nissl-stained sections (260 sections/case*16 cases=4160 total sections). Sections were carefully examined for features of neuropathology by two authors and findings were compared to those by a blinded clinical neuropathologist. Some autistic cases displayed focal regions of disturbance of multiple lamina-specific and neuronal-cell type-specific gene expression markers, sometimes embedded within local areas of normal marker expression. Quantitative analyses compared marker expression between pathological and neighboring regions within autistic cases as well as between autistic and control cases. Results suggest abnormal migration and disorganization across lamina and cell types in autism. This is the largest study of the young autistic postmortem cortex and the first to detail laminar- and cell-type specific gene expression abnormality and cortical disorganization. These neurodevelopmental defects cannot be caused by some speculated postnatal causes of autism, but pinpoint prenatal life as the time of autism onset. The important implications for identifying underlying causes of autism, establishing the age of neurobiological onset and creating animal, cellular and molecular models of autism are discussed.

## 2121/T/Poster Board #670

**Dominant isolated Dandy Walker malformation continuum in a four generation kindred.** *G. McGillivray[1,2,3], M. Fink[2,3,4]*. 1) Murdoch Children's Research Institute, Melbourne, Australia; 2) Royal Children's Hospital, Melbourne, Australia; 3) Royal Women's Hospital, Melbourne, Australia; 4) The University of Melbourne, Melbourne, Australia.

Dandy-Walker malformation (DWM) is heterogeneous. A significant proportion of cases have a chromosomal aetiology. DWM is also a feature of over 80 distinct inherited and sporadic syndromes. Dominant DWM with occipital cephalocele in one family links chromosome 2q36. Most cases of isolated DWM are sporadic and some patients have deletions including ZIC1 and ZIC4 on 3q24. Convincing autosomal dominant pedigrees with isolated DWM have not been described previously. The purpose of this presentation is to document the clinical, imaging and molecular cytogenetic findings in a four generation kindred with familial hydrocephalus. All affected members have variable expression of posterior fossa anomalies falling within the Dandy Walker continuum/spectrum, ranging from isolated elevation of the vermis to a full Dandy Walker malformation. Arrested or shunted hydrocephalus is a feature and affected family members have intellectual abilities ranging from normal to mild disability. Some have behavioural difficulties. The most severely affected individual is male, but another male has mild expression with cerebellar vermis elevation. X-linked inheritance cannot be excluded, but the presence of mildly affected male individuals and affected female individuals is more consistent with autosomal dominant inheritance. Recent re-evaluation of the imaging of the fetal posterior fossa and the embryology of the roof of the rhombencephalon suggests that Dandy-Walker continuum, persistent Blake's pouch cyst (BPC) and mega cisterna magna (MCM) all have the same underlying developmental aetiology. Prognosis is determined by the presence or absence of cerebellar vermian hypoplasia and the presence or absence of associated hydrocephalus related to obstruction of CSF flow into the subarachnoid spaces. Fetal MRI is an important tool in the prognostic assessment of affected pregnancies.

## 2122/T/Poster Board #671

**Association between Autism and Recurrent 17q12 Deletions.** *D. Moreno-De-Luca[1], M. Adam[1], S.M. Myers[2], A. Pakula[3], L. Weik[4], L. Guy[3], K. Uhas[1], N.J. Eisenhauer[2], M.R. Rossi[1], S. Aradhya[5], C.L. Martin[1], D.H. Ledbetter[1]*. 1) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Geisinger Medical Center, Danville, PA; 3) Marcus Autism Center, Atlanta, GA; 4) Children's Hospital of Wisconsin, Milwaukee, WI; 5) GeneDx,Gaithersburg, MD.

A new, recurrent microdeletion of 17q12 (del 17q12) was recently reported in association with renal disease (in pediatric patients and a stillbirth) or diabetes in patients with maturity-onset diabetes of the young type 5 (MODY5). The deletion is 1.5 Mb in size, is mediated by flanking segmental duplications, and contains the HNF1B (TCF2) gene responsible for the autosomal dominant MODY5 form of diabetes. Initial reports indicated that this microdeletion was not associated with mental retardation and no features of autism disorders were noted. As part of the ISCA Consortium (International Standard Cytogenomic Array), which is a group of clinical laboratories performing whole genome copy number array analysis using a standardized array design, we studied a subset of 7752 patients, from which approximately 20% have a diagnosis of autism or autism spectrum disorders. We identified four males and one female, ranging in age from 1 to 12 years old, with the same 1.5 Mb deletion of 17q12 as recently reported in renal disease or diabetes. All four males were referred for array testing with a primary indication of autism or autistic features, and the female was referred for developmental delay. Additionally, all patients share phenotypic features such as speech delay, macrocephaly, onychodystrophy, and different degrees of renal anomalies. None of them show signs of diabetes yet, which is not surprising given their young age. Parental testing has been completed for four individuals and demonstrated that the deletion was de novo in all of them. Microsatellite testing is being performed to identify the parental chromosome from which the deletion arose, and tri-color FISH studies are underway to evaluate the presence of an inversion polymorphism that corresponds to the deleted interval. This region of proximal 17q overlaps with an autism linkage peak identified in multiple studies, predominantly associated with "male-only" families (i.e., families in whom all affected individuals are males), strongly suggesting a sex specific risk allele for autism. Interestingly, all four male patients described here had autism or autistic features. These data raise the possibility that the 17q12 microdeletion may be associated with an increased risk of autism, especially in males.

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## 2123/T/Poster Board #672

**Identification of proteins that interact with the large intracellular loop of the nAChR α5 subunit: impact of the Chrna5 D398N polymorphism (rs16969968).** *T.A. Precht[2], J.A. Laughlin[2], J.A. Stitzel[1].* 1) Department of Integrative Physiology, Univ. of CO, Institute for Behavioral Genetics, Boulder, CO; 2) University of Colorado, Institute for Behavioral Genetics, Boulder, CO.

The interaction of nicotine with neuronal nicotinic acetylcholine receptors (nAChRs) is mostly responsible for the physiological and addictive aspects of smoking. Recent evidence has demonstrated that commonly occurring SNPs in CHRNA5, the gene that encodes the α5 nicotinic receptor subunit, in the human population can confer increased risk for nicotine addiction and other smoking-related phenotypes. One of the variants in CHRNA5 that repeatedly has been found to be associated with smoking-related phenotypes is the non-synonymous SNP rs16969968. This SNP converts an aspartic acid (D) residue at position 398 in the α5 nAChR subunit, which is highly conserved across vertebrate species, to an asparagine (N). In the brain, the α5 subunit predominantly assembles with α4 and β2 subunits to form an α42β22α5 nAChR. Functional studies have shown that α4β2α5N398 nAChRs are significantly less responsive to agonist stimulation than are α4β2α5D398 nAChRs, indicating this SNP dramatically decreases the function of this brain-expressed nAChR. This decrease in function was not due to a change in sensitivity to agonist, nor was it due to alteration in or differential expression of the receptor. What remains unclear is how this single amino acid change in the α5 subunit within the large intracellular loop between transmembrane domains 3 and 4 (M3M4) yields a less functional receptor upon agonist stimulation. Previous studies with both the α4 and β2 subunits have identified a wide variety of intracellular protein interactions with the M3M4 loops of these subunits that alter receptor function. Because this SNP eliminates the negative charge of the aspartic acid residue it is possible that protein-protein interactions will be altered, and this might lead to a less functional intact receptor. We intend to identify protein-protein interactions between the M3M4 intracellular loop of α5 and cytosolic proteins and determine whether the Chrna5 D398N SNP alters any identified interaction. For these experiments we have fused the α5 M3M4 loop containing either the D398 residue or the N398 residue to Glutathione-S-Transferase (GST) and will perform a pulldown experiment. Mass spectrometry will be used to identify proteins whose interactions with the α5 M3M4 loop are dependent upon the Chrna5 D398N polymorphism. Supported by NIDA grant DA017637 and WashU grant CA089392.

## 2124/T/Poster Board #673

**Defining the autism-epilepsy phenotype.** *K. Hamilton[1], R. Tuchman[2], R.K. Abramson[3], H.H. Wright[3], J.R. Gilbert[1], M. Pericak-Vance[1], M. Cuccaro[1].* 1) Miami Inst Human Genomics, Univ Miami Miller Sch Med, Miami, FL; 2) Department of Neuorology, Univ Miami Miller Sch Med, Miami, FL; 3) Department of Neuropsychiatry, Univ SC Sch Med, Columbia, SC.

Autism and epilepsy are common complex disorders. Conservative estimates suggest that ~25%-30% of individuals with autism have a comorbid seizure disorder or epilepsy. Despite ample description of the co-occurrence of autism and epilepsy, a clear understanding of why some children with autism develop epilepsy or why children with autism and epilepsy have a worse outcome has eluded the field. Comorbid features such as epilepsy offer clues as to possible genetic underpinnings for both autism and epilepsy. Given that both autism and epilepsy are clinically heterogeneous, a useful starting point for dissecting the complex genetic etiology of these co-occurring disorders requires definition of the autism-epilepsy phenotype (AE). To define the AE phenotype we identified all cases of autism with co-occurring epilepsy and complete data (n=67) and a comparison group of autism only (AUT) patients (n=412). Logistic regression (LR) modeling was used to determine which variables best differentiated AE and AUT cases. LR model selection procedure used multiple clinical variables including domain scores from the Autism Diagnostic Interview-Revised (ADI-R; Social, Verbal, Nonverbal, Repetitive, and Evidence of Abnormal Behavior), age at single words, age at first phrases, age first walked, age at symptom recognition and gender. Significance threshold for entry into the model was set at 0.10. Inspection of the Vineland Adaptive Behavior Scale composite score (VABS), an indicator of developmental level, revealed that AE cases were significantly lower than AUT controls ($p<0.001$). All subsequent analyses were adjusted for developmental level. Also, the prevalence of females was higher in the AE group vs. the AUT control group ($p<0.005$). In our LR model, four variables distinguished AE cases from AUT: VABS composite, age at symptom recognition, ADI-R Repetitive Behavior Domain score, and Evidence of Abnormal Behavior score. This study defines features unique to the AE phenotype, three of which are developmental in nature. Further, these features suggest a more severe pattern of developmental problems which is consistent with observations that individuals with co-occurring autism and epilepsy have a worse outcome. Our results, while preliminary, set the stage for additional analytic approaches (e.g., structural equation modeling and cluster analysis) as well as biologic and genetic studies to more precisely define the AE phenotype.

## 2125/T/Poster Board #674

**Deafening fever!** *S. Marlin[1, 4], Y. Nguyen[2], L. Jonard[3,4], N. Loundon[2,4], I. Rouillon[2], E. Garabedian[2,4], F. Denoyelle[2,4], D. Feldmann[3,4].* 1) Genetique, INSERM U587, Hosp Armand Trousseau, Paris, France; 2) ENT Unit, Hosp Armand Trousseau, Paris, France; 3) Biochemistry and Molecular Biology Unit, Hosp Armand Trousseau, Paris, France; 4) Reference Center for “Genetic Deafness”, Hosp Armand Trousseau, Paris, France.

Transient deafness associated with an increase in core body temperature is a rare and enigmatic phenomenon. Here, we report a novel mutation in the gene OTOF causing a temperature dependent deafness. Mutations in OTOF, which encodes otoferlin, have been previously reported to cause DFNB9, a nonsyndromic form of deafness caracterized by severe to profound, prelingual hearing impairment and auditory neuropathy. In DFNB9 patients, auditory brainstem responses (ABRs) are absent or severely distorted but otoacoustic emissions (OAE) are preserved. Otoferlin is a calcium sensitive protein that is involved in presynaptic vesicles exocytosis at the auditory inner hair cell ribbon synapse. Three siblings (ages 10, 9 and 7 years old) of a consanguinous family were found to be affected by a profound and transient deafness only present when they become febrile. Audiometric tests revealed an auditory neuropathy. As the family genotype was consistent with linkage to the DFNB9/OTOF region, we underwent a molecular analysis of the 48 exons and of the intron-exon boundaries of OTOF. This study revealed a novel mutation p.Glu1803del (E1803del) in exon 44 of OTOF. It was found to be homozygous in the patients and segregates with the hearing impairment in the family. The new mutation described here is located in the C2F domain of the protein and is associated with an unusual phenotype compared to previous reported cases of patient affected by OTOF mutations. This result open the way for new researches on the physiopathology of auditory neuropathy.

## 2126/T/Poster Board #675

**Chromosome 15 linkage analysis of two repetitive behavior phenotypes in Utah pedigrees with Autism Spectrum Disorders.** *D. Cannon, W. McMahon, K. Allen-Brady, J. Miller, R. Robison, H. Coon.* Department of Psychiatry, University of Utah School of Medicine, Salt Lake City, UT.

Because Autism Spectrum Disorder (ASD) is a heterogeneous phenotype, it has been argued that more specific ASD traits should be included in genetic research. Repetitive and stereotyped behavior, a broad ASD trait, comprises at least two more specific traits measured by different Autism Diagnostic Interview-Revised (ADI-R) items, viz. Repetitive Sensory-Motor Actions (RSMA) and Insistence on Sameness (IS). IS and RSMA differ in their phenotypic correlates and familiality. We investigated in large multiplex pedigrees linkage of both IS and RSMA on chromosome 15 and compared those linkage peaks with peaks we previously reported with ASD in the same pedigrees. Subjects were members of 70 pedigrees having at least two family members with ASD. A total of 653 subjects were genotyped, 192 of whom had a study diagnosis of ASD. RSMA included hand and finger mannerisms, unusual sensory interests, repetitive use of objects, complex mannerisms, and rocking. IS included difficulties with minor changes in personal routine or environment, resistance to trivial changes in environment, and compulsions/rituals. Genotyping services were provided by the Center for Inherited Disease Research (CIDR) using the 6K single nucleotide polymorphism (SNP) linkage panel. We performed parametric and non-parametric linkage analyses and repeated the analyses for each scale based on residual scale scores derived by using the other scale as a covariate. These residual analyses tested the extent to which linkage signals were due to variance unique to IS and RSMA phenotypes. There were two peaks. At 15q13.1-q15.1, RSMA had a max HLOD of 3.93 (NPL=4.54, marker=rs904951), and IS had a max HLOD of 2.00 (NPL n.s., marker=rs965471). At 15q21.1-q22.2, only IS was linked with a max HLOD of 1.88 (NPL=2.60, marker=rs11856). Not only was there no RSMA signal at 15q21.1-q22.2, but the IS residual signal was much stronger (HLOD=3.03, NPL=3.10) than the IS raw score signal, indicating the shared variance between IS and RSMA actually dampened the IS signal at 15q21.1-q22.2. Our findings illustrate the benefit of linkage analysis using homogeneous phenotypes. Two peaks in our previous linkage analysis of ASD affected status appear to be supported by different aspects of repetitive behavior.

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## 2127/T/Poster Board #676

**Interstitial 6q deletions may include a novel locus for idiopathic generalized epilepsies.** *E. Andermann[1,2,3], D. Amrom[1,2], J. Lavoie[4,5], M. Veilleux[2,6], F. Andermann[2,6,7].* 1) Neurogenetics Unit, Montreal Neurological Hospital and Institute, Montreal, PQ, Canada; 2) Department of Neurology & Neurosurgery, McGill University; 3) Department of Human Genetics, McGill University; 4) Cytogenetics Laboratory, Montreal Children's Hospital; 5) Department of Pathology, McGill University; 6) Epilepsy Service and Seizure Clinic, Montreal Neurological Hospital and Institute; 7) Department of Pediatrics, McGill University.

Purpose: Chromosome (chr) 6q deletion syndrome encompasses three distinct phenotypes corresponding to proximal (6q11-16), interstitial (6q15-25) and terminal deletions (6q25-qter). Epilepsy has rarely been reported in 6q interstitial deletions. We report the clinical, epilepsy, and array CGH data in a woman with interstitial 6q deletion, febrile seizures and absences, and compare it to our previously reported patient with interstitial 6q deletion and adult onset of myoclonic epilepsy (Andermann E et al., Epilepsia 2008,49(Suppl.7):309-310). Methods: Detailed review of medical records; oligonucleotide microarray of genomic DNA; and fluorescence in situ hybridization (FISH) to confirm the deletions. Results: A 22-year-old woman had febrile seizures at age 3, speech delay at age 4 and later absence attacks. Evaluation showed mild mental deficiency, generalized epileptiform discharges, and at age 13 mild left occipital periventricular leukomalacia. At age 22, she had upslanting eyes, notched eyebrows, micrognathia, microcephaly, mild mental retardation, and absences poorly controlled by valproic acid and topiramate. Karyotype at 4 years revealed a chr6q deletion. Microarray analysis detected a single copy loss of 360 oligonucleotide probes at 6q22.1-22.33 (12.8 Mb, at least 32 OMIM genes), confirmed by FISH. A 31-year-old man had a febrile seizure at age 8 months and developmental delay. At age 14, two hair whorls, small forehead with low frontal hairline, head circumference at P3, mild retrognathia, hyperextensible joints, and mild quadriparesis were recorded. In his late twenties, he developed stimulus-sensitive myoclonic jerks that increased in frequency. Evaluation showed no ictal or interictal epileptic abnormalities, and mild cerebellar vermian atrophy. Karyotype revealed a chr6q22.1-22.2 deletion. Microarray analysis detected a de novo single copy loss of 164 oligonucleotide probes at 6q21-22.31 (7.6 Mb, at least 18 OMIM genes), confirmed by FISH. Conclusions: Chr6q interstitial deletions may have a different epilepsy phenotype (febrile seizures, absences and myoclonus) from that seen with 6q terminal deletions. In one reported GEFS+ pedigree with 15 affected individuals, a novel locus has been mapped to chr 6q16.3-22.31 (Poduri A et al. Epilepsia 2008, 49(Suppl.7): 318-319). Our results suggest that this region might be narrowed to 6q22.1-22.31, and may represent a new locus for idiopathic generalized epilepsies.

## 2128/T/Poster Board #677

**High prevalence of abnormal genomic gains/losses in adults with unexplained intellectual disability.** *J. Jirikowic[1], C. Wells[1], M. Springer[2], K. Swisshelm[2], M. Taylor[1].* 1) University of Colorado Denver, Aurora, CO; 2) Colorado Cytogenetics Laboratory, Denver, CO.

**Purpose:** Array comparative genomic hybridization (aCGH) is now a widely used tool for the evaluation of intellectual disability. Meta-analysis of published data show positive findings in 10% of patients based largely on studies of modest-sized pediatric clinics or from larger clinical referral laboratories where the patient population is also biased towards pediatric cases. Adults with static, unexplained intellectual disability have not been systematically studied with aCGH. Here, we report our experience with aCGH testing on adults with unexplained intellectual disability referred to an adult genetics clinic. **Methods:** In 2006 we began applying clinically available aCGH testing to adults referred with an intellectual disability phenotype. As our laboratory's aCGH testing advanced from 2006-2009, our clinical testing progressed from an early-generation targeted array to a current genome-wide platform, which uses 5,500 clones to achieve an average resolution of 500Kb and targets 114 disease loci. All patients also underwent high-resolution karyotyping and molecular testing for Fragile X syndrome. **Results:** Our population was comprised of 40 patients (16 males, 24 females) with an average age of 35.3 years. All patients had unexplained intellectual disability ranging from mild to severe and only two had documentation of prior normal karyotypes; most had not been evaluated by genetics since childhood or had never undergone a genetic evaluation. Three subjects had abnormalities identified on initial high-resolution chromosome analysis. Twelve of the remaining 37 patients had genomic gains (4) and losses (8) identified only by aCGH. **Conclusion:** Abnormal copy number variation gains and losses detected by aCGH appear are prevalent in adults with unexplained intellectual disability. Our data showing abnormalities in 32% of cases with normal karyotypes suggest that the yield of aCGH in adults with this phenotype may be higher than in pediatric populations.

## 2129/T/Poster Board #678

**Characterization of human iPS cells established from Down syndrome patient-derived fibroblast cell lines.** *M. Hiratsuka[1], N. Uno[2], N. Imaoka[2], S. Masuda[2], K. Ueda[1], Y. Kazuki[2], M. Osaki[2], K. Higaki[3], K. Takahashi[4], S. Yamanaka[4], M. Oshimura[1,2].* 1) Molecular and Cell Genetics, Tottori University, Yonago, Japan; 2) Biomedical Science, Graduate School of Medical Science, Tottori University; 3) Functional Genomics, Research Center for Bioscience and Technology, Tottori University; 4) Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan.

Down syndrome ( DS ) is caused by a whole, or occasionally partial, trisomy 21 resulting in complex and variable phenotypes. The most common phenotype of DS is characterized by mental retardation and late onset Alzheimer disease neuropathology. The mechanism by which trisomy 21 leads to DS phenotypes remains to be elucidated, although two hypotheses, developmental instability theory and gene-dosage hypothesis, are proposed. We have previously created chimeric mice from ES cells containing a single human chromosome 21 and demonstrated that this mouse could be used as a DS-model mouse with various phenotypic abnormalities including abnormal behavior. Furthermore, it has been demonstrated that these ES cells showed a higher incidence of apoptosis during early neural differentiation in vitro, in which some genes responsible for this phenomenon were identified. To validate whether this phenomenon also occurs in human cells, we established iPS clones ( DS-iPSs ) from four DS patient-derived fibroblast cell lines ( Coriell Institute ) by means of Yamanaka Protocol with either four or three factors. DS-iPSs, selected under the morphological criterion, showed the expression of pluripotent markers determined by both immunohistochemistry and RT-PCR analyses. DS-iPSs also exhibited differentiation potential, assessed by the methods of both embryoid body ( EB ) and teratoma formation. We are now under investigation of neural differentiation potential and apoptosis induction during this differentiation in DS-iPSs. Neural differentiation is performed by either co-culture with the mouse PA6 stromal cell line ( SDIA, stromal-derived inducing activity ) or EB formation. The possibility of trisomy 21 enhancing apoptosis of neural precursor cells will be discussed.

## 2130/T/Poster Board #679

**Large scale re-sequencing of autosomal synaptic genes reveals a high rate of de novo deleterious mutations in non-syndromic mental retardation.** *J. Michaud[1], F. Hamdan[1], J. Gauthier[2], S. Dobrzeniecka[2], A. Piton[2], H. Daoud[2], D. Spiegelman[2], M. Côté[2], E. Henrion[2], O. Diallo[2], C. Marineau[2], R. Lafrenière[2], P. Drapeau[3], J.-C. Lacaille[4], G. Rouleau[1,2].* 1) Research Center, CHU Sainte-Justine, Montreal, Quebec, Canada; 2) Research Center, Centre Hospitalier Universitaire de l'Université de Montréal, Montréal, Quebec, Canada; 3) Department of Pathology, Université de Montréal, Montréal, Québec, Canada; 4) Department of Physiology, Université de Montréal, Montréal, Quebec, Canada.

Mental retardation (MR) is a neurodevelopmental disorder affecting ~1-3 % of the general population. A large proportion of patients have the non-syndromic form of the disorder which is characterized by the absence of associated morphological, radiological or metabolic features. Very little is known about autosomal dominant non-syndromic MR (NSMR) genes, mainly because MR results in lower reproductive fitness, decreasing the likelihood of identifying families that are amenable to linkage analysis. In this study, we hypothesized that de novo mutations in genes implicated in synapse function represent an important cause of autosomal dominant NSMR. To identify such mutations, we re-sequenced the coding exons (and their intronic junctions) of 290 autosomal synaptic genes, selected based on functional criteria, in 95 patients with NSMR. We mainly chose sporadic cases with available healthy parents to increase our chance of finding de novo mutations. In total, we identified 12 de novo mutations, including 4 truncating, 2 splicing, and 6 missense mutations, in 9 different genes. Interestingly, de novo truncating mutations in SYNGAP1, which were considered pathogenic based on genetic and functional evidences, were identified in 3 patients, suggesting that haploinsufficiency of this gene may represent a common cause of NSMR. The other 3 truncating / splicing mutations, which were found in STXBP1 (n=2) and SHANK3 (n=1), were also predicted to be pathogenic. SYNGAP1 and SHANK3 have been shown to influence synaptic plasticity whereas STXBP1 is required for neurotransmission. More work is needed to determine whether the missense mutations have any clinical consequences. In conclusion, our study points to a high rate of de novo pathogenic mutations in NSMR, with at least 6 % of the studied patients carrying such mutations.

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## 2131/T/Poster Board #680

**Characterization of the Lphn3 mutant mouse; a model for ADHD and addiction vulnerability?** *D. Wallis[1], D.S. Hill[2], L.C. Abbott[3], R.H. Finnell[1,2], P.J. Wellman[4], B. Setlow[4].* 1) Texas A&M Institute for Genomic Medicine, Texas A&M Health Science Center, Houston, TX; 2) Center for Environmental and Genetic Medicine, Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, TX; 3) Department of Veterinary Integrative Biosciences, College of Veterinary Medicine, Texas A&M University, College Station, TX; 4) Behavioral and Cellular Neuroscience Program Department of Psychology Texas A&M University College Station, TX.

Vulnerability to addiction and ADHD are frequently comorbid, suggesting a common etiology. The LPHN3 gene has already been correlated in linkage and association studies to both addictive phenotypes and ADHD independently. Lphn3 is a member of the LPHN subfamily of G-protein coupled receptors. LPHN3 has been implicated in modulating neurotransmitter release, although the endogenous ligand is unknown. We have utilized a gene-trap embryonic stem cell line to generate mice mutant for the Lphn3 gene. We report initial phenotypic characterization and analysis of this mutant. We have begun our studies by evaluating the Lphn3 expression pattern. We find Lphn3 in the adult amygdala, hypothalamus, hippocampus, subcommissural organ, and olfactory bulb. We have also evaluated differential gene expression between mutant and wildtype male littermates at postnatal day 0 in whole brain RNA. We have evaluated expression of genes commonly considered ADHD candidate genes through TaqMan gene expression assays as well as evaluated genes identified as "neurogenesis and neural stem cell marker" genes through the use of SABiosciences RT-PCR-array. Most notably, we find changes in dopamine and serotonin receptors and transporters (Dat1, Drd4, 5Htt, 5Ht2a), changes in neurotransmitter metabolism genes (Th, Gad1), as well as changes in neural developmental genes (Nurr, Ncam,). Additional preliminary data indicate that the null mice have a hyperactive phenotype in the open field. We are following up with additional behavioral characterization, as well as brain region-specific analyses of dopamine and serotonin levels, which appear to be increased in the dorsal striatum of the mutants. Alterations in energy metabolism of monoamines have been posited as a core deficit in ADHD. Such alterations in these mutants likely affect cognition, motor inhibition, and vulnerability to addiction.

## 2132/T/Poster Board #681

**Genetic Analysis of Korean Patients with Spinocerebellar Ataxia.** *G. Kim[1,2], J. Lee[1], S. Choi[1], B. Lee[1,2], H. Yoo[1,2].* 1) Med Gen Clinic & Lab, Asan Med Ctr, Seoul, Korea; 2) Genome Research Center for Birth defects and Genetic Diseases, Asan Medical Center, Seoul, Korea.

Spinocereballar ataxia (SCA) is characterized by progressive degeneration and dysfunction of the cerebellum, brainstem, and spinal cords. Autosomal-dominant SCA is a genetically heterogeneous disease entity. To date, more than 25 subtypes of SCA have been identified according to their causative chromosomal loci. Genetic testing for 13 subtypes (SCA1,2,3,5,6,7,8,10,12,13,14,17 and 27) are commercially available. In this study, we retrospectively analyzed cumulative data on molecular genetic testing results of SCA1, 2, 3, 6, and 7 in Korean patients. During the period of 1999 to 2008, 638 unrelated Koreans with the presumptive diagnosis of SCA have been referred to Medical Genetics Clinic & Laboratory in Asan Medical Center for genetic testing. The age at the test provided was 45±15 years old. TNR (trinucleotide repeat) copy number was determined by PCR and direct sequencing of the number of (CAG)s using ABI3130 Genetic analyzer. Totally, 128 patients (20.1%) were positive for the test; SCA1 (5, 3.9%), SCA2 (38, 29.7%), SCA3 (30, 23.5%), SCA6 (39, 30.5%), and SCA7 (16, 12.5%). The copy number of SCA1 was 45±8.53, SCA2 42±3.13, SCA3 71±5.39, SCA6 23±1.53 and SCA7 50±11.37. The TNR copy was inversely correlated with the onset age in SCA2, 6 and 7 (P=0.011, 0.001 and 0.043, respectively), but not in SCA 1 and 3. In summary, SCA 1,2,3,6 and 7 were genetically confirmed in 20 % of the Korean patients referred with presumptive clinical diagnosis of SCA, among which SCA 6, 2 and 3 were common subtypes in order. Excluding SCA 1 and 3, inverse correlation between age and TNR copy number was observed in most subtypes. Further study to explain these discrepancies among SCA subtypes and genetic testing of other subtypes of SCA for unclassified patients are necessary.

## 2133/T/Poster Board #682

**Investigating autosomal recessive cerebellar ataxias: clinical, biochemical and neuroimaging studies of 252 Brazilian patients.** *C. Lourenco[1,2], K. Dantas[2], C. Funayama[2], C. Sobreira[2], J. Pina-Neto[1], W. Marques Jr[2].* 1) Dept Med Gen, Univ Sao Paulo, Ribeirao Preto, Brazil; 2) Department of Neurology, Univ Sao Paulo, Ribeirao Preto, Brazil.

Autosomal recessive cerebellar ataxias (ARCAs) comprise a heterogeneous group of inherited neurodegenerative disorders that affect the cerebellum, the spinocerebellar tract and/or the sensory tracts of the spinal cord. They lead to progressive cerebellar ataxia in association with other neurological or extra-neurological signs. The epidemiological features and the relative frequency of such disorders are quite unknown yet. We prospectively studied 252 suspected ARCA patients from Brazil between 2005 and 2008. All patients were evaluated in the neurogenetics clinics with a standard evaluation, neuroimaging studies (CT scan, brain MRI with spectroscopy), ophtalmological and auditory evaluations, neurophysiological studies (EEG, ERG, BAER and EMG/NCV), hormone and biochemical tests, muscle biopsy with respiratory chain mitochondrial analysis, screening for inborn errors of metabolism (enzyme studies, peroxisomal and sterol panels, cholestanol dosage, organic acids, sulfatides and aminoacids chromatography, GAGs analysis), molecular studies for the FRDA expansion and, when indicated, nerve/skin biopsy for EM studies and karyotype. A conclusive diagnosis was established for 196 patients. The most frequent causes for the recessive ataxias seen in our cohort were Friedreich ataxia, vanishing white matter disease, Joubert syndrome (and related disorders), ataxia with oculomotor apraxia types I and II, mitochondrial disorders and neurolipidoses (Niemann-Pick type C and Tay-Sachs B1 variant). ARCAs are rare disorders witb a wide differential diagnosis. Recognition of the most frequent genetic causes of ARCA can lead to a sequential evaluation capable of establishing a definitive diagnosis in the majority of patients, avoid expensive testing and prevent delays in the initiation of treatment when available. .

## 2134/T/Poster Board #683

**Parental consanguinity and idiopathic generalized epilepsy in Hamadan, Iran.** *H. Pour-Jafari[1,2], M. Mazdeh[3], B. Pour-Jafari[2].* 1) Molec Med & Gen, Sch Med, Hamadan Univ Med Sci, Hamadan, Iran; 2) Research Center for Molecular Medicine, Hamadan Univ Med Sci, Hamadan, Iran; 3) Neurology Dept, Sch Med, Hamadan Univ Med Sci, Hamadan, Iran.

Idiopathic generalized epilepsies (IGEs) are a group of disorders determined by strict clinical and electroencephalogram (EEG) features proposed by the International League Against Epilepsy (ILAE) classification of epileptic syndromes. Most IGE patients display a complex pattern of inheritance. In this report the family history and types of paternal marriages in a groups of patients with IGE, in a referral center in Hamadan, Iran were studied and the importance of consanguinity and family history in the occurrence of this type of disease was investigated. In a cross sectional study all patients with epileptic seizer referred to neurology clinic, Farsh'chian Hospital, Hamadan, Iran, were investigated during a period of 6 months. In total, 95 unrelated patients (25 males and 70 females) were diagnosed with idiopathic generalized epilepsies adopted with ILAE classification. Their average age at the time of study was 25.44 (13 y min and 65 y max). The sex ratio (M/F) was 0.36. The types of their disease, family history and parental consanguinity were studied. The results were compared with the same indexes among general population in Hamadan, a city located in the west of Iran. The types of epilepsy among our cases in order of higher to lower frequencies were: Tonic-Clonic (82.1%), Myoclonic (13.7%), Absence (3.2%) and Tonic (1.1%) respectively. Family history was positive in 50.5 per cent of patients. Parental consanguinity was present in 34.7 per cent of cases. The most frequent type of consanguinity was first cousins (34.7%). The percentage of consanguinity marriages in the parents of epileptic individuals was statistically different from general population (chi2=16.14, p=0.000). Based to the results, it seemed that genetic factors, e.g. monogenic mutations, must be considered in idiopathic epilepsies. The results have confirmed that positive family history and paternal consanguinity lead genetic counselors to the role of genetic factors in such families. Also the importance of preventive programs such as school education and pre-marriage genetic counseling should be mentioned by the health organizations in developing countries.

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## 2135/T/Poster Board #684

**Detection of large deletions/duplications in *NOTCH3* in CADASIL patients using MLPA.** *E.M.J. Boon[1], M.J. Pont[1], D. van Heusden[1], M.J. Vollebregt[1], P. Lakeman[2], M. Elting[2], S.A.M.J. Lesnik Oberstein[1], H.B. Ginjaar[1].* 1) Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) Clinical Genetics, VU University Medical Center, Amsterdam, Netherlands.

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is an adult-onset cerebrovascular disorder caused by mutations in the extracellular EGF-like domains of the *NOTCH3* gene. To date only pathogenic missense mutations and small deletions in *NOTCH3* have been described. To investigate whether large deletions or duplications play a role in CADASIL, we designed a multiplex ligation dependent probe amplification (MLPA) kit for all coding exons of *NOTCH3*. Sixty-one patients with a clinical suspicion of CADASIL were screened using direct sequencing and MLPA. In one patient a compound heterozygous mutation was detected consisting of a missense mutation in exon 13 and a deletion of exon 3-16 of the *NOTCH3* gene. These results show that deletions and/ or duplications are probably rare in CADASIL, but can now be detected using our home made MLPA kit and might play a role in the pathogenesis of CADASIL.

## 2136/T/Poster Board #685

**Single-Step Molecular Determination of Deletional and Non-Deletional Prader-Willi and Angelman Syndromes by Methylation-Specific Quantitative Melting Analysis.** *S.S. Chong[1,2,3], W. Wang[2], H.Y. Law[4].* 1) Department of Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; 2) Children's Medical Institute, National University Hospital, Singapore, Singapore; 3) Molecular Diagnosis Center, Department of Laboratory Medicine, National University Hospital, Singapore, Singapore; 4) DNA Diagnostic and Research Laboratory, Department of Pediatric Medicine, KK Women's and Children's Hospital, Singapore, Singapore.

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are clinically distinct neurological disorders caused by lack of expression of oppositely imprinted genes in chromosomal region 15q11-13. The loss of expression can be due to parent-specific segmental deletions or can arise from non-deletional mechanisms such as uniparental disomy of chromosome 15 (UPD15) or defects in imprinting. Most of the current diagnostic methods for PWS/AS require separate amplification and detection steps, while current homogeneous assay strategies cannot distinguish between deletional and non-deletional PWS/AS. We have developed a single-step methylation-specific PCR (ms-PCR) assay to analyze methylation differences and copy number changes in PWS/AS. In this assay, bisulfite-modified DNA was subjected to duplex amplification of the *SNRPN* gene and a control amplicon from the *LIS1* gene, in the presence of the DNA binding dye EvaGreen. Qualitative melting curve analysis (MCA) distinguishes the maternal and paternal *SNRPN* amplicons based on their sequence differences after bisulfite modification. Quantitative ratio analysis of the maternal or paternal *SNRPN* peak height relative to the *LIS1* control fragment allows discrimination between deletional and non-deletional PWS and AS. Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) was performed in parallel for comparison purposes. Results obtained from this relative quantitative melting curve analysis (RQ-MCA) strategy were completely concordant with the MS-MLPA genotyping results for all deletional and non-deletional PWS and AS samples. RQ-MCA represents a simple, rapid and robust alternative to MS-MLPA for the detection of, and discrimination between, deletional and non-deletional forms of PWS and AS.

## 2137/T/Poster Board #686

**Development of a predictive gene classifier for autism spectrum disorders based upon differential gene expression profiles between cases and controls.** *V.W. Hu.* Biochemistry and Molecular Biology, The George Washington University Medical Center, Washington, DC.

Autism is a neurodevelopmental disorder which is currently diagnosed solely on the basis of abnormal behavior as well as observable deficits in communication and social functioning. Although a variety of autism candidate genes have been identified on the basis of genetic analyses, none have been shown to be unequivocally diagnostic for idiopathic autism or to account for more than a few % of autism cases. DNA microarray analysis was employed to obtain the gene expression profiles of lymphoblastoid cell lines (LCL) of 87 autistic male individuals who were divided into 3 phenotypic subgroups (Hu et al., Autism Research, 2:78-97, 2009) based on cluster analyses of severity scores on the Autism Diagnostic Interview-Revised (ADI-R) assessment instrument (Hu and Steinberg, Autism Research, 2:67-77, 2009). We compared these expression profiles against that obtained from LCL of 29 nonautistic male control subjects. Using these datasets, we then utilized gene classification and cross-validation analysis software (TM4:MeV) to identify sets of differentially expressed genes that have a high statistical probability of predicting cases and controls. We have identified panels of selected genes (less than 30) which correctly classify samples according to affected/unaffected status with an accuracy exceeding 90%. When autistic samples are subtyped according to ADI-R cluster analyses prior to the gene expression and classification analyses, the accuracy of correct assignment to cases and controls exceeds 98%. High throughput quantitative nuclease protection assay of a subset of "classifier" genes (n= 14) for one of the ASD subtypes further confirms the ability of the selected differentially expressed genes to identify autistic and control subjects with an accuracy of ~80%. We suggest that such limited sets of genes may serve as useful biomarkers for diagnosis of idiopathic autism.

## 2138/T/Poster Board #687

**Peripheral blood transcriptome biomarkers to predict epilepsy during early latent period.** *N. Vi[1], A. Bragin[2], A. Mazarati[3], L.C. Kudo[1], S.L. Karsten[1,4].* 1) NeuroInDx, Inc., Signal Hill, CA; 2) Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA; 3) Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA; 4) Neuroscience, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center.

Status epilepticus (SE) is a common cause of hippocampal neurodegeneration and synaptic reorganization that may or may not lead to the development of epilepsy. No effective prognostic tools exist to reliably predict whether chronic epilepsy would develop in post-SE patients. Therefore it is not known which post-SE patients should undergo antiepileptogenic preventive therapy, and for whom the therapy would be effective. We attempted to identify prognostic peripheral biomarkers of epilepsy using a microarray-based approach for the analysis of expression changes in the blood of two rat models of chronic epilepsy. Adult Wistar male rats (postnatal day 90) were subjected to SE induced by either systemic pilocarpine (n=25) or intrahippocampal kainic acid (KA; n=19). Peripheral blood was collected before status epilepticus to establish a baseline and one, three, seven and thirty days after SE. Isolated RNA was used for global transcriptome analysis using Agilent Rat Whole Genome microarrays. After SE, animals underwent continuous monitoring with both EEG and video for the occurrence, frequency and severity of spontaneous recurrent limbic seizures for six weeks. Seizure syndrome was retrospectively correlated with the changes in blood transcriptome profile. Identified gene expression changes were confirmed using semi quantitative RT-PCR and tested for effectiveness in independent group of post-SE kainic acid rats. Microarray data analysis revealed nearly 100 genes in each model associated with later occurring epilepsy. About 20% of the identified genes were regulated similarly in both models. This gene set is an initial class predictor/biomarker associated with later occurring chronic seizures. To further evaluate the effectiveness of the identified biomarkers, an additional group of post-SE animals (kainic acid model) was tested using custom designed prognostic microarray. We have demonstrated that the molecular signature preceding the development of epilepsy is present in the peripheral blood transcriptome, allowing the design of a prognostic biomarker chip that can be used to both screen and diagnose potential epilepsy patients, and to prospectively evaluate the effectiveness of antiepileptogenic therapy.

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## 2139/T/Poster Board #688

**Single-tube methylation-specific PCR screen for *FMR1* CGG repeat structure and expansion in fragile X syndrome.** *C. Teo[1,2], C.G. Lee[3,4,5], S.S. Chong[1,2,6]*. 1) Department of Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; 2) University Children's Medical Institute, National University Hospital, Singapore, Singapore; 3) Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; 4) Division of Medical Sciences, Humphrey Oei Institute of Cancer Research, National Cancer Center, Singapore, Singapore; 5) DUKE-NUS Graduate Medical School, Singapore, Singapore; 6) Department of Laboratory Medicine, National University Hospital, Singapore, Singapore.

Fragile X syndrome (FXS) is the most common heritable cause of mental retardation. It is predominantly caused by hyperexpansion and hypermethylation of a CGG trinucleotide repeat in the 5' untranslated region of the *FMR1* gene on chromosome Xq27.3. There are 4 allelic classes of *FMR1* CGG repeats: normal alleles (5 - 44 repeats), gray zone alleles (45 - 54 repeats), premutation alleles (55 - 200 repeats), and full mutation alleles (more than 200 repeats). Full mutation alleles are associated with FXS, while unmethylated premutation alleles have been associated with premature ovarian failure (POF) in females and with late-onset fragile X-associated tremor ataxia syndrome (FXTAS) in males, both of which are clinically distinct from FXS.

The most commonly used methods for analysis of *FMR1* repeats comprise of direct PCR amplification across the repeats, which is unreliable beyond the gray zone range due to the GC-rich nature of this locus, supplemented with Southern blot analysis to detect large repeat expansions, which is labor-intensive and time-consuming. Newer methylation-specific PCR (ms-PCR) methods require multiple reactions and cannot reliably detect all premutations. We describe a novel single-tube strategy involving fluorescent duplex ms-PCR and capillary electrophoresis that reliably distinguishes between all 4 allelic classes of CGG repeats in males and females. Triplet-primed PCR was performed simultaneously on methylated and unmethylated alleles of genomic DNA that had been subjected to sodium bisulfite conversion. The assay was validated on a panel of well-characterized male and female reference DNA samples with repeat lengths spanning the entire size spectrum. All genotype classes were accurately and easily determined based on their unique electropherogram patterns. Importantly, this approach reliably detects the presence of all premutation and full mutation alleles. The electropherogram patterns also inform on the repeat structures of the normal and gray zone alleles, which may aid in genetic counseling. Furthermore, this assay is able to detect skewed X-inactivation in premutation and full mutation females, which may be helpful in correlating with clinical findings. This simple single-tube strategy can serve as a rapid screening assay not only for FXS, but also for POF and FXTAS.

## 2140/T/Poster Board #689

**Virus-mediated gene silencing as a possible cause central nervous system disorders.** *T.C. Pereira, M.L.L. Moraes, I. Lopes-Cendes.* Dept of Medical Genetics, School of Medicine, State University of Campinas, Campinas, SP, Brazil.

Rationale: Human herpesvirus 6B (HHV-6B) was recently associated with a specific type of epilepsy in some pacients; these same individuals showed a significant reduction of EAAT2 messenger RNA. However the mechanism through which EAAT2 is repressed is unknown, as well as whether it is the cause or consequence f HHV-6B replication in affected cells. This is an interesting finding since several viruses can trigger a phenomenon known as virus-mediated gene silencing (VIGS), in which the pathogen represses a certain transcript through siRNAs. Objective: We set out to analyze whether some viruses could promote silencing through VIGS of certain genes expressed in the central nervous system (CNS). Methods - Pairs composed of viral genomes/genes were analysed using softwares (blast; strand analysis, gene runner) to identify viral regions potentially able to promote silencing of the corresponding gene. Results: To date, we have performed 36 complete surveys (3 viruses versus 12 genes). Although six of these presented no evidence of VIGS, most of them (29) showed regions (i.e. siRNAs) with potential for promoting VIGS. Remarkably, one pair: HHV-6B and doublecortin (DCX) showed a region with full complementarity. In addition, we found that the Huntington disease (HD) gene is complementary to regions in several viruses searched. Conclusions: Our computational survey provides evidence for VIGS of DCX by HHV-6B, which should now be investigated through molecular assays for confirmation. As for the HD data one can speculate that VIGS could be one of the postulated non-genetic factors acting as modifiers of the disease phenotype which could be influencing clinical characteristics such as age at onset.

## 2141/T/Poster Board #690

**Gene-Environment Interactions in Parkinson's Disease: CYPs and Pesticides.** *S.L. Rhodes[1], J.M. Bronstein[2], B. Ritz[1].* 1) Dept Epidemiology, UCLA Sch Public Hlth, Los Angeles, CA; 2) Dept Neurology, UCLA Sch Medicine, Los Angeles, CA.

Enzymes of the cytochrome (CYP) P450 superfamily metabolize drugs, endogenous molecules, and exogenous chemicals. Metabolic activity of CYPs varies between individuals and has been attributed to different haplotypes. We investigated CYP2 variants with demonstrated differences in metabolic activity for association with Parkinson's disease (PD) and for impact on PD-pesticide associations in the population-based Parkinson's Environment and Genes (PEG) study. PEG is unique in its assessment of pesticide exposure: an unbiased GIS-based computer model incorporates state-mandated reports of commercial agricultural pesticide use, historical land use maps, and residential addresses to estimate pesticide-specific exposure from 1974 to 1999 for each subject. 361 cases and 368 controls were genotyped for poor metabolizer (PM) variants: CYP2C9*2 and *3, CYP2C19*2 and *3, and CYP2D6*3 and *4. Maneb, paraquat, chlorpyrifos, and diazinon were investigated for interaction with CYP PM genotypes in age, sex, smoking adjusted logistic regression. No 'main effect' associations were observed between CYP PM variants and PD. 1.5- to 2-fold increases in risk were observed for the combination of maneb and paraquat (Mb/Pq; p=0.0045), chlorpyrifos (p=0.01), and diazinon (p=0.07), but not for paraquat alone (p=0.9). We observed no change in PD risk (OR=0.90 [0.11-7.18] p= 0.9227) by Mb/Pq exposure for subjects with the PM genotype of CYP2D6, but observed an increase in risk for subjects exposed Mb/Pq and possessing the wild type (WT) genotype (2.73 [1.58-4.75] p=0.0003) when compared to subjects exposed to neither pesticide. Similar associations were seen for CYP2C9 and CYP2C19 WT with Mb/Pq exposure (2.02 [1.21-3.38] p= 0.0074; 1.89 [1.11-3.21] p=0.0195, respectively). We observed suggestive negative association for CYP2D6 PM genotype (0.53 [0.11-2.55] p=0.4289) and significant risk association for the WT genotype (1.88 [1.28-2.76] p= 0.0014) in subjects exposed to the organophosphate (OP) chlorpyrifos, but not the OP diazinon, compared to unexposed subjects of the same genotype; this association remained after adjusting for Mb/Pq. These findings are in contrast to prior studies. Our frequency of CYP2D6 PM is low (5%) and our interaction analyses likely under-powered. Our results suggest that mechanisms underlying pesticide-gene interactions in PD are complex. Future GxE investigations should concentrate on high quality, unbiased exposure assessment over appropriate time periods.

## 2142/T/Poster Board #691

**Neuropathy target esterase mutations in individuals with sporadic ALS.** *S. Rainier[1], B. Bentley[1], T. Siman-Tov[1], T. Tobin[1], J. Moore[1], R.H. Brown, Jr.[2], J.K. Fink[1,3].* 1) Department of Neurology, University of Michigan, Ann Arbor, MI; 2) Neurology, University of Massachusetts School of Medicine; 3) the Geriatric Research, Education, and Clinical Center, Ann Arbor Veteran's Affairs Medical Center, Ann Arbor, MI.

Neuropathy target esterase (NTE) is a widely distributed membrane phospholipase that is implicated in the pathogenesis of both organophosphorus compound induced delayed neuropathy (OPIDN) and autosomal recessive motor neuron disease (NTE-MND). In the case of OPIDN, a disorder that includes progressive spastic paraplegia and peripheral neuropathy, organophosphorylation of NTE's active site serine leads to inhibition of NTE esterase activity and formation of toxic or "aged" NTE. NTE-MND is an autosomal recessive MND characterized by progressive spastic paraplegia and wasting of distal upper and lower extremity muscles and is similar to that of OPIDN. Individuals with NTE-MND have homozygous or compound heterozygous mutations involving NTE's esterase (NEST) domain. Finding that NTE is implicated in both a neurotoxin induced motor neuron disorder (OPIDN) and a form of autosomal recessive MND raises the possibility that abnormalities involving NTE or its biochemical pathways may contribute to other motor neuron disorders including amyotrophic lateral sclerosis (ALS). We investigated this possibility by examining the NTE coding sequence in subjects with ALS. We focused our analysis on the ten exons (exons 22 through 32) that together encode NTE's esterase domain because this was the location of NTE-MND mutations. We used heteroduplex analysis and DNA sequencing to analyze NTE exons 22 through 32 in DNA samples from 100 individuals with apparently sporadic ALS and an equal number of control subjects. Three ALS subjects were found to be heterozygous for novel, amino acid substituting mutations that disrupted inter-species conserved residues in NTE's esterase domain: Q756R, G942A and H1023Y. One of these subjects had an additional heterozygous, novel, amino acid substituting mutation in an exon outside of NTE's esterase domain (V19A). Each of these mutations was absent in at least 105 control individuals and is predicted to be not tolerated by SIFT analysis. Analysis of the biochemical and functional consequences of NTE mutations in ALS subjects is in progress. Our findings support the importance of NTE in motor neuron biology; and support the possibility that genetic variation in NTE is a contributing factor to the development of "apparently sporadic" ALS.

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## 2143/T/Poster Board #692

**Mutation Analysis of GIGYF2 Gene in Chinese Patients with sporadic late-onset Parkinson's Disease.** *JF. Guo[1,3], BS. Tang[1,2,3], L. Wang[1], WW. Zhang[1].* 1) Department of Neurology, Xiangya Hospital, Central South University, Chagsha, Hunan, China; 2) National Laboratory of Medical Genetics of China, Changsha, Hunan 410008, PR China; 3) Neurodegenerative disorders research center, Central South University, Changsha, Hunan 410008, PR China.

Objective:Establish a technical platform by using Polymerase Chain Reaction (PCR) and DNA sequencing to detect the mutations of GIGYF2 gene in Chinese patients with sporadic late-onset Parkinson's Disease, to make clear the mutation characteristics of GIGYF2 gene in Chinese PD patients. Methods:Mutation analysis of GIGYF2 gene in 300 unrelated Chinese patients with late-onset PD was performed using DNA sequencing. Results:We found 8 allele changes of GIGYF2 gene in 300 Chinese patients with sporadic late-onset PD, which were not found in DNA sequencing reports of the 200 controls. They are as follows: c.G766A,c.-C1130A,c.C1417T,c.C1555T,c.GG1474-1475AA, c.C1738A, c.C2935T, c.G3208C. All of them were new mutations according to bdSNP data. The mutation frequency of GIGYF2 gene among Chinese Han patients with sporadic late onset PD is 2.7%. In our research we did not find any mutations which had been reported. Conclusion:The mutations of GIGYF2 gene might be the etiological factors of Chinese sporadic late-onset PD.

## 2144/T/Poster Board #693

**Depression in Alzheimer's disease - the role of life events, epigenetics and genetics.** *P. PROITSI[1], J. MILL[2], JP. POWELL[1].* 1) NEUROSCIENCE, MRC CNR, KING'S COLLEGE LONDON, INSTITUTE OF PSYCHIATRY, LONDON, LONDON, United Kingdom; 2) SGDP, KING'S COLLEGE LONDON, INSTITUTE OF PSYCHIATRY, LONDON, LONDON, United Kingdom.

Many patients with Alzheimer's disease (AD) have depressive symptoms with serious consequences for patients and caregivers. Interactions between stressful life events (SLE), polymorphisms in the serotonin transporter (SERT) and brain-derived neurotrophic factor (BDNF) genes and depression have been described in children and adults, though this has not yet been studied in AD. The expression of the BDNF gene is highly complex with multiple promoters subject to epigenetic modification. BDNF and SERT act in concert to regulate neuronal plasticity and may be both compromised in aging and age-related neurodegenerative disorders making them good candidates for depression in AD. Our aim was to examine the relationships between epigenetic modification in the BDNF gene, polymorphic variation in the BDNF and SERT genes, and SLE in AD patients with depression. The HTTLPR and STin2 polymorphisms of the SERT gene and the Val66Met polymorphism of the BDNF gene were genotyped for ~1000 probable AD patients including 40 autopsy confirmed AD brains (and 5 controls) from the UK with full behavioural data including the Cornell Scale for Depression. Demographic data was used to quantify SLE. Three CpG islands regions from the BDNF promoter were investigated, capturing more than 100 CpGs, using the Sequenom MassARRAY EpiTYPER platform. DNA was extracted from 7 different brain regions (BA8, BA9, BA10, BA46, Entorhinal Cortex, Superior Temporal Gyrus and Occipital Cortex) and methylation analysis was performed on each area and genomic lymphocyte DNA from all 45 samples as well as an additional 150 lymphocyte DNA samples. A significant association was identified between the number of SLE and depression (p=0.005) in the whole dataset. No significant associations between the examined polymorphisms and depression in AD were identified. A very significant association between hypermethylation in a CpG island approximately 800bp upstream the major transcription start site and higher levels of depression ($R^2$=0.679, p=0.002) was seen in lymphocyte DNA. No interaction was identified with BDNF Val66Met polymorphism or/and SLE. Results from patients' brain tissue provide an indication on different methylation patterns of different tissues. Investigation of the interaction of genetics, epigenetics and environment allow us to examine the possibility of detecting a peripheral biomarker for depression and life events in AD.

## 2145/T/Poster Board #694

**Glucocerebrosidase gene mutations and Parkinson disease in Chinese population.** *BS. Tang[1,2,3], JF. Guo[1,3], QY. Sun[1].* 1) Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan 410008, PR China; 2) National Laboratory of Medical Genetics of China, Changsha, Hunan 410008, PR China; 3) Neurodegenerative disorders research center, Central South University, Changsha, Hunan 410008, PR China.

Objective: To evaluate the association between Parkinson disease (PD) and mutations in the glucocerebrosidase gene (GBA) in Chinese population. Background: Mutations in the glucocerebrosidase (GBA) gene have recently been identified as contributing to the development of Parkinson disease (PD) in several populations. Methods: PCR restriction enzyme assay and DNA sequencing of four GBA common mutations (L444P, F213I, R353W and N370S) were carried out in 402 Chinese patients with PD and 413 age- and sex-matched controls. Results: Among the 402 Chinese Parkinson disease patients, we found eleven patients (2.74%) who carried a heterozygous mutant GBA allele. The eleven heterozygotes have the L444P mutation. The average age at disease onset of the eleven patients was 54.64 years, comparable to the disease onset in the total patient group (54.79). All eleven patients carrying a GBA mutation presented with a typical parkinsonian phenotype and had a good or excellent response to levodopa. No heterozygotes were found among the 413 age- and sex-matched controls. We compared the frequency of heterozygotes for GBA mutations among PD patients (11/402) and the control group (0/413) by means of the chi-square test. The difference was statistically significant ( 2=11.456,P=0.001). Conclusions: Our results demonstrate that the GBA gene mutations appear to be a risk factor for Parkinson disease in China .

## 2146/T/Poster Board #695

**Influence of Variations in Two Serotonin Genes and Gender Differences on Learning and Memory.** *E. Vitale[1], P. Khanna[2], C. Myers[2], M.A. Gluck[2].* 1) Institute of Cybernetics, CNR, Pozzuoli (NA) - Italy; 2) Center for Molecular and Behavioral Neuroscience Rutgers University - Newark.

There has been a long-standing debate on whether the basis for individual differences in performing behavioral tasks is rooted in nature or nurture, or more simply put, genetics or environment. In other words, it is still unclear if differences in human learning and memory principally reflect genetic factors, environmental factors or both. We used two polymorphisms of serotonin related genes to address this question. One, a 44-nucleotide insertion/deletion in the promoter region of the serotonin transporter gene (5HTTLPR), already implicated in deficits in different areas of personality measures and learning. The other, a single nucleotide polymorphism of the serotonin 2a receptor gene (His452Tyr), associated with deficits in delayed verbal memory. Our study investigates the relationship of these specific genetic variations to reward and punishment learning, and to personality traits assessed using Cloninger's Tridimensional Personality Questionnaire (TPQ). This study showed that the polymorphism of the serotonin receptor gene does not play a role in underperformance in delayed recall of words. However, males carrying one or more of the Tyr allele scored lower on both the Beck Depression Index and the Harm Avoidance personality trait of the TPQ than males carrying two His alleles. Both genetic and gender differences were found to significantly affect reward learning, where males outperformed females. In addition, individuals carrying both the Long-Tyr and Short-His genotype outperformed individuals carrying both the Short-Tyr and Long-His genotype. Interestingly, the Reward Dependence trait of the TPQ was influenced by the serotonin transporter polymorphism, where females with the long genotype tended to score higher than females with the short genotype. This study shows that both environmental and genetic factors play a major role in differences in learning and memory in healthy individuals.

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## 2147/T/Poster Board #696

**The research on the association between C2264T,G4883C,G7153A variants in LRRK2 gene and sporadic Parkinson's disease in Han population.** *LY. Yao[1], BS. Tang[1,2,3], JF. Guo[1,3], L. Wang[1].* 1) Department of Neurology, Xiangya Hospital,Central South University, Changsha, Hunan, China; 2) National Laboratory of Medical Genetics of China, Changsha, Hunan 410008, PR China; 3) Neurodegenerative disorders research center, Central South University, Changsha, Hunan 410008, PR China.

Background: The LRRK2 mutation and single nucleotide polymorphism (SNP) vary among different ethnicities. Recent researches discovered that certain SNP in LRRK2 gene may increase the risk for Parkinson's disease, and may differentiate patient subgroups and influence future individualized therapeutic strategies. Objective and Method: We conducted a case-control study to determine the prevalence of C2264T(Pro755Leu), G4883C(Arg1628Pro)and G7153A(Gly2385Arg) variants in LRRK2 gene in 401 patients with sporadic PD and 398 unrelated healthy controls in Han population in central part of China, and carried out an association analysis among these three SNPs. We utilized restricted fragments length polymorphism and DNA sequencing methods to identify genotype of these three variants. Result: 1: There is no statistical difference in genotype or allele frequencies of C2264T variant in LRRK2 between sporadic PD patient group and healthy control group(genotype: $\chi2$ =0.657, P=0.686; allele: $\chi2$ =0.654, P=0.687) 2. There is no statistical difference in genotype or allele frequencies of G4883C variant in LRRK2 between sporadic PD patient group and healthy control group(genotype: $\chi2$ =2.02, P=0.248; allele: $\chi2$ =2.02, P=0.248) 3. There is no statistical difference in genotype frequencies of G7153A variant in LRRK2 between sporadic PD group and healthy control group ($\chi2$ =3.71, P=0.156), yet there is statistical difference in allele frequencies of G7153A variant in LRRK2 between sporadic PD patient group and healthy control group ($\chi2$ =3.92, P=0.048, OR=1.66, 95%CI: 1.00-2.76). 4. C2264T, G4883C and G7153A variants in LRRK2 are independent respectively without linkage disequilibrium.(all values of r2 equal to zero) 5. Logistic regression analysis reveals that the regression model admitting age, gender, and genotype of G7153A as independent variables is statistically significant (P<0.05) and age is an independent risk factor for PD(OR=1.997, 95% CI: 1.474-2.706, P<10-3). Conclusion: Neither C2264T(Pro755Leu) or G4883C(Arg1628Pro)in LRRK2 gene is a risk factor for the onset of PD in Han population in central part of China. Both of them are rare in Han population in central part of China. G7153A(Gly2385Arg) in LRRK2 gene is a risk factor for PD in Han population in central part of China. C2264T, G4883C and G7153A variants in LRRK2 gene are independent respectively without linkage disequilibrium.

## 2148/T/Poster Board #697

**Mutant TBP reduces the expression of HSPB1 through abnormal interacting with the NF-Y transcription factor.** *s. huang, M. Roberts, X. li, S. Li.* Department of Human Genetics, Emory University School of Medicine.

Expansion of the polyglutamine (polyQ) tract in human TATA-box binding protein (TBP) causes spinocerebellar ataxia 17 (SCA17),one of the nine polyQ neurodegenerative diseases. It remains unclear how the polyQ tract regulates normal protein function and induces selective neuropathology in SCA17. We generated conditional SCA17 conditional knock-in (KI) mice using mouse endogenous promoters to express mutant TBP at the endogenous level via Cre/loxP recombination system. These mice showed progressive weight loss, neurological symptoms, and neurodegeneration. We found that mutant TBP aggregates co-localized with the components of the NF-Y transcriptional factor in vitro and in transgenic mouse brain. Compared to normal TBP, mutant TBP shows enhanced interaction with one isform of the NF-Y transcriptional factors, NF-YA. Our lab previously reported that, in SCA17 transgenic mice, the small heat shock protein HSPB1, a potent neuroprotective factor, was downregulated. The luciferase reporter assay using mutant TBP and HSPB1 reporter expressed in HEK293 cells showed decrased luciferase intensity. These data indicate that mutant TBP seqesters NF-Y to reduce HSPB1 gene expression and causes transcriptional dysregulation in SCA17 mouse brain, providing insight into the molecular pathogenesis of SCA17. Supported by NIH grants NS045016. Norminator for ASHG trainee awards Xiao-Jiang Li, Membership ID:44111, E-mail:xli2@emory.edu.

## 2149/T/Poster Board #698

**The carboxy-terminal fragment of $\alpha$1A -calcium channel preferentially aggregates in human spinocerebellar ataxia type 6 cerebella.** *T. Ishiguro, K. Ishikawa, H. Mizusawa.* Dept Neurology and Neurological Science, Tokyo Medical & Dental Univ, Tokyo, Japan.

Spinocerebellar ataxia type 6 (SCA6) is an autosomal dominant neurodegenerative disease caused by an expansion of polyglutamine (polyQ) tract in the carboxyl(C)-terminal cytoplasmic domain of $\alpha$1A voltage-dependent calcium channel protein (Cav2.1). It remains controversial whether the main toxicity depends on the loss change of P/Q type Ca channel function, or gain of function mechanism accompanied by accumulation of misfolded protein because of relatively small polyQ expansions compared with other polyQ diseases. We conducted this study to clarify how a small expansion alters Cav2.1 expression pattern in human SCA6 brains. Two new antibodies against the Cav2.1 C-terminus were applied in western blotting and immunohistochemical analyses. First, we showed that C-terminal fragments (CTF) of Cav2.1 (75~85 kDa size) were present in human brains. Second, we could segregate the CTF from purified cytoplasmic aggregates and also found the CTF with polyQ in the nucleus in SCA6 cerebella. On the other hand, we detected the CTF only in cytoplasm in normal control cerebella. In accord with these data, we demonstrated that CTF with small polyQ(Q28) formed oligomers and visible aggregates in cultured cells. We conclude that a small polyQ expansion, within normal length in other polyQ diseases, could confer propensity for protein aggregation on the CTF. SCA6 could have aspects disorders of protein misfolding and oligomer formation similar to other polyglutamine diseases.

## 2150/T/Poster Board #699

**Clinical heterogeniety and gene mutation frequencies in spinocerebellar ataxias with only predominant cerebellar features.** *K. Ishikawa, T. Amino, N. Sato, T. Ishiguro, M. Takahashi, M. Ohbayashi, H. Mizusawa.* Dept Neurology, Tokyo Med & Dental Univ, Tokyo, Japan.

Spinocerebellar ataxia (SCA) is a group of heterogeneous conditions both clinically and genetically. It may not be difficult to predict which gene mutations patients have, when their clinical presentations are typical. However, predicting gene mutations would be difficult on patients without obvious neurological symptoms except for cerebellar dysfunctions (i.e., predominant cerebellar features). This study was conducted to clarify clinical features of patients with predominant cerebellar features useful for predicting gene mutations. Sixty-five consecutive patients who were referred to our facility for genetic testing were studied. After molecular diagnosis, clinical features characterizing SCA subtypes were retrospectively searched. Among enrolled patients, 35% of patients were SCA6, and 30% were the chromosome 16q22.1-linked autosomal dominant cerebellar ataxia (16q-ADCA). Machado-Joseph disease (MJD) was the third frequent (10%) subtype of pure cerebellar syndrome. SCA14, SCA15 or SCA8 were not seen in our cohort. The remaining 25% of patients were patients without known mutations. Clinically, age of onset was higher in 16q-ADCA than SCA6 (SCA6: 45 years vs 16q-ADCA 60 years), and MJD had a mild limitation on upward gaze. We conclude that among patients with predominant cerebellar features, age-of-onset and eye signs could be good predictors for molecular diagnosis.

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## 2151/T/Poster Board #700

**Analysis of SCA2 and SCA3/MJD repeats in Parkinson's disease in mainland China, genetic, clinical, and PET findings.** *W. Junling[1,2], B. Xiao[1,2], X.X. Cui[1,2], J.F. Guo[1,2], L.F. Lei[1,2], X.W. Song[1,2], L. Shen[1,2], H. Jiang[1,2], X.X. Yan[1,2], Q. Pan[3], Long. Zhigao[3], Xia. Kun[3], Tang. Beisha[1,2,3].* 1) XiangYa Hospital Central South University, ChangSha, 86., China; 2) Neurodegenerative Disorders Research Center, Central South University,-ChangSha,86,China; 3) National Lab of Medical Genetics of China, Changsha, 86. China.

Background: Recent reports suggest that CAG triplet expansions of spinocerebellar ataxia type 2 and 3 (SCA2 and SCA3/MJD) genes (ATXN2 and MJD1) are the cause of typical levodopa-responsive Parkinson's disease (PD), several of which were found in patients of Chinese ethnicity. It is unclear whether ethnicity (or founder effects within certain geographical region) alone can explain such an association. Objectives: To investigate the prevalence and clinical feature(s) of SCA2 and SCA3/MJD in patients with Parkinsonism in a Mainland Chinese population and to find the difference in the SCA2 and SCA3/MJD mutations between ataxic and parkinsonian phenotypes. Patients and Methods: CAG triplet repeat expansions of ATXN2 and MJD1 genes were analyzed in a cohort of 452 Mainland Chinese patients affected by typical Parkinsonism, including 386 sporadic and 66 familial forms. Cloning and direct sequencing of the expanded allele was performed in patients positive for the ATXN2 and MJD1 expansion. Striatal dopamine transporter (DAT) was evaluated in one SCA2 and one SCA3/MJD-positive family members using carbon (C 11) [11C]-radiolabeled-CFT positron emission tomography (PET). Results: We found two patients in one familial PD (FPD) family (1.5%) and two sporadic PD patients (0.5%) with expanded CAG repeats in the ATXN2 locus, The expansions ranged from 36 to 37 repeats and were interrupted by CAA as (CAG)n(CAA)(CAG)8 in all the ATXN2 expansion-positive patients. Meanwhile, we found four patients in two FPD families (3%) and another three sporadic PD patients (0.8%) in the MJD1 locus, The expansions ranged from 58 to 73 repeats and were not interrupted by CAA or other variants. All patients had levodopa-responsive Parkinsonism without obvious cerebellar signs. [11C]-CFT PET in affected members in SCA2 and SCA3/MJD families showed decrements of 11C-CFT uptake in all patients listed above. Conclusions: A mutation in SCA2 or SCA3/MJD is one of the genetic causes of PD, specifically FPD, in Mainland China. Parkinson's disease patients, especially those with a family history of PD are strong candidates for routine screenings of SCA2 and SCA3/MJD mutations. [11C]-radiolabeled-CFT PET can provide a useful way to evaluate the degree of nigrostriatal dopaminergic damage in SCA2 and SCA3/MJD-related Parkinsonism.

## 2152/T/Poster Board #701

**Development and analysis of an expanded ataxia protein interaction network that contains CACNA1A and Ataxin-7 and their partners.** *J.J. Kahle[1], N. Gulbahce[2,3], J. Lim[1], C.A. Shaw[1], A.L. Barabasi[2,4], H.Y. Zoghbi[1,5,6,7,8].* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX 77030, USA; 2) Center for Cancer Systems Biology and Department of Cancer Biology, Dana-Farber Cancer Institute, 1 Jimmy Fund Way, Boston, MA 02115, USA; 3) Department of Physics and Center for Complex Network Research, Northeastern University, Boston, MA 02115, USA; 4) Center for Complex Network Research and Department of Physics, University of Notre Dame, 225 Nieuwland Science Hall, Notre Dame, IN 46556, USA; 5) Department of Pediatrics, Baylor Col Medicine, Houston, TX 77030, USA; 6) Division of Neuroscience, Baylor Col Medicine, Houston, TX 77030, USA; 7) Program in Developmental Biology, Baylor Col Medicine, Houston, TX 77030, USA; 8) Howard Hughes Medical Institute, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA.

Several inherited Spinocerebellar ataxias (SCA) are caused by expansion of CAG repeats encoding polyglutamine (polyQ) tracts in proteins of diverse functions. Our lab generated a protein interaction network for ataxia-causing proteins using a high-stringency yeast 2-hybrid screen. Two key ataxia-causing proteins, CACNA1A and Ataxin-7, were not well represented in the network because we failed to identify their interacting partners. We hypothesized that using fragments of the coding region for each of these proteins would prove more successful for identifying interacting partners, and that placing these two proteins and their partners in the network would provide insight about their functions. CACNA1A, the alpha1A subunit of the P/Q-type calcium channel has two predominant splice forms: the MPI isoform contains the CAG repeat in-frame and encodes a polyQ tract in the C-terminus, whereas the MPc isoform lacks the polyQ tract. We screened a human brain cDNA library using baits for both isoforms and identified 86 interacting proteins, 15 of which interact exclusively with the MPI isoform. We also generated overlapping protein-fragment baits for Ataxin-7, a component of a chromatin-remodeling complex, and identified 49 interacting partners. We tested over 30%; of the interactions in mammalian cells by co-affinity purification and validated 77%; of them. We integrated CACNA1A , Ataxin-7, and their partners in the ataxia network and found high connectivity of the ataxia-causing proteins. We next used the ataxia network and Medicare patient data to examine gene-disease interactions for proteins that are in the network and diseases that are comorbid with ataxia. We found the relative risk of macular degeneration (MD) is significantly higher (2.35 fold increase) if a patient has ataxia. We therefore scrutinized the ataxia network and discovered a subnetwork that contains two MD associated proteins. These MD proteins interact with proteins not previously studied in the retina. We tested all of the novel components of the subnetwork and found that all are expressed in the retina. This type of analysis on the ataxia network provided insight into the functional relationship of the ataxia proteins, their interacting partners, gene-disease relationships and comorbidity.

## 2153/T/Poster Board #702

**Activation of p38MAPK and decreased expression of HSP70 in transformed lymphoblastoid cells from patients with Machado-Joseph disease.** *H. Tsai[1], C. Chan[1], W. Lee[2], T. Chen[3].* 1) School of Medical Laboratory and Biotechnology, Chung Shan Medical University, Taichung, Taiwan; 2) Feng Chia University, Taichung, Taiwan; 3) National Taichung Institute of Technology, Taichung, Taiwan.

Machado-Joseph disease (MJD, also called spinocerebellar ataxia type 3) is an autosomal dominant neurodegenerative disorder caused by an expansion of unstable CAG repeats in the coding region of MJD gene (chromosomal locus 14q24.3-q31). This disease is also associated with an accumulation of abnormal proteins, including expanded polyglutamine-containing proteins, molecular chaperones, and the ubiquitin-proteasome system. In this study, two MJD lymphoblastoid cell lines (LCLs) were utilized to examine the effects of polyglutamine expansion on heat shock proteins. Interestingly, under basal conditions, Western blot and semi-quantitative RT-PCR analyses showed a significant decrease of HSP70 protein expression in cells containing expanded ataxin-3, as compared with that of the normal LCL. The heat shock protein HSP70 had strong anti-apoptotic properties and functioned at multiple steps of the apoptotic-signaling pathway. The role of p38MAPK which plays an important role in coordinating genetic responses to various stresses, were subsequently analysis from expanded ataxin-3 and control cells by Western blot analysis with a phospho-p38MAPK antibody. Our data revealed that the expression of expanded ataxin-3 resulted in elevation of p38MAPK phosphorylation. The above results suggested that the activation of p38MAPK may be responsible for the increased cell death observed in expanded ataxin-3 expressing cells. Moreover, interfering with p38MAPK phosphorylation could rescue cells from polyglutamine-induced toxicity. Taken together, our results indicate that expanded ataxin-3 which leads to neurodegeneration can significantly impair the expression of HSP70 proteins and ultimately result in an increase of stress-induced cell death.

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## 2154/T/Poster Board #703

**A neuropathological study of two autopsy cases of early-onset spinocerebellar ataxia 6.** *X.J. Wang[1,2], B.S. Tang[1,2], H. Wang[1,2], H. Jiang[1,2], L. Shen[1,2,3].* 1) Xiangya Hospital, Central South University, ChangSha, Changsha, China; 2) Neurodegenerative Disorders Research Center,Changsha,China; 3) National Lab of Medical Genetics of China,Changsha,China.

Background: Spinocerebellar ataxia type 6 (SCA6) is a late onset autosomal dominantly inherited ataxic disorder, and is among the known CAG-repeat diseases. The permanent and progressive character of the cerebellar deficit mainly starting in the third to fifth decade of life led many researchers to consider SCA6 as a 'pure' cerebellar ataxia of late onset. Patients and Methods: Here, we describe a family with spinocerebellar ataxia type 6, characterized by an earlier onset and a more rapid course. Results: In the two patients we observed more severe neurodegeneration in cerebellum, dentate nucleus and olivary nuclei, furthermore, we show the evidences of synaptic modification in the cerebellar cortex. Conclusions: Morphologically, these findings confirm the mechanisms underlying neurodegeneration in inferior olivary complex and dentate nucleus, which interpreted as the retrograde transsynaptic degeneration and the anterograde transsynaptic degeneration secondary to the cerebellar cortical lesion. Reference: 1 Hong Jiang, Beisha Tang, Kun Xia, Yongxing Zhou, Bo Xu, Guohua Zhao, Haiyan Li, Lu Shen, Qian Pan, Fang Cai.2005 Spinocerebellar ataxia type 6 in Mainland China: molecular and clinical features in four families. J Neurol Sci. 236, 25-29. 2 Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, Amos C. Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the alpha 1A-voltage-dependent calcium channel. Nat Genet. 15(1), 62-9. 3 Manto MU. The wide spectrum of spinocerebellar ataxias (SCAs). Cerebellum. 4(1), 2-6. 4 Ishikawa K, Watanabe M, Yoshizawa K, Fujita T, Iwamoto H, Yoshizawa T. Clinical, neuropathological, and molecular study in two families with spinocerebellar ataxia type 6 (SCA6). J Neurol Neurosurg Psychiatry. 67(1), 86-9.

## 2155/T/Poster Board #704

**TP-PCR Revisited.** *J. Warner, M.E. Porteous.* S.E. Scotland Genetic Service, Western General Hosp, Edinburgh, United Kingdom.

In 1996 we published a generally applicable method for the amplification of tandemly repeated tri-nucleotide sequences (1). The original fluorescent "Triplet Repeat Primed PCR" assay was designed to analyse the 3'UTR CTG repeat of the DMPK gene which is expanded in myotonic dystrophy (DM1). Derivations and extensions of this assay have been used with great success in this and other laboratories for the many different tandemly repeated units involved in disease. Nearly all published assays re-use our original tail primer sequences and tail repeat combinations. We present a series of improved DM1 TP-PCR assays with optimal reaction component and primer choices. We outline a general set of primer design rules for the selection of flanking primers, tails and tail primers leading to improved peak ladder length and uniformity for any tandem repeat unit. We demonstrate how TP-PCR copes well with template heterogeneity arising from mitotic instability or mosaicism giving a better estimate of average expansion size than long range PCR methods. We demonstrate how the "boundary lock" effect which gives maxima within the ladder of peaks at true alleles <100 repeats arises. We show how boundary locking can be manipulated to reduce preferential amplification of smaller alleles. Using these simple rules TP-ladders in excess of 1kb in size are achievable. Adoption and extension of these principles should ultimately lead to the demise of the diagnostic Southern blot for the tri-nucleotide repeat disorders. (1) J Med Genet 1996, 33: 1022-1026.

## 2156/T/Poster Board #705

**Alterations in alternative splicing in a knockin mouse model of FXTAS.** *J.N. Galloway[1], R. Willemsen[2], B.A. Oostra[2], D.L. Nelson[1].* 1) Baylor College of Medicine, Houston, TX; 2) Erasmuc MC, Rotterdam, The Netherlands.

Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) is a late onset neurodegenerative disorder which primarily affects male Fragile X premutation carriers. We and others have shown that expanded noncoding CGG containing repeat RNAs are toxic to neurons. The current molecular model for FXTAS suggests the pathogenic RNA can sequester RNA binding proteins as well as other known and unknown cellular proteins into intranuclear inclusions preventing these proteins from performing their normal cellular functions and thus leading to clinical features of FXTAS. This model is very similar to the disease model for the myotonic dystrophies where one of the downstream effects of protein sequestration to pathogenic RNAs is misregulated alternative splicing. Using an expanded knockin mouse model of FXTAS we have performed a biased screen for changes in known splicing events in brain, heart and skeletal muscle. We find that at least two genes, Agrin and Protein tyrosine phosphotase receptor, type F (*Ptprf*) exhibit altered splicing patterns showing increased exclusion of exon 31 and increased inclusion of exon 19a respectively. Additionally, we observe increased expression of *Fxr1* fetal isoform C in expanded skeletal muscle. These results suggest that misregulated splicing events may be the link to disease phenotypes in FXTAS. Ongoing efforts to characterize splicing events on a genome-wide level using microarrays and deep sequencing will help us to have a better understanding of genetic changes which lead to neurodegeneration.

## 2157/T/Poster Board #706

**Correlation of nuclear accumulation of expanded polyglutamine and dysregulation of sodium channel β4 subunit in Huntington disease transgenic mice.** *F. Oyama[1,2], H. Miyazaki[2], M. Yamada[2], M. Kurosawa[2], Y. Kino[2], N. Nukina[2].* 1) Dept Applied Chemistry, Kogakuin Univ, Hachioji, Tokyo, Japan; 2) Lab Structural Neuropathology, RIKEN BSI, Saitama, Japan.

Nuclear accumulation (NA) of the expanded polyglutamine has been identified before the onset of neuropathology and neurological phenotypes in the Huntington Disease (HD) mice. Transcriptional dysregulation is a main pathological feature of HD. However, the role of NA in the transcriptional dysregulation remains unclear. We identified sodium channel β4 subunit (β4) as an early affected gene in HD mice. To assess the correlation between NA formation and dysregulation of β4, we developed several β4 promoter-Venus (a variant of GFP) transgenic mouse lines which showed distinct expression patterns of the transgene. They were crossed with HD mice and analyzed at the presymptomatic stage. In double transgenic mice, we observed that Venus expression was reduced in NA bearing cells, whereas it was preserved in non-NA bearing cells. These results suggest that NA modulates the gene expression of β4 in HD mice through the promoter dependent manner.

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## 2158/T/Poster Board #707

**Analyses of conditional mouse models of SCA3 reveal reversibility of symptoms.** *J. Boy[1], T. Schmidt[1], H. Wolburg[2], M. Böttcher[1], S. Nuber[1], U. Schumann[1], A.F. Mack[3], I. Schmitt[4], F. Zimmermann[5], C. Holzmann[6], A. Servadio[7], T.B. Leergaard[8], F. Odeh[8], J.G. Bjaalie[8], O. Riess[1].* 1) Medical Genetics, University of Tuebingen, Tuebingen, Germany; 2) Institute for Pathology, University of Tuebingen, Tuebingen, Germany; 3) Institute for Anatomy, University of Tuebingen, Tuebingen, Germany; 4) Clinic for Neurology, University of Bonn, Bonn, Germany; 5) Center for Molecular Biology, University of Heidelberg, Heidelberg, Germany; 6) Medical Genetics, University of Rostock, Rostock, Germany; 7) Telethon Institute of Genetic and Medicine, Naples, Italy; 8) Center for Molecular Biology and Neuroscience and Institute of Basic Medical Science, University of Oslo, Oslo, Norway.

Spinocerebellar Ataxia Type 3 (SCA3) is an autosomal dominantly inherited neurodegenerative disorder caused by the expansion of a CAG stretch in the MJD1 gene. In order to analyze whether symptoms caused by ataxin-3 with expanded polyglutamine repeats are reversible, we generated inducible transgenic mouse models using the "Tet-Off system". This system is based on two constructs: The promoter construct controls the expression of the so-called tTA gene product. The binding of tTA to a TR element in the responder construct induces the transcription of the gene of interest. The expression can be blocked by the addition of doxycycline which allosterically inhibits the tTA. For the ataxin-3 responder mouse lines, a full length construct containing an expanded repeat with the pathological length of 77 glutamine repeats was used. The use of two different promoter mouse lines with known expression in the brain (prion protein (Prp) promoter, Ca2+/calmodulin-dependent protein kinase II (CamKII) promoter) allows us to target the transgene expression to different brain regions. Using the CamKII promoter, the trangene is targeted mainly to the frontal brain and mice display first symptoms at the age of six months. However, when we use the Prp promoter, the transgene is widely expressed in the brain with pronounced expression in the cerebellum and the brain stem. In these mice, first motor symptoms can be detected at the age of two months. We then stopped the expression of the transgene by treatment with doxycycline and compared treated and untreated mice at different levels: Performing immunohistochemical analyses of brain sections, we studied the expression of the transgene and neurodegenerative processes in the brain. Behavioural studies revealed that turning off the pathogenic transgene in an early disease stage led to the reversibility of motor symptoms. Comparable approaches in human patients -if available- would therefore require early initiation.

## 2159/T/Poster Board #708

**Expression of mutant huntingtin in astrocytes causes neurological phenotypes in HD mice via glutamate transporter defects.** *J.W. Bradford[1,2], J.Y. Shin[1], M.A. Roberts[1], S. Li[1], X.J. Li[1].* 1) Dept Human Gen, Emory Univ, Atlanta, GA; 2) Program in Genetics and Molecular Biology, Emory Univ, Atlanta, GA.

Huntington's disease (HD) is the most common of nine inherited neurodegenerative disorders caused by an expanded glutamine tract in the N-terminus of the huntingtin (htt) protein. An expansion of over 37 glutamines in htt results in the late onset of HD symptoms, including movement disorders, body weight loss, cognitive deficits, and eventually death 15-20 years following onset. Although mutant htt is expressed ubiquitously in various types of cells, including glia, how mutant htt in glia contributes to the pathology of HD remains unknown. We generated an HD mouse model that expresses N-terminal human mutant htt with either 98 or 160 polyglutamine repeats under the glial fibrilary acidic protein promoter (GFAP), which drives gene expression in astrocytes. Electron microscope analysis showed mutant htt expression only in astrocytes in the brains of these transgenic mice. Astrocytes, which are the most numerous type of glial cell, express glutamate transporters that remove extracellular glutamate to protect neurons against glutamate excitotoxicity. Mice expressing mutant htt in astrocytes show age- and polyglutamine repeat-dependent neurological phenotypes, including body weight loss, rotarod deficits, and early death when compared to WT littermates or GFAP-23Q control mice. Moreover, double transgenic mice expressing mutant htt in both neurons and astrocytes endure more severe neurological symptoms and earlier death than HD mice that express mutant htt primarily in neurons. Using co-immunoprecipitation with primary astrocyte lysate, we found that mutant htt binds more Sp1 transcription factor than WT htt. ChIP analysis further showed reduced association of Sp1 with the promoter of glutamate transporter. Both of these factors may contribute to the decreased expression of glutamate transporter seen in the brains of these mice. These results challenge the classic cell autonomous view of neurodegeneration by implying an important role for glial mutant htt in HD pathology. These findings also suggest that new possibilities for HD treatment might involve astrocytes. Supported by NIH grants AG019206, NS041669, and NS045016.

## 2160/T/Poster Board #709

**An ordered-subset linkage analysis of niacin skin flush response in schizophrenia.** *W.J. Chen[1,2,3], S.S. Huang[1], C.M. Liu[2], Y.J. Lien[1,3], S.H. Lin[1,3], P.C. Hsiao[3], S.V. Faraone[4], M.T. Tsuang[5,6], H.G. Hwu[1,2].* 1) Institute of Epidemiology, National Taiwan University, Taipei, Taiwan; 2) Department of Psychiatry, College of Medicine and National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan; 3) Genetic Epidemiology Core Laboratory, Division of Genomic Medicine, Research Center for Medical Excellence, National Taiwan University, Taipei, Taiwan; 4) the Genetics Research Program and the Department of Psychiatry and Behavioral Sciences, SUNY Upstate Medical University, Syracuse, NY; 5) Department of Psychiatry and the Center for Behavioral Genomics, University of California, San Diego, CA; 6) Harvard Institute of Psychiatric Epidemiology and Genetics, Harvard Departments of Epidemiology and Psychiatry, Boston, MA.

Previous studies suggested that the attenuation of flush response to topically applied niacin was specific to patients with schizophrenia, showed familial aggregation in families of patients with schizophrenia, and was more impaired in both patients and non-psychotic relatives from families with higher familial loading for schizophrenia. This study aimed to evaluate the linkage signal for schizophrenia in genome scan by means of ordered subset analysis on the basis of the impaired niacin flush response. Subjects of this study were part of the participants of the Taiwan Schizophrenia Linkage Study, which collected a nation-wide family sample with at least two siblings fulfilling the DSM-IV criteria for schizophrenia or schizoaffective disorder, depressive type. The genotyping was conducted by the Center for Inherited Disease Research, with 369 microsatellite markers spaced at an average of 9-cM intervals. Among these families, 197 had at least one member with information on the niacin skin test and were included for this study. The mean niacin flush response score averaged over all the family members with the niacin skin test information was used as the covariate for each family. A series of ordered subset linkage analyses was then conducted to increase the homogeneity of the samples by ranking families according to the mean niacin flush response scores in each family, and generate a new maximum nonparametric linkage z (NPL-Z) score on each chromosome for each subset of families. The statistical significance for a subset-derived increase in linkage signal was evaluated using permutations to obtain a chromosome-wide p value. Two chromosomal regions were found to have significant increases in NPL-Z score by ranking families in descending order of niacin flush score, including 5q35.2 (NPL-Z = 3.65, an increase of 2.97, empirical $p = 0.006$) and 15q26 (NPL-Z = 3.08, an increase of 2.11, empirical $p = 0.047$). Another region with significant increases in NPL-Z score was found on chromosome 22q13.1 (NPL = 2.26, an increase of 2.09, empirical $p = 0.043$) by ranking families in ascending order of niacin flush score. Meanwhile, there was little evidence of linkage on these regions in the original genome wide scan for the whole sample. These results suggest that the flush response to niacin may be a marker of underling heterogeneity in schizophrenia and potentially useful to demarcate subgroups of the disorder with different susceptibility genes.

## 2161/T/Poster Board #710

**BACHD transgenic rats with full-length mutant huntingtin exhibit early phenotypic abnormalities.** *H. Nguyen[1], L. Yu[1], S. Metzger[1], J. Ehrismann[1], T. Ott[1], X. Gu[2], M. Gray[2], W. Yang[2], A. Weiss[3], P. Paganetti[3], O. Riess[1].* 1) Department of Medical Genetics, University of Tuebingen, Tuebingen, Germany; 2) Department of Psychiatry & Biobehavioral Sciences, UCLA, Los Angeles, USA; 3) Neuroscience Research, Norvatis Institutes for BioMedical Research (NIBR), Basel, Switzerland.

Our group has previously generated and characterized transgenic rats, which express a fragment of mutant huntingtin (htt), as model of Huntington's disease (HD) in detail. This rat model mirrors many aspects of HD, but it lacks the full-length mutant htt protein and therefore some aspects of the human condition might be imperfectly replicated. In order to overcome this potential disadvantage, we aimed to generate transgenic rats, which express full-length mutant human htt in the same developmental and tissue- and cell-specific manner seen in patients with the disease. To achieve this, bacterial artificial chromosomes (BACs) containing human genomic DNA spanning the full-length gene with 97 CAG/CAA repeats including all regulatory elements were used. Twenty-three founders were analysed for copy number of BAC insertion, integrity of transgene and size of CAG repeats. In F1 transgenic rats of each line the level of RNA and protein expression in brains was measured and the number of integration sites was determined. Western blot result show that the majority of BACHD rat lines expressed full-length htt at various levels. Two lines were then selected for detailed phenotyping. First results already indicate robust and early progressive motor deficits, impaired motor skill learning, reduced activity as well as reduced drinking and feeding and accumulation of N-terminal htt fragments in the line with the highest expression level of mutant htt.

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## 2162/T/Poster Board #711

**Characterization of N-terminal Huntingtin Fragments that Accumulate in the Nucleus.** *L. Smith[1, 2], XJ. Li[1], SH. Li[1].* 1) Department of Human Genetics, Emory University, Atlanta, GA; 2) Graduate program in Genetics and Molecular Biology, Emory University, Atlanta, GA.

Huntington's disease (HD) is an autosomal dominant, late-onset neurodegenerative disorder characterized by the expansion of a polyglutamine (polyQ) repeat located in the N-terminal region of the huntingtin (htt) protein. Wild-type htt consists of less than 36 glutamine repeats whereas the mutant version of the protein has an expanded polyQ repeat of greater than 37 glutamines. Htt is normally a cytoplasmic protein but in HD, N-terminal fragments of mutant htt localize to the nucleus to form inclusions. In the nucleus, mutant htt has been shown to aberrantly interact with various proteins such as the transcription factors Sp1 and TAFII130. The nuclear toxicity of mutant htt is indicated by the fact that mutant htt preferentially accumulates in the nuclei of striatal neurons, which are most vulnerable in HD. However, the mechanism for the nuclear accumulation of mutant htt remains to be investigated. We first aimed to identify the size of the mutant htt fragment that can accumulate in striatal nuclei. We transfected HEK 293 cells with N-terminal htt fragments of various lengths (212, 300 and 500 amino acids). Using immunocytochemistry and nuclear fractionation techniques, we analyzed whether each of the fragments could accumulate in the nuclei. We found that the smaller fragment of 212 amino acids showed more nuclear accumulation than larger fragments of 300 and 500 amino acids. Next we aimed to determine how phosphorylation of the N-terminus may affect nuclear accumulation of htt. We mutated phosphorylatable sites in the N-terminus of htt to create unphosphorylatable and phosphomimic mutants. We expressed these mutants in HEK293 cells and found that phosphorylation enhances nuclear accumulation and aggregation of mutant htt. Supported by NIH grants NS 045016 and NS41669 Nominator for ASHG trainee award: Xiao-Jiang Li (Membership ID 44111), E-mail: xli2@emory.edu.

## 2163/T/Poster Board #712

**Dentatorubral-Pallidoluysian Atrophy (DRPLA) in an Australian family of Chinese descent.** *K.H.C. Wu[1], R. Beran[2], P. Procopis[3], M. Davis[4], A. Colley[1].* 1) Clinical Genetics Dept, Liverpool Hospital, Liverpool, Sydney, Australia; 2) Neurology Dept, Liverpool Hospital, Sydney, Australia; 3) Neurology Dept, Children's Hospital at Westmead, Sydney, Australia; 4) Neurogenetics Unit, Anatomical Pathology Dept, Royal Perth Hospital, Perth, Australia.

**Purpose:** Dentatorubral-pallidoluysian atrophy (DRPLA) is a rare autosomal dominant neurodegenerative disorder caused by CAG trinucleotide repeats in the Atrophin-1 gene on chromosome 12p13.31. To date, only three Chinese kindreds, one in Taiwan and two in Hong Kong, have been reported. Here we report three affected members over two generations in an Australian family of Chinese descent with DRPLA. One member was diagnosed retrospectively from stored sample. "Birth-order effect" previously reported in two Chinese families was not observed. **Methods:**Case report on clinical presentations, electroencephalographic (EEG) and cerebral magnetic resonant imaging (MRI) features, and molecular genetic studies; and compare these with previously published reports. **Results:** The proband had migrated from China when young. He presented at 49 years of age with wide-based ataxic gait, which progressively deteriorated and he was wheelchair bound in six years. During this interval, he developed cognitive decline and aggressive behaviour. His EEG showed no epileptic activity. His MRI showed generalised cerebral atrophy. His eldest son, at 2 years of age (12 years prior to proband's presentation), presented with severe mental retardation and myoclonic epilepsy, was wheelchair-bound in childhood and died at 12 years of age with an unknown cause. The proband's youngest daughter presented, at age 7 years (4 years prior to the index presentation) with learning difficulties. She later developed epilepsy at age 12 years, and startle myoclonus at age 16 years. Her EEG showed generalised epileptiform activities. Molecular genetic studies showed CAG repeats of 58, 70, and 66 in the proband, his son, and his daughter, respectively. The proband's mother is in her 80's and is well. The proband's father died in South American as a young adult. **Conclusion:** Our family demonstrates genetic anticipation between the two generations, and correlation between the disease severity, age of onset and the number of triplet repeats, consistent with the literature. It however does not support the "birth-order effect" proposed to be due to the effect of advancing paternal age on meiotic instability, as demonstrated in two other Chinese families. Further, it may be speculated that the proband's father either had a "high-end" normal allele that subsequently expanded into pathogenic size in the proband, or had a "low-end" pathogenic expansion and therefore did not manifest the disease prior to his death.

## 2164/T/Poster Board #713

**Identification of novel early-onset progressive cerebellar ataxia gene in Finnish Hound dogs.** *K. Kyöstilä[1,2,3], S. Cizinauskas[4], P. Syrjä[2], E. Suhonen[5], J. Jeserevics[4], A. Sukura[2], H. Lohi[1,2,3].* 1) Department of Medical Genetics, University of Helsinki, Finland; 2) Department of Basic Veterinary Sciences, University of Helsinki, Finland; 3) Department of Molecular Genetics, Folkhälsan Institute of Genetics, Finland; 4) Referral Animal Neurology Hospital Aisti, Vantaa, Finland; 5) Small Animal Clinic Kontiolahti, Kontiolahti, Finland.

Human hereditary ataxias belong to a heterogeneous group of disorders characterized by progressive degeneration of the cerebellum and its connections. Although several causative genes have been identified in human ataxias the precise molecular mechanisms that cause the neuronal degeneration are still poorly understood. Recent annotation of the canine genome and development of the related genomic tools for gene mapping provides us a new animal model to identify novel ataxia genes. Ataxia-causing cerebellar cortical abiotrophies (CCAs) have been described in several dog breeds with breed-specific age of onset and disease progression. We report here the identification of a homozygous mutation in a novel ataxia-causing gene segregating with an early-onset progressive CCA disorder in Finnish Hounds (FH). We also provide clinicopathological characterization for the disease. Affected FH puppies develop sings of motor incoordination at the age of 4 to 12 weeks and after this the disease progresses fairly quickly. Magnetic resonance imaging (MRI) shows reduction in cerebellar size in 9 out of 10 clinically examined affected puppies. Histopathological examination reveals marked degeneration and loss of Purkinje cells in the cerebellar cortex. To map the disease locus we genotyped 14 affected puppies and 20 healthy family members using Illumina's canine 24K SNP chip arrays. The genotyping data was analyzed with PLINK software using both case-control and family-based association methods which identified a 1Mb disease associated region at CFA8 ($P_{raw}$ = 1.83E-6, $P_{genome\text{-}wide}$ = 0.00774). The associated region included 5 genes of which one plausible candidate was selected for mutation screening. Sequencing of the coding regions of the gene revealed a homozygous missense mutation in the affected dogs resulting in a single amino acid change in the encoded protein. Mutation and segregation was confirmed by genotyping 241 randomly selected FHs, which indicated a 10% carrier frequency in the FH population in Finland. The identified gene has not been previously associated with inherited ataxias in any species and we are currently sequencing the human ortholog in early-onset ataxia patients with similar clinical symptoms and unknown genetic background. Identification of the novel ataxia gene will cast more light to the pathogenesis of cerebellar neurodegeneration and will improve our understanding of the disease mechanisms in both species.

## 2165/T/Poster Board #714

**The Autism Genome Project: Dissecting the genetic and genomic etiology of autism.** *S. Ennis on behalf of the Autism Genome Project Consortium (AGP).* Medical Genetics, School of Medicine and Medical Sciences, UCD, Dublin, Ireland.

Autism and autism spectrum disorders (ASD) are neurodevelopmental disorders affecting approximately 1 in 150 individuals and characterized by deficits in reciprocal social interaction, communication and patterns of repetitive behaviors and restricted interests. Evidence to date supports high heritability and a complex genetic architecture. The Autism Genome Project (AGP) was formed to facilitate gene identification by uniting investigators and family data. We will present results from genome-wide association (GWA) analyses from close to 3000 families. Thus far we have results from over 1500 ASD families, roughly half of the families we will analyze during this three-year phase of the AGP. At 1500 families, the sample represents the first stage of the two stage process for screening the genome for CNVs and SNP loci affecting risk for ASD. Our principal GWA analysis is the additive model and our principal partitions of the data split along two axes: ancestry narrow (European) versus all; and inclusive diagnostic group (any ASD) versus narrow (autism). At this point our results implicate only one gene from our main analyses, namely MACROD2 ($p < 2.910^{-8}$). Intriguingly, a strong consistent but smaller signal for association for strict/European covers the gene *SLC25A12* on chromosome 2. This gene has been evaluated extensively in autism data sets because it falls in a linkage region and has been shown to be associated with autism in some but not all smaller data sets. However the signal for association occurs across 25 SNPs spanning a 350Kb region that encompasses *DYNC1I2*, *SLC25A12*, *HAT1* and the 5' region/first exon of *MAP1D*. The minimum p-value, $5.510^{-6}$, occurs for rs6731562 in *HAT1*. Other genes are implicated by various splits of the data. For example, for verbal ASD individuals, a p-value of $1.110^{-8}$ occurs in gene *PLD5* at rs2196826 whereas for non-verbal subjects no SNP in this gene generates an association p-value less than 0.01. Another notable result ($8.110^{-8}$) is for paternal transmission of alleles in *RASAL1*, a gene encoding a RAS-GTPase-activating protein and falling on 12q24.13. The largest test statistic and smallest p-value, $8.110^{-8}$, occurs for rs12422659, a synonymous exonic SNP. Insofar as we are aware, however, there is no evidence for *RASAL1* being imprinted. Whether these loci remain intriguing and whether new loci are implicated will be determined by analyses to be presented at the 2009 ASHG meeting by using a larger number of samples.

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## 2166/T/Poster Board #715

**Genome-wide association study on early-onset bipolar disorder.** *S. Jamain[1], S. Cichon[4,5], B. Etain[1,2], T.W. Muhleisen[4,5], A. Georgi[6,7], N. Zidane[1], S. Herms[4,5], M. Mattheisen[4,5], L. Priebe[4,5], F. Mathieu[1], C. Henry[1,2,3], D. Zelenika[8], I. Gut[8], W. Maier[7], M. Albus[6], T. Schulze[6,9], F. Bellivier[1,2,3], M. Rietschel[6,7], M.N. Noethen[4,5], M. Leboyer[1,2,3].* 1) Dept Genetics, INSERM U955, EQ 15, Creteil, France; 2) Faculty of Medecine, University Paris 12, Creteil, France; 3) Dept of Psychiatry, H. Mondor-A. Chevenvier group, Creteil, France; 4) Institute of Human Genetics, University of Bonn, Bonn, Germany; 5) Dept of Genomics, Life ans Brain Center, University of Bonn, Bonn, Germany; 6) Dept of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany; 7) Dept of Psychiatry, University of Bonn, Bonn, Germany; 8) Commissariat à l'Energie Atomique, Genomic Insitute, Centre National de Genotypage, Evry , France; 9) Unit on the Genetic Basis of Mood and Anxiety Disorders, National Institute of Mental Health, National Institutes of Health, US Department of Health and Human Services, Bethesda, MD, USA.

Many genome-wide association studies (GWAS) have been recently performed on bipolar disorder, leading to discrepant results and difficulties in replication between independent samples. One possible explanation could come from a lack of common genetic variation influencing the vulnerability to bipolar disorder in general, but different susceptibility genes might influence clinical subsets of the disease. Thus, we focused our study on potentially more homogenous and more familial sub-forms of bipolar disorder in order to unravel heterogeneity of the classical categorical entity bipolar disorder and to identify genetic susceptibility genes. We carried out a genome-wide association study using 313,952 single nucleotide polymorphisms (SNPs) on 468 patients with early age at onset or familial history of bipolar disorder and 2832 controls. The strongest association was observed on chromosome 15 ($P$=4.4 x $10^{-7}$) for the whole sample. An additional analysis, including only patients with an early age at onset (N=318), identified several regions for which the association was stronger despite a smaller sample size. Among them, two SNPs located on chromosomes 12p12 and 5p13 ($P$=2.7 x $10^{-7}$ and $P$=3.6 x $10^{-6}$, respectively) are located in genes encoding proteins playing a role in phosphoinositide signalling pathway, which is consistent with the hypothesis that several genes in one pathway may be involved in the pathophysiolgy of bipolar disorder. Our results suggest, at least for bipolar disorder, that the phenotype issue remains an important challenge to identify susceptibility genes.

## 2167/T/Poster Board #716

**Association of common variants at 7p21 with Frontotemporal Dementia with TDP-43 Inclusions.** *P.M.A Sleiman[1], V.M. Van Deerlin[2,3], M. Martinez-Lage[2], A. Chen-Plotkin[2,4], L.S Wang[2], G. Schellenberg[2,3], H. Hakonarson[1,5], J.Q. Trojanowski[2,3], V.M.Y Lee[2,3], International Consortium on Frontotemporal Dementias.* 1) Center for Applied Genomics, Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Center for Neurodegenerative Disease Research, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA; 3) Institute on Aging, University of Pennsylvania School of Medicine, Philadelphia, PA; 4) Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA; 5) Division of Pulmonary Medicine and Department of Pediatrics, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine.

Frontotemporal dementia (FTD) is the second most common cause of dementia in individuals under age 65. The most frequent neuropathological correlate of FTD is frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP), many cases of which are familial. Mutations in the progranulin gene (GRN) account for approximately 20% of cases, genetic risk factors for the remaining 80% of cases have yet to be identified. We assembled an international consortium to identify common genetic susceptibility loci for FTLD-TDP by genome-wide association (GWA). The GWA included 515 cases and 2509 controls. Following standard quality control, cases were matched to controls by 'genetic matching' to reduce population stratification and Cochran-Armitage trend tests calculated for each SNP. Three SNPs mapping to a single LD block on chromosome 7p21 reached genome-wide significance (P-value 1.08x$10^{-11}$; OR 0.61) and replicated in a separate cohort of FTLD-TDP cases (P-value 2x$10^{-4}$). In addition to the three genome-wide significant SNPs nine others also showed significant association with FTLD-TDP, all of which mapped to a 68 Kb interval. The 7p21 variants were also shown to contribute to the genetic risk for FTLD-TDP in a subset of the patients who harbored pathogenic progranulin mutations. To further characterize the candidates we carried out expression data in the brain tissue of patients and controls to identify either phenotype associated differential expression or genetic transcriptional regulators. Our data implicate variants at 7p21 as the first common genetic susceptibility factors for FTLD-TDP.

## 2168/T/Poster Board #717

**Identification of Loci Influencing Age-At-Onset in Late-Onset Alzheimer Disease Implicates Variation on Chromosome 12p.** *A.C. NAJ[1], G.W. Beecham[1], E.R. Martin[1], M.A. Slifer[1], E.H. Powell[1], P.J. Gallins[1], I. Konidari[1], P. Whitehead[1], J.R. Gilbert[1], J.L. Haines[2], M.A. Pericak-Vance[1].* 1) Institute for Human Genomics, University of Miami, Miami, FL; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Alzheimer Disease (AD) is the leading cause of dementia among the elderly with 50%; risk of occurring after 85 years of age. AD is also highly genetic (estimated $H^2$~70%), and susceptibility loci for AD may contribute to earlier age-at-onset (AAO) of disease, as do the *APOE* ε3/ε4 alleles. To identify risk loci for late-onset AD (LOAD), we performed a genome-wide association study (GWAS) of AAO of AD and subsequently of risk for LOAD. We analyzed data on 1,169,331 SNPs genotyped and/or imputed among 1,474 AD cases by combining three GWAS datasets, and imputing non-overlapping SNPs. Initially, we tested SNP associations with AAO among all AD cases in the dataset, following up on associations of P<$10^{-5}$ by testing association with LOAD risk among cases and 1,331 cognitive controls. We observed associations with AAO of SNPs near *APOE* (e.g., rs11672085, P=4.12×$10^{-5}$). We also identified a number of signals with P<$10^{-4}$ that clustered on chromosome 12, specifically 26 SNPs near 33.1-33.2Mb, that demonstrated associations of P<$10^{-5}$ with AAO, the strongest being rs10047666 (P=1.40×$10^{-6}$) genotyped in two of three datasets, though none of these SNPs demonstrated statistically significant association (P<0.05) with LOAD risk. These signals fell ~200kb upstream of the synaptotagmin X gene (*SYT10*), which encodes a presynaptic protein that may contribute to memory performance; *SYT10* expression has been associated with spatial memory loss in mice. Additionally, two independent SNPs strongly associated with AAO (rs7970175 on chromosome 12, P=7.65×$10^{-6}$; rs10944728 on chromosome 6, P=7.73×$10^{-6}$) also showed significant or strong associations in subsequent analyses of LOAD risk (rs7970175, P=1.01×$10^{-9}$; rs10944728, P=1.23×$10^{-5}$), however neither SNP is located in/near known biological candidate genes for AD. Combining three GWASs of LOAD, we observed novel associations of multiple SNPs on chromosome 12 with AAO of Alzheimer Disease. In addition, we observed associations of chromosome 6 and 12 SNPs with AAO and with LOAD, however potential roles for these SNPs remain uncertain and merit further investigation.

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## 2169/T/Poster Board #718

**A Novel focal idiopathic epilepsy locus identified in Belgian Shepherd dogs.** *E.H. Seppala[1,2,3], A. But[1,2,3], I. Baranowska[4], M. Berendt[5], C.H. Gulløv[5], S. Cizinauskas[6], A. Short[7], W. Ollier[7], K. Lindblad-Toh[8,9], M. Fredholm[10], H. Lohi[1,2,3], CNG genotyping platform.* 1) Department Medical Genenetics, University of Helsinki, Finland; 2) Department of Basic Veterinary Sciences, University of Helsinki, Finland; 3) Department of Molecular Genetics, Folkhälsan Institute of Genetics, Finland; 4) Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Sweden; 5) Department of Small Animal Clinical Sciences, University of Copenhagen, Denmark; 6) Referral Animal Neurology Hospital Aisti, Vantaa, Finland; 7) Centre for Integrated Genomic Medical Research, School of Translational Medicine, University of Manchester, UK; 8) Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, MA; 9) Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden; 10) Department of Basic Animal and Veterinary Sciences, University of Copenhagen, Denmark.

Idiopathic epilepsies are common genetic disorders in both human and dogs. Most of the known epilepsy genes have been found in rare monogenic Mendelian forms of epilepsy syndromes and the genetic background of common idiopathic epilepsies has remained largely unknown. Epilepsy is the most common neurological disorder in dogs and they provide natural disease models for human epilepsies, with several discovered epilepsy genes being orthologs of the corresponding human epilepsies, suggesting that novel genes are likely to also contribute to the development of human epilepsy. The aim of this study was to map the epilepsy locus in Belgian Shepherds (BS). The prevalence of epilepsy among BS dogs is estimated to be almost 10% and previous studies have shown that a single locus with a large effect on the incidence of seizures is segregating in this population. Majority of the affected BSs suffer from focal idiopathic epilepsy with possible secondary generalization. We performed a genome wide association study by genotyping 40 cases and 44 matched controls from Finland and Denmark. Samples were genotyped on the Affymetrix v2 canine SNP array (50k) and association was analyzed with PLINK software. We mapped the disease locus to 3-Mb locus at CFA37 ($P_{raw}$= 1.34E-06 and $P_{genome\text{-}wide}$= 0.017) containing 25 genes. None of them are known to be associated with epilepsy. By sequencing a non-ion channel candidate gene, a coding variation resulting in Arg>His change was detected. By genotyping additional (n= 97) BSs we found that 73% of the epileptic dogs (n=37) were homozygous for the mutation compared to 23% of the healthy controls (n=60; OR=8.6; 95% CI 3.4-23.1; P=1.53E-6). However, the same variant was also present in 27 other breeds studied suggesting that it is a common polymorphism which is likely in a close LD with an actual disease-causing mutation. Further fine-mapping and sequencing of the associated region continues in 100 epileptic and 110 healthy control BSs and other breeds. These results indicate that we have mapped and confirmed a novel focal idiopathic epilepsy locus in a dog breed and the identification of the mutation will lead to the discovery of a novel non-ion channel epilepsy gene that can be studied in human focal epilepsy patients.

## 2170/T/Poster Board #719

**Genome-wide significant confirmation of SNPs in SNCA and the MAPT region as common risk factors for Parkinson disease.** *T. Edwards[1], W.K. Scott[1], C. Almonte[1], A. Burt[1], E.H. Powell[1], G. Beecham[1], L. Wang[1], S. Zuchner[1], I. Konidari[1], G. Wang[1], M. Pericak-Vance[1], J. Haines[2], J. Vance[1], E.R. Martin[1].* 1) Miami Institute for Human Genomics, Miller School of Medicine, University of Miami and the Dr. John T. Mcdonald Foundation Dept of Human Genetics; 2) Center for Human Genetics Research, Vanderbilt University Medical Center, Vanderbilt University.

Parkinson disease (PD) is a chronic neurodegenerative disorder with a cumulative prevalence of greater than one per thousand. While several rare Mendelian forms of PD have been described, the genetic factors underlying idiopathic PD have been elusive. Three independent genome-wide association studies (GWAS) have previously investigated the genetic susceptibility to PD using various study designs and genotyping platforms. These studies implicated several genes as PD susceptibility loci with strong, but not genome-wide significant, associations. In the current study, we combined and imputed data for joint analysis from two previously published GWAS, obtained from dbGAP, with our new GWAS with 605 cases and 621 controls genotyped at 491,590 SNPs. Genotyped SNPs in SNCA (rs2736990 genome-wide empirical p = 0.0109, OR = 1.29 95%CI 1.17-1.42 G vs. A allele, PAR% = 12%) and the MAPT region (rs11012 genome-wide empirical p = 0.0079, OR = 1.42 95%CI 1.25-1.61 C vs. T allele, PAR% = 8%) were statistically significant at the genome-wide level after adjustment for multiple tests. No other SNPs were close to genome-wide significant in this analysis, though several biologically implicated genes (RORA, NPAS3, WIPF1, DBC1, GFPT2) were replicated in at least two of three data sets at the 0.05 level with consistent direction of effects across independent samples. These genes are being further investigated in a fourth PD dataset. This study confirms that SNCA and the MAPT region are major genes influencing risk for most PD patients as they have been consistently observed to significantly associate with PD here and in other studies, and these exposures combined explain at most 20% of PD cases.

## 2171/T/Poster Board #720

**DISC1 conditioned genome-wide association study of psychosis proneness in a large birth cohort.** *L. Tomppo[1,2], J. Ekelund[1,2,3], D. Lichtermann[4], J. Veijola[5], S. Gabriel[6], MR. Järvelin[7], N. Freimer[8], W. Hennah[1,2,9], L. Peltonen[1,2,6,10].* 1) Institute for Molecular Medicine Finland FIMM, University of Helsinki, Helsinki, Finland; 2) Public Health Genomics Unit, National Institute for Health and Welfare, Helsinki, Finland; 3) Department of Psychiatry, University of Helsinki, Helsinki, Finland; 4) Café Ersatz, Bonn, Germany; 5) Department of Psychiatry, University of Oulu, Oulu, Finland; 6) Broad Institute, Cambridge, MA; 7) Imperial College, London, United Kingdom; 8) UCLA, LA; 9) Medical Genetics Section, University of Edinburgh, Edinburgh, United Kingdom; 10) Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

DISC1 is currently one of the most promising candidate genes for schizophrenia and other major mental illnesses. We have previously detected association between DISC1 and measures of social and physical anhedonia in the large birth cohort of Northern Finland 1966 (NFBC66). We studied four psychometric scales that are intermediate phenotypes for psychosis, and function as proxies for positive and negative aspects of psychosis. Significant association was observed between previously reported combinations of three DISC1 SNPs and the Revised Social Anhedonia Scale (SAS). In the present study, we performed a genome-wide association study conditioned on these previously recognized DISC1 variants in this cohort, for the outcome measures Revised Social Anhedonia Scale and Revised Physical Anhedonia Scale (PHAS). From the original individuals (N = 12 058), 4 561 individuals attended a 31-year follow up and provided data for the present study. The sample has been genotyped using the Illumina Infinum Assay and HumanHap 370K marker set. Carriers of risk and protective DISC1 variants were analyzed separately (N = 3 054 and N = 962 respectively). The remaining individuals not carrying either of the variants were analyzed as one group (N = 545). No markers were significant at the genome-wide level. However, we noted suggestive evidence for association on several chromosomal regions. These included chromosomes 1q23.1 (risk model, best P = 4.38E-06) and 16q22.1 (protective model, best P = 5.30E-06) for SAS and 2q21.3 (risk model, best P = 1.84E-06) and 12q24.21 (neutral model, best P = 2.24E-06) for PHAS. With these genetically more homogeneous stratified samples we are more likely to recognize variants with small effect sizes that are related to psychosis proneness and potentially interact with DISC1. This approach of using quantitative phenotypes assessed in large population cohorts can be a powerful way of identifying genes that predispose to related clinical disorders, in this case psychosis.

## 2172/T/Poster Board #721

**Runs of homozygosity associated with autism in females: novel candidate gene TMEM47 undergoing recent positive selection?** *P. Lin[1,2,3], J. Albert[3], J. Wortman[3].* 1) Dept Psychiatry, National Taiwan Univ Hosp, Taipei, Taiwan; 2) Maryland Psychiatric Research Center, Catonsville, MD, USA; 3) Dept Medicine, Univ of Maryland School of Medicine, Baltimore, MD, USA.

Previous evidence has suggested that several candidate genes associated with risk of psychiatric disorders, such as schizophrenia, have undergone recent positive selection. One of the lines of evidence for recent positive selection of genetic variants is extended homozygous haplotypes (EHH). A common variant with a recent origin may reflect recent positive selection. Such a common variant may be characterized by ancestral haplotypes identical by descent, which may result in EHH. In the current study, we used Affymetrix 500K SNP arrays to search for EHH associated with common variants in 770 female individuals affected with autism spectrum disorder (ASD) and 300 unaffected female siblings. The EHH was defined as at least 100 contiguous SNPs with homozygous genotypes. EHH where deletions were speculated according to the probe intensity ratio data in the same regions were excluded in the analysis. To interrogate the associations between EHHs and ASD, logistic regression analysis with generalized estimation model to adjust for intra-sibship correlation was performed. The results reveal several chromosome regions that contained EHH that were over-represented in either ASD individuals or unaffected siblings. One of the most interesting findings was obtained in a 157.6 Kb region located on chromosome Xp11 that contains the transmembrane protein 47 (TMEM47) gene, which was found to be more frequent in affected individuals than unaffected siblings (OR = 3.04; p-value = 0.0003). Previous studies suggest that the TMEM47 gene might be associated with mental retardation. Additionally, the TMEM47 gene has been found to be highly expressed in some brain regions, such as prefrontal cortex, amygdale, and hypothalamus, etc. We then queried this gene using the webtool HAPLOTTER and found this gene might have been subject to recent positive selection (p-value = 0.023). Taken together, the TMEM47 gene may undergo recent positive selection and play a role in pathogenesis of ASD.

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## 2173/T/Poster Board #722

**Genome-wide association study in Schizophrenia families.** *L. Rodriguez Murillo[1], J.L. Roos[2], J.A. Gogos[3,4], G.R. Abecasis[5], M. Karayiorgou[1].* 1) Department of Psychiatry, Columbia University Medical Center, New York, NY; 2) Department of Psychiatry, University of Pretoria, Pretoria, RSA; 3) Department of Physiology & Cellular Biophysics, Columbia University Medical Center, New York, NY; 4) Department of Neuroscience, Columbia University Medical Center, New York, NY; 5) Department of Biostatistics, Michigan University, Ann Arbor, MI.

Schizophrenia is a severe psychiatric complex disorder characterized by psychotic symptoms that include delusions and hallucinations, apathy, altered emotional reactivity and cognitive deficits as impairments in executive function, attention and working memory. It is estimated that around 1 % of the worldwide population suffer from schizophrenia. Multiple genetic and environmental factors may be interacting to cause the expression and development of the disease. Currently, genome-wide association studies (GWAS) are being used extensively to discover common genetic factors implicated in complex disorders. We performed a genome-wide association study in the Afrikaners, a founder population from South Africa of European descent. In this association study we are using a family-based approach studying families formed by at least one affected individual. Family-based association studies are advantageous over the case-control approach mainly because they are robust to population stratification effects. We genotyped 1376 individuals from 363 families for ~500,000 single nucleotide polymorphisms (SNPs) with the Affymetrix 5.0 GeneChip array. Following stringent quality-control filtering, preliminary analyses performed with LAMP showed SNPs associated with Schizophrenia with P-values in the order of $10^{-6}$. Some of the SNPs with the lowest P-values are located within genes likely to be involved in the disease pathobiology. Furthermore, we performed meta-analysis of our strongest association signals with data publicly available from other GWAS in Schizophrenia.

## 2174/T/Poster Board #723

**Gene variants associated with schizophrenia in Norwegian genome-wide study are replicated in a large European cohort.** *L. Athanasiu[1, 2, 3], M. Mattingsdal[1, 3], A.K. Kähler[1, 2, 3], A. Brown[3, 4, 5], O. Gustafsson[1], I. Agartz[3, 6], I. Melle[1, 3], V.M. Steen[7, 8], S. Djurovic[1, 2, 3], O.A. Andreassen[1, 3], The SGENE consortium.* 1) Department of Psychiatry, Oslo University Hospital - Ulleval, Oslo, Norway; 2) Department of Medical Genetics, Oslo University Hospital - Ulleval, Oslo, Norway; 3) Insitute of Psychiatry, University of Oslo, Oslo, Norway; 4) Department of Biostatistics, University of Oslo, Oslo, Norway; 5) Department of Matemathics, University of Oslo, Oslo, Norway; 6) Department of Psychiatric Research, Diakonhjemmet Hospital, Oslo, Norway; 7) Dr. Einar Martens Research Group for Biological Psychiatry, Department of Clinical Medicine, Univeristy of Bergen, Bergen, Norway; 8) Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway.

Genome-wide association studies (GWASs) have successfully identified susceptibility genes for various complex disorders. However, despite an estimated heritability of 0.8, few common variants have been identified in schizophrenia. Scandinavian populations are generally reckoned well suited for genetic studies, pertaining ethnically homogenous populations, only recently subjected to non-Caucasian immigration. This notion is ensured in our study as all study subjects are born in Norway and the vast majority has two Norwegian-born parents. Furthermore, since the Norwegian health care system is run by the government, participating hospitals in this study are the only psychiatric health care providers in the catchment area. This has enabled inclusion of a representative and unselected patient sample. We undertook a genome-wide association study (GWAS) of schizophrenia in a Norwegian discovery sample of 201 cases and 305 controls (TOP study) with follow-up analysis in a larger European replication sample of 2,663 cases and 13,780 control subjects (SGENE- study). Firstly, the discovery sample was genotyped with the Genome- Wide Human SNP Array 6.0 (Affymetrix Inc, Santa Clara, CA, USA) and 572,888 markers were tested for schizophrenia association. Secondly, we selected the top 1,000 markers in the discovery GWAS, and tested these (or HapMap- based surrogates) for association in the replication sample. Sixteen loci were associated with schizophrenia (nominal Cochrane Mantel Haentzel P value < 0.05 with concurring OR) in the replication sample. Secondly, we combined findings from these two studies in a meta-analysis using Fischer's combined probability test. The strongest evidence for SNP-schizophrenia associations from the meta-analysis (P < 0.00001) was provided for markers rs7045881 on 9p21, rs433598 on 16p12 and rs10761482 on 10q21. The markers are located in *PLAA*, *ACSM1* and *ANK3* respectively. *PLAA* is not previously described as a susceptibility gene, but the 9p21 is an implied schizophrenia linkage region, potentially harboring vulnerability genes. *ACSM1* on 16p12 is also part of implied schizophrenia linkage region, but the locus is not previously associated with schizophrenia. The association of *ANK3* with schizophrenia is intriguing in light of recent associations of *ANK3* with bipolar disorder, and supports the hypothesis of an overlap in genetic susceptibility between these psychopathological entities.

## 2175/T/Poster Board #724

**More is worse? Genome-wide expression analysis in Williams syndrome.** *L. Dai[1], R. Weiss[2], U. Bellugi[3], D. Mills[4], J. Korenberg[1].* 1) Brain Inst, University of Utah, Salt Lake City, UT; 2) Department of Human Genetics, University of Utah, Salt Lake City, UT; 3) Salk Inst for Biol. Studies, La Jolla, CA; 4) School of Psychology, Bangor University, Gwynedd, United Kingdom.

Williams syndrome (WS) is a rare disorder associated with striking deficits in visual-spatial function, relatively preserved language and a "gregarious" personality, Caused by the deletion of ~25 genes on chromosome 7, WS provides a unique opportunity to link these genes to developmental and functional defects in WS. In a previous study, we applied genome-wide expression analyses to a cohort of 19 subjects with WS and 8 normal controls using Affymetrix Exon 1.0 ST arrays. These arrays allow high resolution exon and gene-level analysis by comprehensive coverage of the genome, including empirically supported and predicted transcribed sequences. We found that rank products analysis revealed a 25-50% reduction in expression levels across WS region transcripts as among the most significant genome-wide reductions between WS and normal. In the current report, we expand the study to a cohort of 34 subjects with WS and 18 normal controls. We first confirmed 179 WS region exons as differentially expressed, and validated the fold change of 8 genes in the WS region by qRT-PCR. Unexpectedly, we found subsets of genes located outside the WS region are over or under-expressed. The top 25 differentially expressed genes outside WS deletion region were validated using qRT-PCR. These results redefine WS as due in part to a deletion and consequent gene over- or under-expression acting through development and in the adult, but also reveal a subset of genes that act downstream of the deletion and represent the first steps to a understanding the network interactions that may mediate the biology WS phenotypes. To further discover the underlying biological pathways, we applied Gene Set Enrichment Analysis (GSEA) and identified, besides the genes in WS region, gene sets involved in cytoskeleton-actin, cell adhesion, membrane trafficking and other cytoskeleton signal pathways. Interestingly, most of the genes in cytoskeleton-actin pathway are over-expressed in WS. They play important roles in neuroblast migration, particularly in the rostral migratory stream, the source of adult neurogenesis, and at the dendritic spine, which suggest a striking dataset leading to models for synaptic function and treatments. In summary, expression analysis of WS implies for the first time the possible genome-wide network effects that are due to the deletion of WS region genes, and we may gain insights into the biological mechanisms of the altered development and adult function in WS.

## 2176/T/Poster Board #725

**Genome-wide association study in the Amish indicates non-APOE genetic effects for dementia.** *A. Davis[1], L. Jiang[1], R. Laux[1], L. McFarland[1], P. Gallins[2], L. Caywood[2], M. Creason[2], D. Fuzzell[2], C. Knebusch[2], C. Jackson[3], W. Scott[2], M. Pericak-Vance[2], J. McCauley[2], J. Haines[1].* 1) Dept CHGR, MPB, Vanderbilt Univ, Nashville, TN; 2) Miami Institute for Human Genomics, University of Miami, Miller School of Medicine, Miami, FL; 3) Scott and White, Temple, TX.

Late Onset Alzheimer's disease (LOAD) is the leading cause of dementia in the elderly. While evidence for an underlying genetic etiology of AD is strong, it has proven to be quite challenging to elucidate. The APOE gene accounts for less than half of the susceptibility and thus other genetic factors are likely to be involved. Genetic heterogeneity is a major complicating factor hindering further gene identification. To overcome this problem and maximize our power to identify AD risk genes, we are studying the genetically isolated and well-defined Amish populations of middle Ohio and northern Indiana. To date we have enrolled over 1850 Amish individuals with 132 of these having either probable or possible dementia. We performed a genome-wide association study (Affymetrix Human SNP Array 6.0) and successfully genotyped 830 Amish individuals (125 with AD). Following QC, 453,389 SNPs were analyzed. We performed parametric and nonparametric 2-pt linkage analysis (Merlin) after using PedCut to divide our large pedigree into smaller, more computationally feasible sub-pedigrees. A lod score of 4.5 was calculated under a recessive model on chromosome 6 at rs4235857, which is ~93 Kb from the EPHA7 gene, known to be involved in the development of the nervous system. Five additional SNPs (4 of which were identified under a recessive model), on chromosomes 3, 6, and 9, had lod scores > 4.0 assuming either a dominant or recessive model. Under a dominant model 4 additional SNPs had lod scores >3.6, and under a recessive model 11 additional SNPs had lod scores >3.6. Because of the relatedness of these individuals, we employed a novel test of association using the MQLS test (Thornton & McPeek). The lowest MQLS p-value (1.7 x 10-7) was calculated for rs6468852 on chromosome 8 ~57 Kb from ATP6V1C1, which encodes a subunit of vacuolar ATPase. Three other SNPs (chromosomes 3, 9, and 11) had p-values <1 x 10-6, and an additional 90 SNPs had p-values <1 x 10-4. Further detailed analysis will clarify these initial results, which suggest novel non-APOE genetic effects for LOAD in our Amish dataset.

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## 2177/T/Poster Board #726

**Genome-wide association study of schizophrenia using STR markers.**
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Schizophrenia is a devastating neuropsychiatric disorder with a multifactorial background. To search for schizophrenia susceptibility loci, we conducted a case-control study using about 30,000 STR markers distributed across the entire genome (average spacing 108 kb; average heterozygosity 0.67). To overcome type I errors due to multiple testing, we designed three sequential steps of screening using independent pooling sample sets. The first screening using pooled samples of 157 case-control pairs revealed 2,966 (10.6%) markers to be significantly associated with the disorder ($p < 0.05$). After the second and third screening steps using pooled samples of 150 pairs each, 52 markers remained to be associated with the disorder. Total 1,536 tagging SNPs in the vicinity of about 200 kb from the 52 positive STR markers were used for an additional genotyping of all screening samples. Significant association was observed for 167 SNPs. We selected 31 SNPs based on levels of $p$ values and conducted a confirmatory association study using large independent samples (2444 - 2475 cases and 2451 - 2479 controls). One SNP located upstream of *SLC23A3* showed the marginal $p$ value (0.054) and significant association with the disorder ($p = 0.004$) in cumulative samples (2891 - 2922 cases and 2946 - 2977 controls). Additional 6 SNPs near the relevant one were employed for genotyping of the cumulative samples. One SNP located between *SLC23A3* and *C2orf24*, and another within *C2orf24* showed significant association with the disorder ($p = 0.026$ and 0.012, respectively). These results suggest *C2orf24* as a candidate for schizophrenia susceptibility in the Japanese population.

## 2178/T/Poster Board #727

**Genome-wide association study for depressive symptom.**
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Background: Depressive disorder is currently one of the leading causes of disease burden in the world. Despite the high prevalence and social impact of depressive disorder, a little is known about the genetic and pathophysiological mechanism. The aims of this study were 1) to identify the association between SNPs and depressive symptom by genome-wide approach with high resolution chip, and 2) to find a new candidate gene for depressive symptom. Methods: The study samples were drawn from the Korean Genome and Epidemiology Study which is an ongoing prospective community-based epidemiology study. In order to measure depressive symptom, we used the Beck Depression Inventory (BDI) score, which is the most widely used self report instrument for measuring the severity of depression. We finally obtained completed BDI questionnaires from 3,203 subjects. Individual genotyping was conducted using the Affymetrix Genome-Wide Human SNP array 500K chip. A total of 324,185 SNPs met quality control criteria and were included in the final analysis data set. We used a linear regression analysis to test for association of SNPs with depressive symptoms using PLINK program. Results: In this genome wide association study for depressive symptom, we found that 20 SNPs demonstrated a significant association with significance level of p<10-4. The identified SNPs were located in the following genes: SFPQ, SDK1, ITPR2, ACCN1, NLGN1, DST, MTHFD1L, HUS1, EXT1, GARNL3, COL5A1, LOC644860, and LOC728602. Among these SNPs, three SNPs in ACCN1 and one SNP in NLGN1 were reported in associated with autism. Conclusion: We have identified several SNPs associated with depressive symptom. For confirming these associations, we will investigate the further biologic and functional annotations.

## 2179/T/Poster Board #728

**Genome-wide association study for Parkinson's Disease in the midwestern US Amish.**
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Parkinson's Disease (PD) is the second most common neurodegenerative disorder of adults. Previous evidence has shown that PD is heritable. Mutations in five known genes ($\alpha$-synuclein (PARK1, PARK4), Parkin (PARK2), PINK1 (PARK6), DJ-1 (PARK7), and LRRK2 (PARK8) cause Mendelian inheritance of PD, while consensus regions from GWAS studies implicate MAPT and SNCA. Other regions have been identified but the genes remain unknown. Genetic heterogeneity complicates the verification of likely genes and the identification of other susceptibility genes. Our approach to overcome the problem of heterogeneity is to study a population isolate, the midwestern Amish communities of Ohio. To date we have enrolled over 1850 Amish individuals with 38 of these diagnosed with PD. In this dataset we see a higher average kinship coefficient among our cases (.016) compared to the rest of our dataset (.012), suggesting that PD is heritable in the Amish. We have undertaken a genome-wide association study (Affymetrix-Genome-Wide Human SNP Array 6.0) and genotyped 900 Amish individuals (33 with PD). Through the use of the Anabaptist Genealogy Database (AGDB), we have determined the family structure for our collected individuals. Following QC, 453,389 SNPs and 827 individuals were analyzed. Because of the relatedness of these individuals, we employed a novel test of association using the MQLS test (Thornton & McPeek). Initial results identified 288 SNPs with p-values less than 1 x 10-4. Five SNPs, located on chromosomes 19, 15, and X, reached genome-wide significance level (< 1 x 10 -7). The most significant result was found on chromosome 19 at rs10402506 with a p-value of 9.0 x 10-9. The closest gene, LILRA2 (Leukocyte immunoglobulin-like receptor) is less than 1 Kb away from this SNP. Within the most significant regions, determined by the MQLS results, we searched for stretches of homozygosity preferentially occurring in affected individuals. Three such regions were identified on chr 13 , 15, and X, implicating the TLN2 and GLuR3 genes. Further analysis will confirm these results which indicate possible identification of novel PD-susceptibility genes.

## 2180/T/Poster Board #729

**Genome-wide Association of Cerebrovascular and Neurodegenerative Quantitative MRI Traits in Alzheimer Disease.**
*A. Parrado[1], M. Logue[1], K.L. Lunetta[2], L.A. Cupples[2], C. DeCarli[3], C.T. Baldwin[1], L.A. Farrer[1], MIRAGE Study.* 1) Genetics Program, Boston University, Boston, MA; 2) Department of Biostatistics, Boston University School of Public Health, Boston MA; 3) University of California, Davis Center for Neuroscience, Davis, CA.

Most genetic association studies of Alzheimer disease (AD) have focused on the dichotomous AD diagnosis outcome. However, the AD phenotype is suboptimal because of likely subject misclassification and underlying genetic complexity. With the exception of APOE, genome wide scans of AD conducted to date have not identified significant associations which are sustained in replication studies. Recently, using a candidate gene approach we showed significant associations between SORL1 (a robustly confirmed AD locus) and MRI-based semi-quantitative measures of white matter hyperintensities (WMH), generalized cerebral atrophy (CA) and hippocampal atrophy (HA), thus encouraging use of these endophenotypes for AD. To identify additional genes related to AD, we performed a genome-wide association analysis of WMH, CA, and HA measured in a Caucasian sample of 288 affected and 313 cognitively healthy members of 209 sibships from the MIRAGE Study who were genotyped using the Illumina HumanCNV370-Duo and the Human610-Quad BeadChips. Genotypes for a total of 1.39 million HapMap SNPs were imputed with the MaCH software using the CEU HapMap subjects as a reference panel. Association analyses were performed using Generalized Estimating Equations to account for sib correlations and adjusted for age at MRI, sex, disease duration, genotyping panel version, ancestry measured by genetic principal components, and APOE $\varepsilon 4$ status. Although no results were genome-wide significant, p-values < 1 x $10^{-6}$ were observed for several SNPs with CA (FLI32682), HA (NFIB), and WMH (LOC100131576, GJA9, RHBDL2, LOC100130627, ARHGAP26 and SUSD1). While the veracity of these associations awaits confirmation and the relationships of these loci to AD requires further study, a SNP in NCSTN showed a noteworthy association with HA (p=3.2 x $10^{-6}$, OR 1.9, 95% CI 1.4-2.4). NCSTN encodes the protein nicastrin which binds presenilin and was previously associated with AD in several but not all studies. The hippocampus is the primary early target of neuronal loss in AD. A replication study using an independent group of more than 600 MIRAGE subjects is currently in progress. These results emphasize the potential importance of neuroimaging endophenotypes as biological markers for discovering genes influencing the etiology of AD.

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## 2181/T/Poster Board #730

**An Irish Genomewide Association Study of Schizophrenia.** *B. Riley[1], The Irish Schizophrenia Genomics Consortium, The Wellcome Trust Case/Control Consortium.* 1) Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth Univ, Richmond, VA; 2) Dept. of Psychiatry, Trinity College Dublin, Dublin, Ireland; 3) Division of Psychiatry and Neuroscience, Queens University Belfast, Belfast, Northern Ireland; 4) Depts. of Genetics, Psychiatry, & Epidemiology, University of North Carolina-Chapel Hill, Chapel Hill, NC; 5) Mental Health Service Line, Washington VA Medical Center, Washington DC, USA; 6) Health Research Board, Dublin, Ireland; 7) Center for Biomarker Research and Personalized Medicine, School of Pharmacy, Virginia Commonwealth Univ, Richmond, VA.

The genomewide association study (GWAS) is a powerful, systematic and unbiased approach to the study of the common disease/common variant (CDCV) hypothesis of complex disorders like schizophrenia. We have assembled a sample of 2357 narrow diagnosis Irish cases and 2000 Irish population controls, and can access 6000 additional UK controls. All cases meet narrowly defined schizophrenia or poor-outcome schizoaffective disorder diagnoses, were assessed directly with a structured clinical interview and had final diagnoses made using consensus criteria. This sample provides ≥80% power to detect allelic odds ratios (ORs) 1.2-1.3 with minor allele frequency (MAF) 0.2-0.3, and is also completely ethnically homogeneous, which should increase its power to detect association further. We have completed 1 million SNP data collection in the second round of the Wellcome Trust Case/Control Consortium, and the SNP genotype dataset is entering analysis at the time of abstract submission. Primary analysis of copy number variation data and of the SNP genotype data with the CA Trend test will be complete for presentation by 10/09. Additional planned analyses (some of which may be complete by 10/09) include 1) weighted FDR analysis using the results of 3 other large GWAS of schizophrenia, 2) analyses of additional phenotypic and clinical data, 3) analysis of evidence for biological or statistical interactions, 4) analyses of pathways and systems and 5) analysis of co-expression.

## 2182/T/Poster Board #731

**Genome-wide association study identifies common variants on two novel loci, α-synuclein, and upstream LRRK2 as genetic risks for Parkinson's disease.** *W. Satake[1,2], Y. Nakabayashi[1], I. Mizuta[1], C. Ito[1], M. Kubo[3], T. Kawaguchi[3], T. Tsunoda[3], M. Watanabe[4], A. Takeda[5], K. Nakashima[6], K. Hasegawa[7], F. Obata[8], T. Yoshikawa[9], H. Kawakami[10], S. Sakoda[2], M. Yamamoto[11], N. Hattori[12], M. Murata[13], Y. Nakamura[3,14], T. Toda[1].* 1) Div Molecular Brain Science, Kobe Univ Grad Sch Med, Kobe, Hyougo, Japan; 2) Department of Neurology, Osaka University Graduate School of Medicine, Suita 565-0871, Japan; 3) Center for Genomic Medicine, RIKEN, Yokohama 230-0045, Japan; 4) Department of Neurology, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba 305-8575, Japan; 5) Division of Neurology, Tohoku University Graduate School of Medicine, Sendai 980-8574, Japan; 6) Department of Neurology, Tottori University Faculty of Medicine, Yonago 683-8504, Japan; 7) Department of Neurology, Sagamihara National Hospital, Sagamihara 228-8522, Japan; 8) Division of Clinical Immunology, Graduate School of Medical Sciences, Kitasato University, Sagamihara 228-8555, Japan; 9) RIKEN Brain Science Institute, Saitama 351-0198, Japan; 10) Department of Epidemiology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima 734-8553, Japan; 11) Department of Neurology, Kagawa Prefectural Central Hospital, Takamatsu 760-8557, Japan; 12) Department of Neurology, Juntendo University School of Medicine, Tokyo 113-8421, Japan; 13) Department of Neurology, National Center Hospital of Neurology & Psychiatry, Kodaira 187-8551, Japan; 14) Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan.

Parkinson's disease (PD) is one of the most common neurodegenerative diseases worldwide, affecting 1-2% of individuals aged ≥65 years. To identify susceptibility variants for PD, we performed a GWAS and two replication studies in a total of 2,011 PD cases and 18,381 controls in the Japanese population. In the GWAS stage, we genotyped 561,288 SNPs on autosomal and sex chromosomes using the HumanHap550 array (Illumina). The GWAS stage included 1,078 cases and 2,628 controls in the Japanese population. After SNP and sample quality control analyses, we used high quality genotype data of 435,539 SNPs in 988 cases and 2,521 controls in the GWAS stage. We observed a close match to that expected under the null distribution (genomic inflation factor $\lambda_{GC}$ = 1.055 for PD). This indicates minimal overall inflation of genome-wide statistical results due to population stratification. For fast-track replication, we selected the 337 most associated SNPs (Cochran-Armitage trend; P ≤0.000533) from analysis of GWAS data and genotyped them in two case-control sample sets (Replication 1 and 2) that were independent of each other: the first sample set (Replication 1) consisted of 612 cases and 14,139 controls and the second one (Replication 2) consisted of 321 cases and 1,614 controls. We identified a novel and strong susceptible locus for PD (Novel Locus 1; P = $1.52 \times 10^{-12}$, OR = 1.30) and designated this region as PARK16. A novel and strong association with PD was also detected (Novel Locus 2; P = $3.94 \times 10^{-9}$, OR = 1.24). The two regions contain interesting genes for PD etiology. We detected a strong disease association at SNCA on 4q22 (rs11931074; P = $7.35 \times 10^{-17}$, OR = 1.37) and LRRK2 on 12q12 (rs1994090; P = $2.72 \times 10^{-8}$, OR = 1.39), both causative genes for autosomal dominant forms of parkinsonism (AD-parkinsonism). The MAPT locus was not identified significant in our study, because, unlike Caucasian, the MAPT H2 haplotype was absent in East Asians. This reflects population differences in the genetic heterogeneity of PD etiology. Our data show 4 genes associated with increased risk of PD, including 2 novel ones. Our data also show involvement of causal genes of AD-parkinsonism in typical PD and highlight population differences underlying genetic heterogeneity in PD.

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## 2183/T/Poster Board #732

**Genome-wide association study of Gilles de la Tourette Syndrome in European ancestry samples and four population isolates.** *J.M. Scharf[1], B.M. Neale[1,2], S. Service[3], A. Tikhomorov[4], J. Fagerness[1], A. Pluzhnikov[4], D. Yu[1], J. Crane[1], M. State[5], J. Tischfield[6], D. Cath[7], G. Rouleau[8], A. Ruiz-Linares[9], C. Mathews[10], C. Sabatti[3], S. Purcell[1,2], N. Freimer[3], N. Cox[4], D.L. Pauls[1], GTS GWAS Consortium.* 1) Psychiatric Neurodevelopmental Genetics Unit, Massachusetts General Hospital, Boston, MA; 2) Program in Medical and Population Genetics, Broad Institute, Cambridge, MA; 3) Center for Neurobehavioral Genetics, University of California, Los Angeles, CA; 4) Department of Human Genetics, University of Chicago, Chicago, IL; 5) Yale Child Study Center,Yale University, New Haven, CT; 6) Department of Genetics, Rutgers University, Piscataway, NJ; 7) Department of Psychiatry, University of Utrecht, Netherlands; 8) Center for the Study of Brain Disorders, University of Montreal, Canada; 9) Department of Genetics, Evolution and Environment, University College London, England; 10) Department of Psychiatry, University of California, San Francisco, CA.

**Introduction:** Gilles de la Tourette Syndrome (GTS) is a highly heritable neuropsychiatric disorder, though no unequivocal GTS susceptibility genes have been identified. Here, results from the first genome-wide association study (GWAS) of GTS in 1,749 cases and 4,410 ancestry-matched controls will be reported. **Methods:** Cases included 983 GTS subjects of European ancestry (EA) as well as 766 GTS subjects from four population isolates (French Canadian (FC), Ashkenazi Jewish (AJ), Antioquia Colombian (CO) and Central Valley Costa Rica (CR). Diagnoses were made using DSM-IV-TR criteria. Case samples and population isolate controls from FC and CO were genotyped on the Illumina Human610Quad Beadchip; EA and AJ controls were previously genotyped on the Illumina Human550K and Human317K Beadchips, respectively. QC and stratified analyses in each population were performed in PLINK. **Results/Conclusions:** Preliminary results are presented based on analysis of 95% of the data. After standard QC to remove poorly performing SNPs and individuals, 1552 cases and 4027 controls remained. To control for population substructure, multi-dimensional scaling was performed and population outliers were excluded. Data were analyzed using logistic regression under an additive model with significant MDS axes included as covariates. Resulting $\lambda_{GC}$ after correction for the first four MDS axes were as follows: EA cases, 1.11; AJ 1.02; FC 1.02; CO 1.01; CR 1.04. Preliminary association analysis identified 8 loci with genomic control-adjusted p-values in the $10^{-7}$-$10^{-9}$ range (seven in the European ancestry sample and one in the Ashkenazi sample). The most significant autosomal locus in the EA sample was located 10 kb downstream of *CACNA1B* (OR 1.47; p=5x$10^{-8}$), which encodes the pore-forming subunit of the N-type calcium channel. In the AJ sample, the strongest signal was found in a 1 Mb gene desert on chromosome 8; the closest gene, *KCNU1*, encoding a voltage-gated potassium channel, lies 250 kb proximal to this locus (OR= 3.0; p=9.5 x $10^{-9}$). In the FC and CR/CO populations, no loci had GC-adjusted p<$10^{-6}$. Meta-analysis across the five populations identified multiple, additional loci with concordant signals across the populations, though none reached genome-wide significance. Results of analyses from the complete case sample will be presented as well as from imputation of all HapMap SNPs. Additional genotyping in a replication sample will be needed to confirm these results.

## 2184/T/Poster Board #733

**A Genome-wide Association Study of Quantitative Traits Related to ADHD.** *A.K. Smith[1], J.F. Cubells[1,2], D.J. Cutler[2].* 1) Psychiatry & Behavioral Sci, Emory University , Atlanta, GA; 2) Dept of Human Genetics, Emory University , Atlanta, GA.

The discovery of candidate genes is often challenging, especially when the etiology of a phenotype is as complex as the development of childhood psychopathology. Still, an emerging literature supports the heritability of several behavioral traits and disorders commonly diagnosed in children, including Attention Deficit Hyperactivity Disorder (ADHD). This study performed a genome-wide association analysis on the publically available GAIN dataset on ADHD, to identify SNPs associated with the dimensions of the Strength and Difficulties Questionnaire (SDQ), which assesses emotional symptoms, inattention/hyperactivity, conduct problems, peer problems, and prosocial behavior. Samples with call rates <90, duplicate samples, and subjects with heterozygosity inconsistent with gender on the X chromosome were eliminated. SNPs were eliminated if they had: a genotyping error rate >1.5 in any one of three genotype classes, a missing data rate >10 for any genotype class, an overall missing data rate of >1/5 the minor allele frequency or any departure from HWE significant at p<0.0001 in the parents of the probands. After removing the SNPs that did not meet QC criteria, 474,959 SNPs were examined using FBAT. Notably, associations were observed between emotional problems and 17 SNPs (6.70x$10^{-7}$ < p < 0.041) in *diacylglycerol kinase, beta* (*DGKB*), which is expressed intensely in the caudate putamen and participates in dopamine-mediated regulation of movements and learning. Twenty-six SNPs in *neuronal PAS domain protein 3* (*NPAS3*), a gene previously associated with biopolar disorder and schizophrenia and involved in hippocampal neurogenesis, were associated with hyperactivity (6.8x$10^{-5}$ < p < 0.047). Conduct problems were associated with 8 SNPs (1.9x$10^{-6}$ < p < 0.022) in *death-associated protein kinase 1* (*DAPK1*), an epigenetically regulated gene associated with Alzheimer's disease. Twenty SNPs in *SRY (sex determining region Y)-box 5* (*SOX5*), which postmitotically regulates migration, postmigratory differentiation, and subcortical projections of neurons, were associated with peer problems (1.7x$10^{-5}$ < p < 0.045). Also, 16 SNPs in *cadherin 4* (*CDH4*), a gene involved in brain segmentation and neuronal outgrowth, were associated with prosocial behavior (5.5x$10^{-6}$ < p < 0.050). This approach has resulted in the identification several genes that warrant further investigation into their associations with child behavior and the development of childhood psychopathology.

## 2185/T/Poster Board #734

**Genome-wide association study of schizophrenia in a Chinese population.** *H.C. So[1], R.Y.L. Chen[1], E.Y.H. Chen[1], E.F.C. Cheung[4], S.S. Cherny[1,2,3], T. Li[5], P.C. Sham[1,2,3].* 1) Department of Psychiatry, University of Hong Kong, Hong Kong; 2) Genome Research Centre, University of Hong Kong, Hong Kong; 3) The State Key Laboratory of Brain and Cognitive Sciences, University of Hong Kong, Hong Kong; 4) Castle Peak Hospital, Hong Kong; 5) Division of Psychological Medicine and SGDP Centre, Institute of Psychiatry, King's College, London, UK.

Schizophrenia is a severe psychiatric disease with a lifetime risk of approximately 1%. Although the heritability of schizophrenia is high, very few susceptibility genes have been identified to date. Genome-wide association study (GWAS) has proved to be a powerful tool in dissecting the genetic basis of complex diseases. To date, most GWAS on schizophrenia were conducted on Caucasians. However, allele frequencies, linkage disequilibrium patterns and genetic or environmental backgrounds may differ among populations, rendering some variants more readily discovered in one population than the other. We have performed a GWAS on schizophrenia with 400 cases and 1311 controls in a Chinese Han population. Samples were genotyped using the Illumina Human610-Quad BeadChip. After quality control procedures, the dataset consisted of 477354 SNPs from 396 cases and 1286 controls. In total 67 SNPs achieved nominal p-values less than 1E-4. We also extracted the association results of SNPs lying within 30 candidate genes listed in the SZgene database. The QQ plot however shows no evidence of enrichment of significant variants within these candidate genes. The most significant SNP in the candidate gene analysis belongs to the DISC1 gene with a p-value of 8.21E-4. We plan to perform a replication study in an independent sample of family trios from Chengdu, China. In addition to selecting SNPs reaching the highest significance levels in the whole-genome scan, we also plan to select top SNPs in the linkage hotspots suggested by a recent meta-analysis. We will adopt a more liberal threshold for SNPs lying within linkage regions than the other SNPs in the genome, as the former group probably has stronger prior evidence for association.

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## 2186/T/Poster Board #735

**A genome-wide association study of obsessive-compulsive disorder.**
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Introduction: Obsessive-Compulsive Disorder (OCD) is a complex genetic illness. This first genome-wide association study (GWAS) of OCD included European ancestry cases & family trios from international sites of the OCF Genetics Collaborative. Methods: Cases included 1558 OCD subjects of European ancestry as well as 477 OCD affected probands & their parents. Diagnoses were made using DSM-IV-TR criteria. Case & familial trio samples were genotyped on the Illumina Human610Quad Beadchip; 3,212 European ancestry 'iControl' samples were previously genotyped on the Illumina Human550K Beadchip. QC & stratified analyses were performed in Plink. TDT & case-control samples were weighted by their power to detect association. GPC based non-centrality parameters were employed. A weighted Z approach was used to transform P into Z-values for combining the two samples. Results/Conclusions: Preliminary results are presented. After standard QC to remove poorly performing SNPs and individuals with low call rate (<97%), excess heterozygosity, sex discordance, or unexpected duplications/cryptic relatedness, 1558 cases remained. Further checks for familial relationship confirmation and Mendel errors left 477 trios in 458 nuclear families. In addition, 3,212 iControl samples with >5% minor allele frequency, <5% missingness at SNP and individual levels, HWE and IBD sharing thresholds of p=10-3 HWE & 0.15 remained. Multi-dimensional scaling (MDS) was performed & population outliers were excluded. Data were analyzed using logistic regression under an additive model with significant MDS axes included as covariates. The resulting genomic control $\lambda$ from the case-control component was 1.084 & for the familial trio (TDT) sample was 1.039. Combining these two datasets yielded a $\lambda$ of 1.09. Preliminary, genomically controlled association analyses identified two loci with p-values in the 10-7 range & 12 loci with p-values in the 10-6 range. Four of the top 14 associated genes are involved in glutamate neurotransmission (GRK4, GRID2, GRIN2B and DLGAP1), consistent with current theories regarding OCD pathophysiology. Furthermore, GRIN2B & genes in the GRK & DLGAP families were previously reported in candidate gene studies of OCD. Although the results of this first GWAS study of OCD were promising, with multiple biologically plausible associated loci, results fell short of genome-wide significance. Additional genotyping in independent samples will be necessary to confirm these findings.

## 2187/T/Poster Board #736

**Genome-wide patterns of genetic variation in individuals with autism spectrum disorders from Croatia.** *L. Wang[1,4], D. Hranilovic[7], K. Wang[6], L. Yurcaba[5], Z. Petkovic[8], N. Gidaya[2], B. Jernej[9], H. Hakonarson[3,6], M. Bucan[2,4].* 1) Pathology and Lab Medicine, University of Pennsylvania, Philadelphia, PA; 2) Genetics, University of Pennsylvania, Philadelphia, PA; 3) Pediatrics, University of Pennsylvania, Philadelphia, PA; 4) Penn Center for Bioinformatics, University of Pennsylvania, Philadelphia, PA; 5) Neuroscience Graduate Group, Biomedical Graduate Studies, University of Pennsylvania, Philadelphia, PA; 6) Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA; 7) Faculty of Science, University of Zagreb, Zagreb, Croatia; 8) Psychiatric Hospital for Children and Youth, Zagreb, Croatia; 9) Laboratory for Neurochemistry and Molecular Neurobiology, Institute Rudjer Boskovic, Zagreb, Croatia.

Genome-wide association studies of complex diseases require genotype analysis of a large global population samples, including cases and control subjects. However, individuals with different ethnic origins may differ in genetic variation, leading to spurious associations. As a first step in the analysis of susceptibility to autism spectrum disorder (ASD) in Croatia, we characterized genetic variation in a sample of 103 subjects with ASD and 200 control individuals. All subjects were genotyped using the Illumina HumanHap550 BeadChip platform. Distance-based phylogeny analysis together with several previously published populations revealed that Croatian subjects cluster, as expected, with Southern Europeans; in addition, individuals from the same geographic region within Croatia cluster together. Croatian subjects are separated from a sample of 600 healthy control subjects of European origin from North America, while Croatian ASD cases and controls are well mixed. A comparison of runs of homozygosity indicated the number and the median length of regions of homozygosity was higher for ASD subjects than for controls. The analysis of this small sample of 103 ASD individuals did not reveal a genome-wide significant ASD susceptibility loci, although among 728 rare CNVs found in Croatia cases and controls, 118 rare CNVs in ASD candidate regions were found in affected subjects. Moreover, three CNVs, including deletion of ACCN1 on Chr 17q11.2-q12, deletion of upstream region of LRRC4C on Chr 11p12-p11.2, and HSFX1 duplications on Chr X, were detected in affected individuals from Croatia and in a large cohort of 900 ASD cases from the USA, but not in corresponding control subjects.

## 2188/T/Poster Board #737

**Pathway analysis of multiple GWAS datasets implicates genes in the extracellular matrix - receptor interaction pathway with autism risk.** *B.L. Yaspan[1], J.S. Sutcliffe[1], M.A. Pericak-Vance[2], J.L. Haines[1], the Autism Genome Project.* 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Miami Institute for Human Genomics, University of Miami, Miami, FL.

Autism is a common neurodevelopmental disorder characterized by deficits in language, reciprocal social interaction and patterns of rigid compulsive behaviors. Twin and family studies indicate that autism has a predominantly genetic etiology, and the presence of broader autism phenotype features in first degree relatives further supports heritability. Substantial progress has been made identifying rare copy number variants associated with autism, but most common allelic associations fail replication; possibly a function of locus heterogeneity and small effect sizes at individual loci. An exception is the recently reported association at 5p14.1, implicating cell adhesion molecules CDH9 and CDH10. As rare variants in autism tend to cluster in specific pathways, we hypothesized that common alleles of modest effect might also cluster in specific pathways to collectively affect risk. We conducted a pathway-based analysis using the KEGG database on the recently published Collaborative Autism Project (CAP) GWAS (487 trios) for exploration and on the larger Autism Genome Project (AGP) GWAS (1442 trios) for replication. If multiple genes in a pathway have an additive effect, statistical association for each SNP might be modest. Thus, known genes (±50kb) containing 2+ SNPs with nominal $p \leq 0.05$ and an $r^2 \leq 0.8$ were included in the analysis (n= 8884 Ensembl gene IDs; 185 KEGG pathways). We tested for enrichment (En) of observed: expected number of genes in each pathway. After multiple testing correction, 10 pathways were significantly overrepresented; strongest being the extracellular matrix-receptor interaction (ECM-RI) pathway (En=2.09, p=1.12E-6). We applied similar SNP and gene inclusion methods to the AGP dataset. Both strict and autism spectrum proband strata were highly significant when testing the ECM-RI pathway (strict En= 5.81,p=4.20E-13;spectrum En=4.54;p=2.17E-10). Further analysis of both datasets showed related signaling pathways focal adhesion and cell adhesion molecules (with CDH9, CHD10) were also significant (adj p from 0.003-1.86E-12). Ongoing analyses will explore the effect of potential biases such as gene and pathway size on these results. However, these preliminary results suggest that variation within genes of the ECM-RI pathway may collectively conspire to increase risk of autism and warrant further investigation.

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## 2189/T/Poster Board #738

**Genome-wide survey for genetic loci underlying noise phobia in herding breed dogs.** *J.S. Yokoyama[1], M.L. Chang[1], K.L. Overall[2], N. Branson[2], S. Juarbe-Diaz[2], D.J. Dyer[2], S.P. Hamilton[1].* 1) Neurogenetics Laboratory, Department of Psychiatry and Institute for Human Genetics, University of California, San Francisco; 2) Center for Neurobiology and Behavior, Department of Psychiatry, University of Pennsylvania.

**Purpose**: The domestic dog is an excellent model for the study of complex behavioral phenotypes. Dogs exhibit naturally occurring behavioral pathologies that are analogous and probably homologous to human psychiatric disorders. Noise phobia, characterized by panic responses to sounds such as thunder, gunshots, and fireworks, is clinically similar to specific phobia in humans, is enriched in certain breeds, and can adversely affect quality of life for both dogs and owners. The distribution of noise phobia within and between canine families and populations suggests an underlying genetic basis for this pathology. The history and homogeneity of individual breeds facilitate efforts to detect associations between genes and complex behaviors that would be challenging in human populations. **Methods**: We conducted a genome-wide survey for SNPs associated with noise phobia in a sample of 183 dogs across 27 breeds using the Affymetrix Canine v2.0 array. Our sample included multigenerational pedigrees of Border Collies and Australian Shepherds that segregate noise phobia and a subset of unrelated cases and controls from several breeds. We filtered markers using standard quality control statistics and assessed phenotypes for noise phobia using a behavioral questionnaire that was developed by our group. We performed allelic association tests between single breed cases and controls and examined cases and controls across multiple breeds via Cochran-Mantel-Haenszel association test, allowing stratification by genetic background to avoid confounding. **Results**: Our case-control analyses yielded associations of markers and affected status on chromosomes 7, 10, 26, 27 and 34 that were supported by preliminary regional fine-mapping. All analyses supported similar findings and withstood permutation to correct for multiple comparisons. **Conclusion**: We have discovered several genomic regions of large genetic effect associated with noise phobia in our sample, suggesting that multiple genetic loci underlie this disorder across breeds. This is the first genome-wide association study in non-human animals focusing on a complex behavioral disorder, and the first to survey any species for markers associated with specific phobia. Future goals include expanding the sample, replicating findings in new samples, incorporating family data into our analyses, and exploring the implications of our results for human psychiatric research.

## 2190/T/Poster Board #739

**Genome-wide screen for rare copy-number variants in Amyotrophic Lateral Sclerosis.** *H. Blauw[1], J.H. Veldink[1], M.A. Van Es[1], P.W.J. Van Vught[1], L.a. Kiemeney[2], S.H. Vermeulen[2], K. Estrada[3], F. Rivadeneira[3], A.G. Uitterlinden[3], S. Cronin[4], O. Hardiman[4], W. Robberecht[5], P.M. Andersen[6], R.A. Ophoff[7,8], L.H. Van Den Berg[1].* 1) Neurology, UMC Utrecht, Utrecht, Netherlands; 2) Department of Epidemiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 3) Department of Epidemiology, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands; 4) Department of Neurology, Beaumont Hospital, Dublin, Ireland; 5) Department of Neurology, University Hospital Leuven, University of Leuven, Leuven, Belgium; 6) Institute of Clinical Neuroscience, Umeå University Hospital, Umeå, Sweden; 7) Department of Medical Genetics and Rudolf Magnus Institute, University Medical Center Utrecht, Utrecht, The Netherlands; 8) UCLA Center for Neurobehavioral Genetics, Los Angeles, USA.

Amyotrophic Lateral Sclerosis (ALS) is an invariably fatal neurodegenerative disease, caused by the selective death of motor neurons in the cerebral cortex and spinal cord. There is no curative treatment. The majority of cases are sporadic and the etiology is considered to be multifactorial, with both environmental and genetic components contributing to disease susceptibility with an estimated heritability of 0.38-0.85. Copy-number variants (CNVs) contribute to genetic and phenotypic variation and can occur as frequent copy number polymorphisms (CNPs) in the population or as rare events with an allele frequency of <1%;. It is thought that these rare structural variants are generally highly penetrant events with deleterious effects. Indeed, several CNV loci containing rare CNVs have been associated to disease phenotypes such as autism and schizophrenia. In this study, we sought to identify rare genomic loci that show association for ALS susceptibilty. 1881 ALS cases and 8760 controls were included. SNP intensity data were derived from different datasets using Illumina 300k, 370k and 550k arrays, and to make the different sets comparable we only used probes common to all arrays. We first applied Loess normalization to the intensity data and used PennCNV software for CNV calling. We applied strict quality control measures based on the per sample data quality and filtered data based on CNV call characteristics. We tested each gene for association with overlapping CNV segments. The gene that yielded the lowest p value was *DPP6* (dipeptidyl peptidase 6): p = 0.0035 (Fisher's exact test). The frequency of CNVs detected in cases was 0.5%;, while the frequency in controls was 0.1%;. The effect was almost entirely due to duplications: when analysing duplications and deletions separately, $p_{duplications} = 0.006$, while $p_{deletions} = 0.32$. *DPP6* encodes a dipeptidyl peptidase-like protein which is predominantly expressed in the brain. Interestingly, an intronic SNP within *DPP6* was significantly associated with ALS susceptibility in three independent association studies. Our results do not survive Bonferroni correction as such, so caution is warranted when examining these results. We are currently making efforts to replicate these findings in independent study populations.

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## 2191/T/Poster Board #740

**Biology of autism revealed through analysis of complex genotypes in GWAS data.** *C. Harris, J. Burns.* Exagen Diagnostics, Inc., Houston, TX.

**Background:** Autism spectrum disorders (ASD) are among the most prevalent and most heritable of neurodevelopmental disorders. While studies have identified many genetic loci as being associated with ASD, the complex genetics underlying the disease remains to be unraveled. We propose generally that reliance on univariate measures of association limits our understanding of complex genetic disease. Moreover, we specifically demonstrate that, for a particular ASD GWAS dataset, an analysis that seeks to identify associated complex genotypes yields results significantly overlapping those compiled from previous studies. **Methods:** Marshall et al. (Am J Hum Genet. 2008 Feb;82(2):477-88) collected genome-wide family SNP data, a portion of which, consisting of the genotypes for approximately 500,000 SNPs for each of 335 ASD cases and 232 parent cases, was made publicly available (http://www.ncbi.nlm.nih.gov/geo/ accession GSE9222). A genetic algorithm-based search was used to identify combinations of SNPs across the affected and parent samples that best discriminated the two groups. Over 5 billion 5-SNP combinations were evaluated. The significance of these was assessed using a permutation test. Reasonable throughput required the use of high performance computing resources. **Results:** We identified 25,116 significant (p<.05) 5-SNP combinations consisting of 4,296 unique SNPs. Genes overlapping or near these SNPs are significantly enriched for genes previously implicated in ASD. Of the 154 genes listed in the AutDB database (http://www.mindspec.org/autdb.html) of genes reported to be associated with autism, 57 were identified by our analysis (p=2.0X10-13). The SNP set was also evaluated using the DAVID functional annotation tools (Dennis G, et al. Genome Biol. 2003;4(5):P3). Significantly enriched biological process and cellular component terms include 'cell adhesion' (p=5.2X10-7, all DAVID p-values Benjamini-corrected), 'axongenesis' (p=8.3X10-4), 'synaptic transmission' (p=1.6X10-2), 'synapse' (p=2.1X10-4) and 'postsynaptic membrane' (p=1.1X10-3). Additional DAVID tools enable identification of associated pathways and tissues. The 'neuroactive ligand-receptor interaction' and 'axon guidance' KEGG pathways were identified (p=4.0X10-4 and p= 3.0X10-3, respectively), as was 'fetal brain tissue' (p=7.9X10-5). A univariate analysis identified 5 SNPs as associated with ASD. In contrast, these did not overlap with AutDB genes and were not enriched for any DAVID terms.

## 2192/T/Poster Board #741

**Genome-wide association study of migraine in Australian twins.** *D.R. Nyholt[1], S.D. Gordon[2], B.P. McEvoy[4], M. Campbell[3], A. Henders[2,3], A.C. Heath[5], G.W. Montgomery[3], N.G. Martin[2].* 1) Neurogenetics Lab, QIMR, Brisbane, Australia; 2) Genetic Epidemiology Lab, QIMR, Brisbane, Australia; 3) Molecular Epidemiology Lab, QIMR, Brisbane, Australia; 4) Queensland Statistical Genetics Lab, QIMR, Brisbane, Australia; 5) Department of Psychiatry, Washington University School of Medicine, St. Louis, MO.

Typical migraine, is a frequent, debilitating and painful disorder that normally affects people during their most productive years (up to 25% of females and 7.5% of males). The World Health Organization recently identified migraine among the world's top 20 leading causes of disability, with an impact that extends far beyond the suffering individual, to the family and community. Although migraine is highly prevalent in our society, its aetiology remains relatively obscure and there are no laboratory based diagnostic tests that identify those who suffer from the disorder. Twin studies indicate that migraine has a significant genetic component, with heritability estimates of 33-65%. Therefore, in an effort to identify the molecular mechanisms underlying the disorder, we are performing a genome-wide association (GWA) study of migraine in a large sample of Australian twins. Although genotyping is still underway, preliminary GWA results utilising 1088 unrelated migraine cases and 2849 unrelated controls have already identified two novel loci reaching genome-wide significance. Up-to-date results from our migraine GWA encompassing genotypes from both additional individuals and imputation, along with our experience in combining data across multiple projects, arrays and platforms will be presented.

## 2193/T/Poster Board #742

**Genome-wide association analysis of sleep length reveals new genes and pathways controlling sleep homeostasis.** *H.M. Ollila[1,2,3], T. Partonen[2], E. Kronholm[2], S. Männistö[2], J. Lönnqvist[2], V. Salomaa[2], L. Peltonen[2,4], M. Perola[2], T. Porkka-Heiskanen[1], T. Paunio[2,3].* 1) Department of Physiology, Institute of Biomedicine, University of Helsinki, Helsinki, Finland; 2) National Institute for Health and Welfare; 3) Department of Psychiatry, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland; 4) Wellcome Trust Sanger Institute, Cambridge, UK.

The genetic component in sleep homeostatic and circadian regulation is high. Disturbances in sleep have also been linked to mood disorders like major depression or bipolar disorder. Furthermore, especially short sleep predisposes to metabolic syndrome and may induce weight gain. However, the genes affecting sleep pattern or mood are not yet well understood. In order to further elucidate the genetic component behind sleep length and seasonal variation we performed a genome-wide DNA association analysis of 2200 Finnish individuals from the national Health2000 project. The individuals were genotyped with altogether over 600 000 single nucleotide polymorphism (SNPs)by Illumina 610 K and characterized for total sleep length per night (TSL) as well as evaluated for depressive symptoms and variation in mood due to seasonal changes. In addition to straight-forward search for association of the single polymorphsisms, Wewe also studied whetherexamined if there were enriched associations of variants from genes single genes from certain molecular pathways were enriched in our dataset. We found several new genes and variants that showed statistically significant association to TSL, which affect sleep length, while some of the genes from sleep regulation also gave suggestive signals for association. Interestingly, the gene ontology analysis showed that sleep length and seasonal changes in mood share common molecular pathways, which belong to brain development, neural, synapse and tyrosine kinase signaling pathways. Altogether we have identified new genes for sleep homeostasis and found potential candidate genes for studying the pathological aspects of both sleep disturbances and mood disorders.

## 2194/T/Poster Board #743

**Genome-wide linkage using the Social Reciprocity Scale (SRS) in Utah autism pedigrees.** *H. Coon[1], M. Villalobos[1], R. Robison[1], D. Cannon[1], K. Allen-Brady[1], M.F. Leppert[2], J. Miller[1], W. McMahon[1].* 1) Dept Psychiatry, Univ Utah, Salt Lake City, UT; 2) Dept Human Genetics, Univ Utah, Salt Lake CIty, UT.

Autism Spectrum Disorders are phenotypically heterogeneous, characterized by impairments in communication and social behavior, and the presence of repetitive behavior and restricted interests. Dissecting the genetic complexity of ASDs may require phenotypic data reflecting more detail than is offered by categorical clinical diagnosis. We present genome-wide results for 64 multiplex and extended families ranging from 2 to 9 generations with phenotypes measured by the Social Reciprocity Scale (SRS). The SRS is a continuous, quantitative measure of social ability giving scores that range from significant impairment to above average ability, therefore allowing us to use information from 518 pedigree subjects, including affected and unaffected relatives. When analyzed as a qualitative trait, results replicated a previous affected-only genome scan using clinical affection status on these families, with findings on chromosomes 7q31.1-q32.3 (HLOD=2.55), 15q13.3 (HLOD=4.09), and 13q12.3 (HLOD=2.23). Additional positive qualitative results were seen on chromosomes 3, 6, and 10. When analyzed as a quantitative trait, results replicated a previous scan of quantitative SRS scores, with results on chromosome 11p15.1-p15.4 (HLOD=2.77) and chromosome 8q13.3 (NPL score=2.56). Additional positive quantitative results were seen on chromosomes 9, 14, 16, 17, and 19. Our results suggest that the information from the SRS provides a useful tool to identify chromosomal regions that may contain autism susceptibility loci.

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## 2195/T/Poster Board #744

**Brain and peripheral gene expression changes in rats after two weeks of exposure to antidepressant drugs.** *K. Doudney[1], J.A. Harley[2], X.Y. Deng[1], M. Allington[1], H.J. Waldvogel[3], R.L.M. Faull[3], P.R. Joyce[2], M.A. Kennedy[1].* 1) Department of Pathology, University of Otago, Christchurch, New Zealand; 2) Department of Psychcological Medicine, University of Otago, Christchurch, New Zealand; 3) Department of Anatomy with Radiology, Faculty of Medical and Health Sciences, The University of Auckland, Auckland 1023, New Zealand.

Antidepressants are an effective treatment for sub-classes of mood disorders including major depressive disorder, although we still don't know the molecular basis of their action. One approach to understanding the molecular effects of these drugs is to expose cultured cells and rodent models to antidepressants and observe protein or RNA expression changes as a measure of the biochemical and genetic processes involved. We have identified a number of antidepressant responsive genes and proteins in proteomic analyses of embryonic stem cell derived neural cells and rat hippocampus (1,2). Additionally, microarray analysis in these systems, and in the serotonergic cell line RN46A, have provided further antidepressant-specific gene expression differences (3,4). To further evaluate these observations, we have compared the immunohistochemical signals of several candidate proteins including PHB, PVALB, HIF1A, PDIA3 and SPR in treated and untreated rat brain sections from animals exposed to paroxetine, citalopram, haloperidol and vehicle only. We present preliminary data showing the differential expression patterns of PHB, PVALB and PDIA3. In parallel with this immunohistochemical study, we are also exploring potential differences in peripheral gene expression patterns in these animals by microarray analysis of cDNA extracted from total peripheral blood. Arrays have been completed on all animals and the results are currently being analysed. Data from these experiments should add to our knowledge of the molecular actions of antidepressants, and provide potential peripheral gene expression markers that may be used to guide or further explore clinical responses to antidepressant therapy. 1. McHugh, P.M. et al. (2009). Proteomic analysis of rat hippocampus exposed to the antidepressant paroxetine. J Psychopharmacol. Apr 3. [Epub ahead of print] 2. McHugh, P.M. et al. (2009). Polymorphisms of sepiapterin reductase gene alter promoter activity and may influence risk of bipolar disorder. Pharmacogenetics and Genomics 19, 330-337 3. McHugh, P.M. et al. (2008). Downregulation of Cyclin D1 (Ccnd1) and Hairy Enhancer of Split 6 (Hes6) in rat hippocampus after chronic exposure to the antidepressant paroxetine Acta Neuropsychiatrica 20, 307-313 4. Glubb, D.M. et al. (2009). Expression and association analyses of promoter variants of the neurogenic gene HES6, a candidate gene for mood disorder susceptibility and antidepressant response. Neurosci. Let.(Accepted).

## 2196/T/Poster Board #745

**A combination of gene expression and tissue microarrays for the identification of human disease biomarkers: application for amyotrophic lateral sclerosis.** *S.L. Karsten[1], L.C. Kudo[2], L. Parfenova[1], N. Vi[2], K. Lau[1,3], J. Pomakian[4], H.V. Vinters[4], M. Wiedau-Pazos[3].* 1) Neuroscience, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA; 2) NeuroInDx, Inc., Signal Hill, CA; 3) Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA; 4) Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA.

The identification of functionally relevant human disease modifying genes and proteins is essential for the design of novel therapeutic approaches. Advances in genomics and proteomics permitted the analysis of thousands of molecules in parallel to quickly identify disease relevant genes and proteins. However, this process still faces multiple challenges. Among them are global differences between animal models and human disease, high discordance between DNA and protein expression data, and a lack of experimental models which would represent human complex diseases accurately. The complexity and inaccessibility of brain tissues create additional obstacles when dealing with neurological disorders. To overcome some of these limitations, we developed an integrative approach that takes advantage of multiple animal models, post-mortem human material and a combination of high-throughput technologies to identify novel molecular markers of a human neurodegenerative disorder, amyotrophic lateral sclerosis (ALS). We used laser capture microdissection coupled with microarray based gene expression analysis to identify transcriptome changes that occur specifically in spinal cord motor neurons or surrounding glial cells in two different mouse models of familial motor neuron disease, SOD1G93A and TAUP301L transgenic mice. The experiments were performed at the presymptomatic stage to exclude identification of false positives due to reactive changes in the advanced disease stage. Identified gene expression changes were mainly model-specific. Then, to investigate the relevance of identified genes in sporadic ALS (SALS), corresponding protein products were examined by high throughput immunoassays using tissue microarrays constructed of human SALS postmortem spinal cord tissues. This large scale gene and protein expression study identified several new SALS biomarkers including Ccl19, Cnga3, Crb1, Mmp14 and established that TAU- and SOD1-induced motor neuron degeneration have distinct molecular mechanisms.

## 2197/T/Poster Board #746

**Puromycin sensitive aminopeptidase (PSA) as a modifier of amyotrophic lateral sclerosis that acts on SOD1.** *L. Parfenova[1], K. Lau[1,2], N. Vi[3], J. Pomakian[4], H.V. Vinters[4], L.C. Kudo[3], S.L. Karsten[1].* 1) Neuroscience, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA; 2) Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA; 3) NeuroInDx, Signal Hill, CA; 4) Pathology and Lab. Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA.

Accumulation of abnormally folded proteins and peptides is a key feature of many neurodegenerative diseases, including Alzheimer's disease, Huntington disease and Amyotrophic Lateral Sclerosis (ALS). Puromycin sensitive aminopeptidase (PSA or NPEPPS) was recently identified as a novel modifier of TAU-induced neurodegeneration with neuroprotective effects via direct proteolysis of TAU protein (Karsten et al, 2006; Sengupta et al, 2006). Another recent report identified PSA as the major peptidase that digests polyglutamine sequences that may be an underlying cause of several polyglutamine diseases including Huntington's disease (Bhutani et al, 2007). These evidences suggest that PSA may be part of a natural neuroprotective mechanism that acts on potentially neurotoxic substrates through proteolytic activity. Here, we report the first evidence of PSA'sinvolvement in the progression of familial and sporadic ALS through interaction with SOD1 (Cu/Zn superoxide dismutase-1). Mutant SOD1 aggregation is observed in familial ALS, in SOD1 transgenic mice, and in some sporadic ALS cases (Boillee et al, 2006; Shaw and Valentine, 2007). To examine the effects of PSA overexpression in vivo, we generated multiple independent lines of PSA transgenic mice using bacterial artificial chromosome (BAC) mediated technology. Immunohistochemical analysis of these mice revealed that SOD1 protein expression is decreased in the brain stem and spinal cord in the adult. On the other hand, Western blot analysis performed on the protein lysates extracted from spinal cord of SOD1-G93A transgenic mice demonstrated that PSA is significantly ($P<0.01$) reduced in the spinal cord of presymptomatic (2-month old) and symptomatic (4-month old) animals. Further, a high-throughput immunohistochemical analysis on human tissue microarray containing sporadic ALS (SALS) postmortem CNS tissue samples showed significant decrease of PSA protein expression in the SALS motor neurons. Our work revealed a potential involvement of PSA in SOD1 metabolism and possibly ALS progression. An ongoing research on the physical interactions between PSA and SOD1 proteins is in progress.

## 2198/T/Poster Board #747

**ADHD in children with Autism Spectrum Disorders: Effect of family history, age, gender, and race.** *R. Abramson[1], A. Hall[2], S. Ravan[1], M. Cuccaro[3], J. Gilbert[3], M. Pericak-Vance[3], H. Wright[1].* 1) Dept Neuropsychiatry & Behav, USC Sch Med, Columbia, SC; 2) Dept. Communication Sciences, USC Sch Public Health, Columbia, SC; 3) Miami Inst. Human Genomics, U Miami School Med, Miami, FL.

**Introduction**: Attention Deficit Hyperactivity Disorder (ADHD) symptoms are reported by parents in a subgroup of children with Autism Spectrum Disorder (ASD). ADHD symptoms are currently considered a part of ASD. ADHD can be inherited (Biederman, 2005). Few studies examine differences in children with ASD+ADHD and a family history of ADHD (FH+) versus children with ASD+ADHD and no family history of ADHD (FH-). This study examines ADHD in children with ASD+ADHD (FH+) and children with ASD+ADHD (FH-). **Purpose**: To evaluate ADHD symptoms in children with ASD+ADHD (FH+) versus children with ASD+ADHD (FH-) by gender, race, and age using the SNAP IV(Bussing et al 2008). **Methods**: Children (n= 285, ages 4-21) with idiopathic ASD (PDD=6, Autism (AUT)=232, Asperger's (ASP)=53) diagnosed by ADI-R criteria(Lord, 1994) and expert clinicians were enrolled from a genetic study. The SNAP IV (a parent report measure) was used to determine the presence of ADHD symptoms in the sample. It has 3 scales: inattention (IN), hyperactivity (HYP) and Oppositional Defiant Disorder symptoms (ODD). Three categories of first degree relatives were included: (1) no ADHD, (2) suspected ADHD, and (3) diagnosed ADHD. **Results**: ANOVA indicated significant differences among AUT+ ADHD family history groups (none, suspected, diagnosed) on IN ($F(2,226)= 4.918$, $p= 0.008$), HYP ($F_{(2,226)}=4.068$, $p=0.018$) and ODD ($F_{(2,226)}=4.307$, $p=0.015$) with higher scores for ADHD FH+. ANOVA showed higher scores for children with ASP+ADHD than AUT+ADHD on IN ($F_{(1,284)}=13.140$, $p=0.000$), HYP ($F_{(1,284)}=6.015$, $p=0.015$, and ODD($F_{(1,284)}=72.970$, $p=0.000$). A 2x2 gender and race ANOVA showed significant main effects on HYP by gender ($F_{(1,238)}=5.044$, $p=0.026$) and race ($F_{(1,238)}=8.570$, $p=0.004$) and a gender by race interaction ($F_{(1,238)}=4.376$, $p=0.038$) and a main effect on IN by gender ($F_{(1,238)}=8.013$, $p=0.005$) and a gender by race interaction ($F_{(1,238)}= 5.859$, $p=0.016$). Overall, nonwhites had lower scores than whites on HYP and girls had lower HYP and IN scores. The interaction showed the nonwhite females scored lower on HYP than the other groups and lower on IN than the male groups. As expected, children age 4-11 had higher HYP scores ($F_{(1,289)}=6.748$, $p=0.010$) and lower ODD scores, $F_{(1,238)}=6.970$, $p=0.009$. **Conclusions**: ADHD symptoms in children with ASD differ in severity based on family history of ADHD, race, gender, and age. These factors need to be further examined as the FH+ subgroup was 18.1% of the sample.

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## 2199/T/Poster Board #748

**The effect of family history, age, and gender on parent report of ADHD behaviors in children with ASD.** *A. Hall[1], R. Abramson[2], S. Ravan[2], M. Cuccaro[3], J. Gilbert[3], M. Pericak-Vance[3], H. Wright[2].* 1) Communication Sciences & Disor, Univ South Carolina Sch Public Health, Columbia, SC; 2) Dept Neuropsychiatry, USC Sch. Med, Columbia, SC; 3) Miami Inst. Human Genomics, U Miami School Med, Miami, FL.

**Introduction**: Hyperactivity, impulsivity and inattention are behavioral features of a subset of individuals with Autism Spectrum Disorders (ASD). However, a diagnosis of Attention Deficit Hyperactivity Disorder is not made if ASD is present (DSM-IV-TR). ADHD can be inherited (Biederman, 2005). Few studies thus far have examined the differences in ADHD behaviors in children with ASD and a family history of ADHD (FH+) versus ADHD behaviors in children with ASD and no family history of ADHD (FH-). This study examines ADHD behaviors in children with ASD+ADHD (FH+) and children with ASD+ADHD (FH-). **Purpose**: To evaluate ADHD symptoms of hyperactivity/impulsivity in children with ASD+ADHD (FH+) versus children with ASD+ADHD (FH-) by gender, and age as measured by the Aberrant Behavior Checklist (ABC) (Aman 1985a,b) **Methods**: Children (n=348, ages 4-21) with idiopathic ASD diagnosed by ADI-R criteria (Lord, 1994) and expert clinicians were enrolled from a genetic study. Hyperactivity/impulsivity is measured by the Aberrant Behavior Checklist subscale for hyperactivity (ABC-H). Two categories of first degree relatives were included: (1) no ADHD (ADHD FH-, n=241) and (2) suspected/diagnosed ADHD (ADHD FH+, n=107). **Results**: ANOVA indicated significant differences among age groups (4-11 vs. 12 and over) on the ABC-H subscale, $F_{(1,363)}=12.628$, p= 0.000. Children ages 4-11 had significantly higher ABC-H scores than those 12 yrs and above, (x=19.93 and x=15.40 respectively). A 2x2 (gender and family history) ANOVA showed a significant main effect of family history ($F_{(1,347)}=13.979$, p=0.000), and a significant gender by family history interaction effect ($F_{(1,347)}=11.653$, p=0.001). The results indicate ADHD FH+ parents reported more hyperactivity/impulsivity in their children than ADHD FH- parents reported. The interaction effect was quite interesting. It suggested girls from ADHD FH- had significantly lower hyperactivity/impulsivity scores than the boys from ADHD FH+. There were no significant differences among the ADHD FH+ girl group and the two male groups. **Conclusions**: ADHD symptoms in children with ASD differ in severity based on family history of ADHD, gender, and age. The age effect was expected. The significant interaction between gender and family history was not expected. While the ABC-H results by gender differ from the SNAP IV, both measures indicate the importance of family history of ADHD in assessing children with an ASD.

## 2200/T/Poster Board #749

**Web-based family recruitment for autism genetic study.** *H. Lee[1], B. Merriman[1], J. Piggot[2], PA. Law[3], JN. Constantino[4], SF. Nelson[1].* 1) Dept Human Gen, Univ California, Los Angeles, Los Angeles, CA; 2) Dept Child and Adolescent Psychiatry and Psychology, Univ California, Los Angeles, Los Angeles, CA; 3) Dept of Medical Informatics, Kennedy Krieger Institute, Baltimore, MD; 4) Dept Psychiatry, Washington University in St.Louis, St.Louis, MO.

The genetic basis of autism has been well established over the last 20 years, based on twin studies and the high relative risk of autism in siblings. Numerous linkage, association, and candidate gene studies have been performed to search for the underlying genes, yet at most a few percent of the genetic component of risk has been identified. This suggests that autism is caused by a large number of distinct genetic risk factors, each with relatively small explanatory power, either in the form of many rare variants, or common variants with small genetic relative risk. In order to have good power to identify such factors, we need tens of thousands of affected individuals. At present, only 3-4,000 European descent autism families are currently available for genetic study. The present project proposes to double this autism sample set in one year. The slow sample collection rate is largely due to the difficulty and expense of clinic-based assessment of the phenotype. The current project employs several novel resources and findings to overcome the obstacle of a lengthy, costly recruitment process. First is the Interactive Autism Network(IAN) registry that has over 10,000 families with autistic children of whom less than 5% have ever participated in any genetic studies, even though they indicate willingness to participate. This database is growing at 300 individuals per month, providing us with a resource for recruitment. Second is that there is strong evidence that the reports given by the parents on their children's affection status are highly correlated with more rigorous clinical assessment. IAN has implemented two standard parent-report questionnaires, SRS (Social Responsiveness Scale) and SCQ (Social Communication Questionnaires). Third is that blood collection can be readily done almost anywhere in the US. Combining these three factors will allow us to perform light phenotyping and rapid blood sample collection for genetic studies. We have launched our first phase of the study to sample a small number of families from IAN registry, perform thorough clinical assessment and compare with the IAN parent-report. Second phase of the study, soon to be launched, will test the blood collection mechanism. We view this web-based recruitment as a complementary and synergistic approach to the current comprehensive assessment approaches and will be a critical component in the comprehensive search for genetic variants contributing to autism risk.

## 2201/T/Poster Board #750

**A Unified Theory of Autism Revisited: Linkage Evidence Points to the IL1RAPL1 Gene using a High-Risk Subset of AGRE Families.** *K. Allen-Brady, D. Cannon, R. Robison, W.M. McMahon, H. Coon.* Utah Autism Research Project, Department of Psychiatry, Univ Utah, Salt Lake City, UT.

Zhao et al. (Proc Natl Acad Sci 2007;104:12831-6) hypothesized that incidence of autism in males could be explained by essentially two types of family structures: low risk autism families with de novo mutations that account for the majority of autism cases, and high-risk multiplex families, where the risk to male offspring approximates 50% consistent with a dominant model and high penetrance, and explains only a minority of autism cases. Using the Autism Genetic Resource Exchange (AGRE) dataset, Zhao et al. identified 86 high-risk families with likely dominant transmission. As genotype data is now available for many members of the AGRE resource, the objective of this abstract was to determine if dominant linkage evidence for an autism predisposition gene exists in these specific 86 high-risk families. HumanHap550K Illumina SNP data was available for 92% of 455 total family members in these 86 high-risk families. We performed a linkage analysis using a pruned subset of markers where markers in high linkage disequilibrium were removed. We observed a single suggestive peak (HLOD 2.08) under a dominant model on chromosome Xp21.3 encompassing part of the IL1RAPL1 gene. Mutations or deletions in IL1RAPL1 have been previously reported in three families with autism. In our study, fourteen families contributed nominally (p<0.05, HLOD > 0.588) to the chromosome X peak. Through follow-up of these 14 families we hope to shed further light on the role of IL1RAPL1 gene and autism. Furthermore, these results demonstrate the potential of using family structure to identify, localize, and further our understanding of autism predisposition genes.

## 2202/T/Poster Board #751

**Genome-wide linkage analysis of bipolar disorder in a Latin American population isolate confirms previous candidate regions on chrs 1p31, 16p12 and 21q21-22 and identifies a novel locus on chr 12q.** *B. Kremeyer[1], J. Garcia[2], H. Muller[1], M.W. Burley[1], I. Herzberg[1], E. Boucher[1], J. Pivard[1], M.V. Parra[3], C. Duque[3], J. Vega[3], V. Reus[4], G. Bedoya[3], C. Lopez[2], J. Ospina-Duque[2], N. Freimer[5], A. Ruiz-Linares[1,3].* 1) Genetics, Evolution and Environment, University College London, London, United Kingdom; 2) Departamento de Psiquiatria, Facultad de Medicina, Universidad de Antioquia, Medellin, Colombia; 3) Laboratorio de Genetica Molecular (Genmol), Facultad de Medicina, Universidad de Antioquia, Medellin, Colombia; 4) Department of Psychiatry, University of California, San Francisco, USA; 5) Center for Neurobehavioral Genetics, Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles, USA.

Bipolar disorder (BP) is a severe and complex psychiatric condition characterised by alternating episodes of depression and mania, which ranges among the top ten causes of morbidity and life-long disability world-wide. There is ample epidemiological evidence for a genetic contribution to the aetiology of the disorder; however, progress in the identification of disease-causing variants has been slow, most likely due to significant genetic and clinical heterogeneity. The study of a rigorously phenotyped pedigree collection from a population isolate can help reduce this heterogeneity. We have previously performed a whole-genome linkage scan on six pedigrees segregating severe BP from the well-characterised population isolate of Antioquia, Colombia (Herzberg et al. 2006, Hum Mol Genet 15:3146-53). We recently collected genotypes for the same set of 382 autosomal microsatellite markers in nine additional Antioquian BP pedigrees. Here, we report the analysis of the combined pedigree set, which altogether comprises 90 cases of BP-I, 22 cases of major depression (MD), and one case each of BP-II and schizophrenia. All diagnoses were made via a best estimate procedure. Linkage analysis under a narrow model considering only BP-I as affected ("narrow model") was conducted using both parametric and non-parametric approaches. To explore possible aetiological overlaps across different psychiatric diagnostic categories, non-parametric analyses were also conducted for a broader phenotype comprising BP-I, BP-II and MD ("broad model"), as well as for a psychosis as a phenotype ("psychosis model"). Under both narrow and broad phenotypic models, the most significant signal was found for chromosome 12ct-q14 (NPL = 2.55/2.35, respectively). This locus has not previously been identified as a candidate region for BP, making this a novel and interesting finding. Additional signals with LOD/NPL scores > 2 were found on chrs 1p22-31 (narrow and broad models) and 21q21-22 (narrow model). Importantly, both regions have repeatedly been implicated in BP susceptibility; the present study strengthens these findings. Analysis under the psychosis model resulted in NPL scores > 2 for candidate regions on chr 2q24-31 and 16p12-q12. The finding on chr 16p is noteworthy because the same locus has been highlighted in the Wellcome Trust CCC GWAS study of BP (WTCCC 2007, Nature 447:661-78), making it an interesting candidate for further follow-up.

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## 2203/T/Poster Board #752

**Meta-analysis of 15 genome-wide linkage scans of smoking behavior.**
*S. Han[1], J. Gelernter[1,2,3,4], BZ. Yang[1,4].* 1) Department of Psychiatry, Division of Human Genetics, University School of Medicine, New Haven, CT 06511, USA; 2) Department of Genetics, University School of Medicine, New Haven, CT 06511, USA; 3) Department of Neurobiology, University School of Medicine, New Haven, CT 06511, USA; 4) VA CT Healthcare Center 116A2; 950 Campbell Avenue; West Haven, CT 06516.

A genetic contribution to smoking behavior, presumably through the mechanism of nicotine dependence (ND), is well established. To identify loci that increase the risk for smoking behavior, many genomewide linkage scans have been performed using various smoking behavior assessments. A number of putative susceptibility loci have been identified, only a few of which have been replicated in independent studies. In current study, we used genome scan meta-analysis (GSMA) to identify potential risk loci which are independent of specific smoking behavior assessments by pooling all available independent genome scan results on smoking behavior. Additionally, to minimize locus heterogeneity, subgroup analyses of the smoking behavior measured by Fagerstrom test for nicotine dependence (FTND) and maximum number of cigarettes smoked in a 24-hour period (MaxCigs24) were performed. A total number of 15 genome scan results were available for analysis, including 3404 families with 10,253 subjects. Overall, the primary 30 cM bin width GSMA across all smoking behavior identified suggestive linkage in chromosome 17q24.3-q25.3 ($P_{SR}$=0.001). Ten regions (2p, 3q, 5q, 6q, 7q, 11p, 12p, 16p, 17q and 20q) with nominal significance ($P_{SR}$ < 0.05) achieved "aggregate" genomewide significance ($P$=0.044), indicating some or all of these 10 regions were likely to harbor risk loci for smoking behavior. Subgroup analysis of FTND did not identify any genomewide suggestive or significant linkage. Subgroup analysis of MaxCigs24 identified a genomewide significant linkage in 20q13.12-q13.32 ($P_{SR}$=0.00041, $P_{OR}$= 0.048), where a strong ND candidate gene, *CHRNA4*, is located. Although genomewide association design has greater power to detect weak effect common variants, linkage analysis has the advantage of detecting diverse genetic effects that segregate in families, including multiple rare variants within one locus or copy number variations. The regions identified in the current study might deserve close attention and will be helpful to facilitate candidate gene identification or to target resequencing studies in the future.

## 2204/T/Poster Board #753

**Executive dysfunctions in nonpsychotic relatives of patients with schizophrenia in different familial loadings: heritability and recurrence risk ratio analyses.**
*S. Lin[1,2], C. Liu[3], S. Liu[3,4], T. Hwang[3], M. Hsieh[3], S. Faraone[5], M. Tsuang[6,7], H. Hwu[1,3,8], W. Chen[1,2,3].* 1) Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan; 2) Genetic Epidemiology Core Laboratory, Division of Genomic Medicine, Research Center for Medical Excellence, National Taiwan University, Taipei, Taiwan; 3) Department of Psychiatry, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan; 4) Far Eastern Memorial Hospital, Taipei, Taiwan; 5) Departments of Psychiatry and Neuroscience and Physiology, SUNY Upstate Medical University, Syracuse, NY, USA; 6) Department of Epidemiology and Psychiatry, Harvard Institute of Psychiatric Epidemiology and Genetics, Harvard Medical Center, Boston, Massachusetts, USA; 7) Department of Psychiatry, Center for Behavioral Genomics, University of California, San Diego, La Jolla, California, USA; 8) Department of Psychology, College of Science, National Taiwan University, Taipei, Taiwan.

Impaired performance on the Wisconsin Card Sorting Test (WCST) has been found in patients with schizophrenia, and poorer performance was also found in their relatives compared with controls with a small effect size. However, these analyses did not address the magnitude of familial aggregation directly and whether different familial loadings of schizophrenia would affect this estimate. This study was aimed to examine whether there was familial aggregation on a variety of performance indexes of the WCST in the families of patients with schizophrenia by means of the heritability estimates and recurrence risk ratio analyses, and the estimates were compared between those from simplex families and those from multiplex ones. Participants were patients with schizophrenia and their nonpsychotic first-degree relatives from 149 simplex families (149 probands, 205 parents, and 77 siblings) and 583 multiplex families (985 probands, 601 parents, and 283 siblings) as well as 440 healthy controls. A computerized version of the WCST was administered for each subject and nine performance indexes were calculated according to the WCST manual. The adjusted z scores of the WCST indexes were obtained with adjustments for sex, age, and education against a group of 440 healthy controls. Both the heritability for continuous scores and the recurrence risk ratios for binary scores with a series of cut-off points in the nonpsychotic relatives of patients with schizophrenia were estimated. The heritabilities ranged from 0% (Nonperseverative Errors and Learning to Learn) to 17% (Perseverative Errors) in simplex families and from 0% (Learning to Learn) to 21% (Perseverative Responses) in multiplex families. The greatest risk ratios were 4.4 in simplex families and 6.3 in multiplex families using the cut-off point of -1 for Failure to Maintain Set. Most risk ratios estimated from multiplex families were higher than their counterparts from simplex families, and the differences were better revealed on the indexes of Perseverative Responses and Perseverative Errors. Our findings suggest that there exist a small-to-modest familial aggregation on the WCST performance in families of patients with schizophrenia, and higher familial loading of schizophrenia is associated with both a greater heritability and greater recurrence risk ratio. Nevertheless, the small magnitude of the familial aggregation in the WCST performance may limit its application as an endophenotypic marker for schizophrenia.

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## 2205/T/Poster Board #754

**Consanguinity and increased risk for Schizophrenia in Egypt.** *H. Mansour[1,2], W. Fathi[2], L. Klei[1], K. Chowdari[1], A. Watson[1], A. Eissa[2], A. Yassin[2], H. Salah[2], S. Tobar[2], H. El-Boraie[2], H. Gaafar[2], M. Elasy[2], I. Ibrahim[2], N. Ibrahim[1], K. Kandil[1], W. El-Bahaei[2], M. Alatrouny[2], F. El-Chennawi[3], B. Devlin[1,4], V. Nimgaonkar[1,4].* 1) Department of Psychiatry, University of Pittsburgh School of Medicine, Western Psychiatric Institute and Clinic, Pittsburgh, Pennsylvania; 2) Department of Psychiatry, Mansoura University School of Medicine, Mansoura, Egypt; 3) Department of Clinical Pathology, Mansoura University School of Medicine, Mansoura, Egypt; 4) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.

Background: Prior studies have suggested increased consanguinity rates may exist among families of patients with psychoses in certain Middle Eastern populations. Our earlier studies showed elevated parental consanguinity rates among patients with bipolar I disorder (BP1) compared with independent Egyptian controls. We extended our anlayses to include patients with Schizophrenia (SZ). Methods: A case-control study involved 75 patients with SZ, 126 screened adult controls, and available parents was conducted at Mansoura University Hospital, Egypt. The control sample was enlarged from our BP1 study. The inbreeding coefficient/ consanguinity rate was estimated from family history data ('self report') followed by DNA analysis using randomly selected short tandem repeat polymorphisms (STRPs, n = 63) ('DNA-based' rate). Results: Self reported consanguinity rates were elevated among SZ patients (consanguinity rates, SZ: 0.43, controls: 0.15, OR 4.2, 95% CI 2.15, 8.18; p = 0.0001, 1 d.f.). These differences were confirmed using estimates for coefficients of inbreeding from DNA-based analyses (mean inbreeding coefficients ± standard error of the means, cases: 0.058 ± 0.007, controls: 0.022 ± 0.003, test statistic=-6.76, p = 6.6 E-12). Conclusion: Self reported consagunity confirmed by DNA analyses showed elevated parental consanguinity rates are among Egyptian SZ patients in the Nile delta region, compared with controls. These results are compatible with recessively inherited risk factors. The consistent results with BP1and SZ raise public health concerns.

## 2206/T/Poster Board #755

**Loci on four autosomes modify age-at-onset of Alzheimer's disease in early-onset PSEN2 families.** *E.E. Marchani[1], T.D. Bird[1, 2, 3], E.J. Steinbart[4,5], G.D. Schellenberg[6], E.M. Wijsman[1, 7, 8].* 1) Division of Medical Genetics, Unversity of Washington, Seattle, WA; 2) Department of Neurology, Veterans Affairs Puget Sound Health Care System, Seattle, WA 98108; 3) Department of Neurology, University of Washington, Seattle, WA; 4) Division of Gerontology and Geriatric Medicine, University of Washington, Seattle, WA; 5) Geriatric Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA; 6) Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia PA; 7) Department of Biostatistics, University of Washington, Seattle, WA; 8) Department of Genome Sciences, University of Washington, Seattle, WA.

Alzheimer's disease (AD), the most prevalent form of dementia, has a complex genetic etiology. APOE is the only gene consistently associated with late-onset AD, but the risk allele is neither necessary nor sufficient to cause the disease. Three additional genes (APP, PSEN1, PSEN2) are associated with rare early-onset forms of the disease (EOAD). These genes only describe a fraction of the variance in age-at-onset (AGE) within families, and other analyses have suggested that additional loci remain to be found. We increased our power to detect these additional modifier loci by analyzing AGE among 11 Volga German EOAD families representing 269 individuals. These families share a single PSEN2 mutation, thus eliminating mutation heterogeneity, and their early AGE reduces the amount of missing data typical of AD data sets. We performed a genome-wide scan on AGE among these families using Bayesian oligogenic segregation and linkage analysis. This approach allowed us to analyze a censored trait while inferring missing data, controlling for segregating PSEN2 and APOE variants, and varying the number and type of quantitative trait locus models proposed. Empirical significance of results was obtained using marker resimulation. Four linkage signals were found to be empirically significant: chromosome 17p (p= 0.0002), chromosome 1q (p<0.001), chromosome 7q (p=0.017), and chromosome 11p (p=0.017). Simultaneous analysis of these four chromosomes maintained strong evidence of linkage to chromosomes 17p, 1q, and 7q. Inclusion of APOE as a covariate proved essential to detect these linkage signals, as all signals decreased when it was excluded from the model. The four regions all coincide with previous linkage signals, associated SNPs, and/or candidate genes identified in other AD study populations. This type of replication establishes several candidate regions for further analysis and emphasizes the oligogenic nature of this disease.

## 2207/T/Poster Board #756

**Genomewide linkage analysis on comorbid depression and cocaine dependence.** *B. Yang[1,4], S. Han[1], HR. Kranzler[5], L. Farrer[6], J. Gelernter[1,2,3,4].* 1) Dept Psychiatry, Yale Univ Sch Med, West Haven, CT; 2) Dept Genetics, Yale Univ Sch Med, West Haven, CT; 3) Dept Neurobiology, Yale Univ Sch Med, West Haven, CT; 4) VA CT Healthcare Center, West Haven, CT; 5) Univ CT Health Center, Farmington, CT; 6) Boston Univ School Med, Boston, MA.

Both illicit drug abuse and depression are common psychiatric disorders in the United States. According to the 2006 National Survey on Drug Use and Health, 6 million Americans age 12 and older had abused cocaine in any form. Major depressive disorder currently affects ~14.8 million American adults. High comorbidity between substance use disorders, including cocaine dependence, and depression in clinical samples has been reported widely. There are many factors involved; the shared genetic liability that contributes to this comorbidity is understudied. To explore this issue, we conducted a multipoint non-parametric linkage analysis for major depressive episode (MDE), and comorbid MDE and cocaine dependence (MDE+CD) in 2,128 individuals from 384 African American (AA) and 350 European American (EA) families who were genotyped for 5,863 SNP markers at CIDR. In AAs, we identified one region on chromosome 7 at 183.5 cM showing suggestive linkage for MDE with a peak lod score of 2.82. A genomewide significant linkage lod score of 3.54 (genomewide empirical p=0.017) was obtained at the same map position for the phenotype of MDE+CD. In EAs, we found evidence for suggestive linkage to MDE on chromosomes 3 (peak lod score = 2.02 at 1.0 cM) and 16 (peak lod score = 1.83 at 76.3 cM). Notable linkage peaks for MDE+CD were also identified in this group of families on chromosomes 5 (peak lod score = 2.25 at 14.3 cM), 10 (peak lod score = 2.18 at 153.5 cM), 16 (peak lod score = 2.11 at 76.3 cM), 3 (peak lod score = 1.84 at 1.0 cM) and 18 (peak lod score = 1.78 at 109.7 cM). In summary, we report a genomewide linkage scan of MDE and comorbid CD and MDE in the two major US populations on the basis of a dense SNP map. These findings can provide useful information to the search for genes jointly influencing comorbid MDE and CD in the AA and EA populations.

## 2208/T/Poster Board #757

**MicroRNA expression in hipocamppal tissue from patients with temporal lobe epilepsy.** *D.B. Dogini[1], T.C. Pereira[1], C.S. Rocha[1], C.L. Yasuda[2], H. Tedeschi[2], E. de Oliveira[2], C.V. Maurer-Morelli[1], F. Cendes[2], I. Lopes-Cendes[1].* 1) Dept Medical Genetics,Campinas, FCM - UNICAMP, Sao Paulo, Brazil; 2) Dept Neurology, Campinas, FCM-UNICAMP, São Paulo, Brazil.

MicroRNAs are a new class of small RNA molecules (21-24 nucleotide-long) that negatively regulate gene expression either by translational repression or target mRNA degradation. It is believed that about 30% of all human genes are targeted by these molecules. miRNAs are involved in many important biological processes including cell differentiation, embryonic development and central nervous system formation. We carried out the present study in order to investigate the possible role of miRNA regulation in temporal lobe epilepsy (TLE). Total RNA was isolated with Trizol of hippocampal tissue from 4 patients with TLE who underwent selective resection of the mesial temporal structures for the treatment of clinically refractory seizures. In addition we used control samples from autopsy (n=4) for comparison. RNA samples were used in real-time PCR reactions with TaqMan MicroRNA assays (Applied Biosystems) to quantify 157 miRNAs in human hippocampal tissue. Bioinformatics analyzes identified three miRNAs species which were differently expressed in patients as compared to controls: let7a was over expressed in patients (4 fold increased), miR-29b and miR-30d were down- regulated in patients (2.5 fold and 0.5 fold decreased, respectively). Possible target genes for let-7a are NME6 and NCAM1 (which would be down-regulated in patients); for miR-29b is MCL-1 and for miR30d are CTNND2, LGI1 and SON (which would be up-regulated in patients). We have identified three miRNA species differently expressed in tissue form patients with TLE. Gene functions related to the possible miRNA targets are involved mainly with cell proliferation, neurogenesis, cell adhesion and apoptosis. Our results reveal new targets which should be explored in additional studies addressing miRNA regulation in TLE.

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## 2209/T/Poster Board #758

**An Animal Model to Investigate the Genetics of Hyperthymic Temperament.** *R. Plaetke[1], W. Byerley[2], S. Gosling[3].* 1) Department of Anthropology, University of Alaska, Fairbanks, AK; 2) Department of Psychiatry, University of California, San Francisco, CA; 3) Department of Psychology, University of Texas, Austin, TX.

Signs & symptoms of bipolar disorder, often described on qualitative scales, are now considered to be parts of continua that belong to traits also occurring in well individuals. Already Kretschmer[1] proposed such a relationship. However, Akiskal[2] included genetics & the aspect of evolution: *"The affective temperaments represent the most prevalent phenotypic expression of the genes underlying bipolar disorder: the disorder itself is an aberration & exists simply because the genes themselves, ..., are useful for evolutionary ends &, in principle, should be demonstrable in other species."*Similarly, Kraepelin[3] described a manic temperament that he originally termed *"constitutional excitement."* He considered the manic temperament as a *"link in the long chain of manic-depressive disposition"* that in its less severe form could still be considered normal. Akiskal et al[3,4] characterized the most discriminatory features of what they named the *"hyperthymic temperament,"* representing the softest expression of Kraepelin's manic type. This temperament, occurring in a variety of well people, is characterized as being exuberant, upbeat, over-energetic, & overconfident. Based on Akiskal's hypothesis, we propose a study to investigate the genetics of hyperthymic temperament in an animal model, the Alaskan Husky (sled) dog. Chromosomal regions & candidate genes identified in this animal model, can be considered to be "biomarkers" for bipolar disorder in humans. This study is unique in its design: **(1)** It aims for determining genes & chromosomal regions by analyzing normally occurring phenotypic expressions in a dog population to investigate the etiology of a psychiatric disorder in humans. **(2)** Dogs will be investigated in a non-invasive & observational way, & in their natural environment. **(3)** A behavioral assay measuring temperament in dogs will be developed by transferring temperament definitions in humans to an animal model, & therefore uses Darwin's[6] theory of the continuum of traits in humans & animals as its framework. We will present study rational, design, goals, & preliminary results supporting our approach. **References:** **1.** Kretschmer 1936, Physique & Character. **2.** Akiskal 2003, American Psychiatric Association 156th Annual Meeting. **3.** Kraepelin 1921, Manic-Depressive Insanity & Paranoia. **4.** Akiskal et al 1979, Psychiatr Clin North Am 2:527. **5.** Akiskal et al 1998, J Affect Disord 59 (Suppl 1):5s. **6.** Darwin 1872, The Expression of the Emotions in Man & Animals.

## 2210/T/Poster Board #759

**A role for the Insulin pathway in an inducible Drosophila model of Alzheimer's disease.** *O. Sofola, F. Kerr, L. Partridge.* Genetics, evolution and environment, University College London, London, United Kingdom.

Alzheimer's disease (AD) is the leading cause of dementia in the ageing population. Alois Alzheimer observed plaques and tangles in the brains of AD patients. The plaques are comprised of amyloid beta and the tangles of tau protein. Several studies have been initiated to understand the underlying mechanism of disease progression and possible biological pathways in AD. We are primarily focused on the role of the Insulin pathway in Alzheimer's disease (AD), due to the strong association between diabetes and AD, the effect of diet on AD, and the involvement of GSK-3 (a component of the Insulin pathway) in AD. In a C.elegans model of AD, daf-2 (Insulin receptor) RNAi in worms expressing amyloid abeta 42 reduced the paralysis and increased the life span of abeta expressing worms. We plan to determine the effect of insulin pathway on AD in Drosophila. Importantly, we will express the abeta protein in the brain of adult flies using an inducible ELAV-GAL4 system, in the presence or absence of intact insulin signalling, and compare the pathology of these flies. This will enable us gain insight of the involvement of the Insulin pathway in AD, in the ageing brain. Our results show that adult expression of abeta leads to toxicity in these flies by altering their motor performance ability. Furthermore, preliminary results suggest that by disrupting a component of the insulin pathway, we are able to delay toxicity in these flies. We are confirming these results and plan to investigate the underlying molecular mechanisms involved.

## 2211/T/Poster Board #760

**Gene Screening in two French-Canadian Families with Primary Lateral Sclerosis.** *V.V. Belzil[1], P.N. Valdmanis[1,2], J. St-Onge[1], N. Dupre[3], G.A. Rouleau[1].* 1) Center of Excellence in Neuromics of Universite de Montreal, CHUM Research Center and the Department of Medicine, University of Montreal, QC; 2) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 3) Departement de sciences neurologiques, CHAUQ Enfant-Jesus, Quebec City, Quebec, Canada.

**Background**: Primary lateral sclerosis (PLS) is a neurodegenerative disease characterized by a progressive spinal and bulbar spasticity due to the degeneration of upper motor neurons. Generally, the lower extremities are first affected, followed by the upper extremities and the bulbar muscles. The neuronal degeneration of PLS is confined to long descending pathways with no involvement of lower motor neurons, as opposed to amyotrophic lateral sclerosis (ALS) involving both upper and lower motor neurons. Although most cases of PLS are sporadic, we previously described a new autosomal dominant syndrome in a French-Canadian family of 10 individuals affected with PLS. A locus on chromosome 4 between the telomere and marker D4S2928 was identified after performing a 550 marker genome scan and confirming a disease haplotype present in all 10 affected individuals. This region spans 10.2 Mb and encompasses about 130 genes. Interestingly, a second small family with PLS has been recently identified with two affected individuals. **Objective**: The goal is to identify and characterize the gene responsible for a familial form of primary lateral sclerosis (PLS) located at the PLS1 locus. **Methods**: The 14 most interesting candidates in the PLS1 locus have been prioritized and screened. One of the candidate was the *huntingtin* gene, playing a role in protein trafficking, vesicle transport,post-synaptic signalling, transcriptional regulation, and apoptosis. Markers encompassing the PLS1 locus were also tested for the newly identified family. **Results**: All the exons of the 14 genes were screened without the detection of a coding mutation. S35 genotyping for the *huntingtin* gene revealed that none of the patients had an expanded CAG repeat which is causative for Huntington's disease. A copy number variant test was also performed for *huntingtin*, and no major deletion or duplication was identified. RNA for *huntingtin* was also analyzed without the detection of significant variations. Three members of the second family seemed to share the same haplotype as the affected members of the first family. **Conclusions**: More genes in the locus are currently being screened and linkage analysis will soon be performed for the second family, which would help narrow the candidate interval. The identification of mutations in a gene responsible for PLS will help provide insight into the causes and the mechanisms involved in PLS.

## 2212/T/Poster Board #761

**REGULATION OF ALZHEIMER'S PRESENILIN-1 MUTATIONS BY AATF IN NEURONAL APOPTOSIS.** *Q. Guo, J. Xie.* Department of Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104.

Most autosomal dominant inherited forms of early onset Alzheimer's disease (AD) are caused by mutations in the presenilin-1 (PS-1) gene on chromosome 14. PS-1 is an integral membrane protein localized mainly in endoplasmic reticulum (ER). We have found in transfected neural cells as well as in neurons from PS-1 mutant knockin mice that PS-1 mutations may sensitize neurons to apoptotic death via calcium-dependent pathways. Apoptosis antagonizing transcription factor (AATF) was initially identified as an interaction partner of DAP-like kinase, a member of the DAP (Death Associated Protein) kinase family of pro-apoptotic kinases. We now report that levels of AATF expression in cortical neurons were significantly altered in neuronal cells transgenic for Alzheimer's mutant PS-1. In these neurons, RNAi-mediated silencing of AATF exacerbated, while overexpression of AATF ameliorated mitochondrial dysfunction, calcium dysregulation, oxidative damage, and apoptotic death induced by mutant PS-1. AATF also reduced production of Abeta accumulation, a pathological hallmark of AD. Recently, we performed in vitro phosphorylation assays, and found that AATF is a phosphorylation substrate of Akt1/PKB$\alpha$, a serine/threonine protein kinase that plays important roles in promoting cell survival and inhibiting apoptosis. Furthermore, we found that phosphorylation of AATF by Akt1 significantly ameliorated apoptotic cell death. These results suggest that AATF is a novel regulator of cellular actions of PS-1 mutations, and that phosphorylation of AATF by Akt1 may represent a critical mechanism of signal transduction mediated by Akt1 in regulating neuronal survival in AD.

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## 2213/T/Poster Board #762

**Puromycin Sensitive Aminopeptidase reduces TAU protein levels in vivo.** *L.C. Kudo[1], K. Lau[2,3], N. Vi[1], L. Parfenova[2,3], M. Hui[4], M. Gray[5], X.W. Yang[5], M. Wiedau-Pazos[2], K.S. Hui[4], S.L. Karsten[2,3].* 1) NeuroInDx Inc., Signal Hill, CA; 2) Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA; 3) Neuroscience, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA; 4) Nathan S.Kline Institute for Psychiatric Research, Orangeburg, NY; 5) Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine at UCLA, Los Angeles, CA.

Recently, we have identified a novel pathway of TAU protein processing involving puromycin sensitive aminopeptidase (PSA), and demonstrated the ability of PSA to protect from tau-induced neurodegeneration in vivo in the fly (Karsten et al, 2006; Sengupta et al, 2006). The role of human PSA as a TAU aminopeptidase was confirmed in a cell-free system. To examine the effects of PSA overexpression on the progress of TAU-induced neurodegeneration in vivo in the mammalian system, we generated multiple independent lines of PSA transgenic mice and crossed them with a TAU-P301L transgenic mouse model (Lewis et al, 2000). PSA mice were generated using bacterial artificial chromosome (BAC) mediated technology. PSA transgenic mice are born normal, breed normally and do not reveal any gross anatomical or behavioral abnormalities, suggesting that low levels of PSA activation might not have any deleterious physiological effects. Expression and activity of PSA are elevated in the brain of hemizygous PSA mice at 2- to 3-fold. In addition, PSA mice do not show any changes in enkephalin turnover/ degradation, supporting the notion that PSA is not an enkephalinase. PSA/ TAU double transgenic mice were characterized at ages 10 weeks, 4 months, 6 months, 9 months and 12+ months. Immunohistochemistry for TAU antibodies raised against phosphorylated TAU C-terminus (V-20, Santa Cruz) and PHF-TAU phosphorylated at Thr181 (AT270, Pierce) showed reduced levels of hyperphosphorylated TAU in the hippocampus, brain stem, cortex, and cerebellum of double transgenic TAU/PSA mice. These combined data suggest that PSA activation can ameliorate accumulation of hyperphosphorylated and toxic tau. Therefore, increasing PSA activity may be a feasible therapeutic approach to inhibit or/and block tau-induced neurodegeneration and thus may provide an effective means to treat tauopathies including Alzheimer's disease.

## 2214/T/Poster Board #763

**A common transcriptional regulatory pathway for genes involved in spastic paraplegia.** *R.D. Nicholls[1,2], W. Zhu[2], K. Hardaway[1], M. Stefan[1,2].* 1) Dept Pediatrics, Birth Defects Labs, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA; 2) Dept Human Genetics, University of Pittsburgh, Pittsburgh, PA.

Hereditary spastic paraplegias (HSP) are a set of clinically diverse neurodegenerative disorders characterized primarily by progressive spasticity in the lower limbs due to axonal degeneration of the corticospinal tract (CST). HSP may be pure or complex, the latter found with additional neurological features such as mental retardation and epilepsy. Dominant inheritance accounts for ~70% of HSP and mutations in one gene, *SPAST* (*SPG4*), are the cause of over 40% of these cases. Nevertheless, to date 45 assigned gene symbols exist for autosomal dominant and recessive, and X-linked, gene loci that are chromosomally linked to HSP pathogenesis but the etiological gene mutations have been identified to date for only 17 or 18 of these. Known spastic paraplegia genes (SPG) encode proteins involved in a multitude of distinct biochemical pathways, attesting to the diverse molecular mechanisms that can affect function and trafficking in long axons, but making selection of new candidate genes difficult. We hypothesize that many SPG loci are co-regulated in order to express the encoded functions in proper CST spatiotemporal patterns. By bioinformatics analyses of human genome sequences we found that the promoters or 5' regulatory regions for 9 of 16 autosomal HSP genes have one or more binding site motifs for the transcription factor (TF) Nuclear Respiratory Factor-1 (NRF1), including *SPG4*, *SPG5*, *SPG6*, *SPG7*, *SPG8*, *SPG11*, *SPG13*, *SPG20* and putative *SPG33*. These sites are evolutionary conserved in all or most eutherian mammals and for some sites, in the marsupial and monotreme. Remarkably, the mammalian *SPG7* promoter is comprised of an array of one to six NRF1 sites and no other conserved TF motifs. We confirmed that NRF1 binds *in vivo* at these regulatory elements using chromatin immunoprecipitation with human and mouse neuroblastoma cell lines. Additionally, siRNA targeting *NRF1* mRNA downregulates expression of these SPG loci as well as luciferase reporter constructs for minimal promoters of the *SPG4* and *SPG6* loci. Since NRF1 sites occur in the promoters of ~5 to 6% of genes across the genome but we find these in ~55% of SPG promoters, we propose that NRF1 coordinates a gene regulatory network (GRN) comprising many of the genes involved in HSP pathogenesis. Further, we propose that identification of NRF1 target genes (and other TFs within the GRN) within large chromosomal domains linked to uncloned HSP loci will improve selection of candidate genes for mutation studies.

## 2215/T/Poster Board #764

**PAR-4-MEDIATED TRANSCRIPTION AND CYTOTOXIC PROPERTIES OF ALZHEIMER'S PRESENILIN-1 MUTATIONS.** *J. Xie, Q. Guo.* Department of Physiology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104.

Most autosomal dominant inherited forms of early onset Alzheimer's disease (AD) are caused by mutations in the presenilin-1 (PS-1) gene on chromosome 14. We have found in transfected neural cells as well as in primary neurons from PS-1 mutant knockin mice that PS-1 mutations sensitize neurons to apoptotic death. Par-4 (prostate apoptosis response-4) is a leucine zipper protein that promotes the death of neurons expressing PS-1 M146V mutation. Par-4-dependent cell-death seems to be mediated by decreased bcl-2 transcription. Overexpression of Par-4 effectively reduced the adaptive increase in Bcl-2 levels in the early stages of neuronal cell death. In pull down assays using specific sequence on the bcl-2 P1 promoter as bait, we found a significantly increased Par-4 binding to the bcl-2 promoter in cortical neurons transgenic for mutant PS-1. Apoptosis antagonizing transcription factor (AATF) is a member of the DAP (Death Associated Protein) kinase family of pro-apoptotic kinases. We found that AATF formed a complex with Par-4 via the leucine zipper domain, leading to a blockade of the pro-apoptotic signaling mediated by Par-4. Binding of Par-4 to the bcl-2 promoter was significantly reduced in neural cells transfected with AATF. These results suggest that Par-4 is directly involved in regulation of bcl-2 transcription in neurons expressing mutant PS-1, and that AATF/Par-4 complex formation may provide a novel neuroprotective pathway in AD.

## 2216/T/Poster Board #765

**Whole genome gene-expression profiling in schizophrenia patients.** *S. de Jong[1], M.P.M. Boks[2], W. Cahn[2], T.F. Fuller[3], S. Horvath[3,4], T. Rietkerk[2], C. Schubart[2], R.S. Kahn[2], R.A. Ophoff[1,3,5,6].* 1) Medical Genetics, University Medical Center, Utrecht, Netherlands; 2) Psychiatry, Rudolf Magnus Institute of Neuroscience, University Medical Center, Utrecht, Netherlands; 3) Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, CA, USA; 4) Biostatistics, School of Public Health, University of California, Los Angeles, CA, USA; 5) Neurobehavioral Genetics, University of California, Los Angeles, CA, USA; 6) Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, The Netherlands.

Schizophrenia is a severe psychiatric disorder characterized by delusions and hallucinations, in addition to a range of negative symptoms. Pharmacological treatment is often not sufficient and induces side effects. This disorder affects about 1% of the population and the genetic contribution is estimated to be around 80%. Therefore, gene-expression can help elucidate genetic mechanisms underlying schizophrenia. In this study, whole-genome gene-expression arrays were performed, using whole blood of schizophrenia patients. Patients were matched to healthy controls. Both medicated (n=200) and unmedicated (n=100) patient groups are included. For most subjects, genome-wide SNP and methylation data is also available. In addition to case-control comparisons using a FDR correction, this data was analyzed using a weighted gene co-expression method. Clustering was performed to create networks of co-expressed genes. This in turn resulted in reconstruction of a limited number of groups of genes ("modules") with highly similar expression profiles. Within the co-expression modules, the most connected genes are driving that group of genes and are considered to be most important. Differences in network structure and connectivity between cases and controls were found and will point to genes of interest in the etiology of schizophrenia. In addition, the effects of medication on gene-expression will be assessed. These effects can give insight into treatment-response and will be of importance in further studies in which medication-naive material is unavailable.

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## 2217/T/Poster Board #766

**The genetic contribution of copy number variants to hereditary peripheral neuropathies.** *J. Huang[1], J. Price[1], G. Montenegro[1], G. Wang[1], X. Wu[2], J. Vance[1], M. Shy[2], S. Züchner[1].* 1) Miami Inst Human Genomics, Univ Miami, Miami, FL; 2) Department of Neurology, School of Medicine, Wayne State University, Detroit, MI.

Hereditary peripheral neuropathies present a group of clinically and genetically heterogeneous entities. All known forms, including the various forms of Charcot-Marie-Tooth disease (CMT) are characterized as Mendelian traits and over 30 genes have been identified thus far. It is well known that copy number variants (CNV) are involved in many diseases, including CMT type 1A. We speculate that hereditary peripheral neuropathy patients without missense mutations might instead carry rare CNVs in known genes, causing the disease phenotype. In this study, we performed the first high-density CNV study in CMT targeting 34 genomic regions harboring known genes for hereditary peripheral neuropathies, including the 17p12 duplication region. We screened a sample of 97 clinically heterogeneous index patients applying custom NimbleGen 4-plex 385K CGH arrays. All results were confirmed by qPCR. We identified six common and rare CNVs, of which three affect coding exons of CMT genes. A novel shorter form of a PMP22 duplication was detected in a CMT1A family previously tested negative in a commercial test. Moreover, we have evidence that this CNV represents a triplication of the PMP22 gene. Two other CNVs in MTMR2 and ARHGEF10 are likely not disease associated. Our results indicate that CNVs are a rare cause for CMT. Their potential relevance as disease modifiers remains to be evaluated. The present study design cannot rule out that specific CMT forms exist where CNVs play a larger role.

## 2218/T/Poster Board #767

**X-linked dystonia-parkinsonism: Effects of disease-specific single nucleotide change 3 (DSC3) within the *TAF1/DYT3* multiple transcript system in a cell system.** *U. Muller[1], T. Herzfeld[1], A. Gaarz[2], M. Grznarova[1], A. Staratschek-Jox[2], J.-L. Schultze[2], D. Nolte[1].* 1) Institut für Humangenetik, Justus-Liebig- Universität, D35392 Giessen, Germany; 2) LIMES-Institut (Life and Medical Sciences Bonn), Universität Bonn, D53115 Bonn, Germany.

X-linked dystonia parkinsonism (XDP) is caused by various disease specific single nucleotide changes (DSCs) within the *TAF1/DYT3* multiple transcript system. This transcript system is composed of 38 exons encoding *TAF1* plus 5 downstream exons (exons d1-d5) (1). While all DSCs, a deletion, and a SVA element observed in XDP patients lie within introns of *TAF1/DYT3*, one, DSC3, is located within an exon (exon d4). Exon d4 can be part of transcripts including *TAF1* exons or be part of a transcript driven by a specific promoter and encoded by d exons (exons d2, d3, d4) only (2). In order to investigate a possible effect of DSC3 at the cellular level we transfected neuroblastoma cells (SK-N-AS) with a construct overexpressing exons d2-d4 with exon d4 including either the wild-type or the mutated allele DSC3. Analysis of RNA extracted from these cells (performed in quadruplicates) on Illumina chips (HumanWG6_V3) revealed 68 genes that are differentially expressed in cells containing wild-type vs. DSC3 constructs. Of these 51 are downregulated at least two-fold in DSC3 vs. wildtype constructs. Seventeen are expressed at higher levels in DSC3 than in wildtype. In silico analysis of these genes using the Allen Brain Atlas revealed that 8 of the dysregulated genes are expressed in the mouse striatum. Potential roles of these genes in XDP are discussed. Although the mechanism of function of the d2-d4 transcript is not known it is intriguing that d4 encoded RNA forms a hairpin structure. This structure is disturbed by DSC3.

1) Nolte D, Niemann S, Müller U: Specific sequence changes in multiple transcript system DYT3 are associated with X-linked dystonia parkinsonism. PNAS 100: 10347-10352, 2003

2) Herzfeld T, Nolte D, Müller U: Structural and functional analysis of the human *TAF1/DYT3* multiple transcript system. Mamm Genome 18: 787-795, 2007.

## 2219/T/Poster Board #768

**Mutational analysis of *FUS/TLS* in familial and sporadic amyotrophic lateral sclerosis in the Japanese population.** *Y. Takahashi, J. Goto, S. Tsuji.* Dept Neurology, Univ Tokyo, Tokyo, Japan.

[Objectives]To elucidate genetic epidemiology of familial and sporadic amyotrophic lateral sclerosis (ALS) with *FUS/TLS* mutations in the Japanese population and to describe clinical presentations of these patients. [Background]*FUS/TLS* has recently been identified as a causative gene for ALS6, an autosomal dominant familial ALS (FALS). It has been reported that the frequency of *FUS/TLS* mutations was approximately 5 % of FALS in the Western population. However, the genetic epidemiology of ALS with *FUS/TLS* mutations in the Japanese population remains unknown. [Materials and Methods] Sixteen genomic DNA samples of familial ALS (FALS) patients were used, where mutations of *SOD1*, *ALS2*, *DCTN1*, *VAPB*, *CHMP2B*, *ANG* and *TARDBP* were excluded using DNA microarray-based resequencing system. The mode of inheritance was autosomal dominant in 11 pedigrees, whereas 5 pedigrees were with affected siblings, in whom 4 were without consanguinity and 1 with consanguinity. In addition, 160 samples with sporadic ALS (SALS) patients and 260 control samples were used for this study. All the exons of *FUS/TLS* were amplified with genomic PCR using specific primers for each exon and further subjected to direct nucleotide sequencing method. Novel nonsynonymous variations were screened in 260 control samples. [Results] Two mutations consisting of a novel mutation K510M in exon 14 and a known mutation R521H in exon 15 were found in 16 families (12.5%) with FALS where mutations of other causative genes have been excluded. In addition, 2 mutations consisting of a novel putative pathogenic mutation G206S in exon 6, where another mutation has been reported, and a known mutation R521C were identified in 160 samples (1.3%) with SALS. The novel mutations were not found in 260 controls (520 chromosomes), located in mutation-clustered regions and conserved among species. The age at onset of patients with K510M, R521H, G206W and R521C were 41, 39, 72 and 39, respectively, raising the possibility that the mutation G206W was less damaging than the other mutations. [Conclusion]Mutations of *FUS/TLS* were relatively frequent in FALS in the Japanese population, indicating that the mutations are broadly identified in different ethnic backgrounds. In addition, mutations were also found in SALS, suggesting mutations with reduced penetrance or *de novo* mutations.

## 2220/T/Poster Board #769

**Inspecting verbal and visual episodic memory in childhood and in adulthood in affected kindreds: A generational paradigm in schizophrenia and bipolar-mood disorder.** *N. Rouleau[1,2], M. Maziade[2], C. Mérette[2], M. Battaglia[3], C. Marino[4], V. Jomphe[2], E. Gilbert[1], A. Achim[2], R.H. Bouchard[2], M.E. Paradis[2], M.A. Roy[2].* 1) School of psychology, Laval University, Quebec, Canada; 2) Centre de recherche Université Laval Robert-Giffard, Québec, Canada; 3) San Raffaele 'Vita Salute' University, Dept of Neuropsychiatric Sciences, Italy; 4) Eugenio Medea Institute, Depat of Child Psychiatry, Italy.

Episodic memory is one of the most prominent neurocognitive deficit observed in patients affected by schizophrenia (SZ) and bipolar/mood disorder (BP). Little is known about the pathological trajectory of memory impairments from childhood to adult clinical status in risk populations. We recently reported that young high-risk offspring (HR) of an affected parent were impaired in both verbal (VEM) and visual episodic memory (VisEM). Over 20 years, we followed up 48 kindreds affected by SZ or BP in the Eastern Québec population. After having characterized the DSM phenotypes, we undertook the phenotyping of cognition in three sub-samples in these kindreds and in controls (N=381): in the younger generations, 60 young HRs of a parent affected by SZ or BP and, in the adult generations, 92 non-affected adult relatives (NAARs) and 40 patients affected by SZ or BP. We used a cross-sectional generational stratification to compare the VEM and the VisEM cognitive profiles of the young non-affected HR offspring to the profiles of adult NAARs and patients. VEM was assessed with the California Verbal Learning Test (CVLT) and VisEM with the Rey complex figure. Compared to normal controls, VEM deficits of moderate to large effect sizes (ES) were observed in HRs (ES= -0.84, $p<0.0001$), in NAARs (ES= -0.59, $p<0.0001$) and in patients (ES= -1.08, $p<0.0001$). Effect sizes of similar magnitude were observed for VisEM in HRs (ES=-0.92, $p<0.0001$) and in patients (ES=-1.01, $p<0.0001$) but not in NAARs (ES=-0.22, NS). The memory impairments displayed by the young HR offspring of these kindreds in the years preceding disease onset were also observable in the adult generations. VEM and VisEM showed distinct generational patterns suggesting that a VisEM impairment in HRs was more likely to be a strong precursor of disease in adulthood whereas a VEM deficit would fit a model of intermediate phenotype (IP), i.e. a trait associated with SZ and BP and present in patients and in NAARs.

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## 2221/T/Poster Board #770

**Gene expression, aging and Alzheimer's disease.** *D. Avramopoulos[1, 2], M. Szymanski[2], R. Wang[1].* 1) Dept Psychiatry, Johns Hopkins Univ, Baltimore, MD; 2) McKusick Nathans Institute of Genetic Medicine, Johns Hopkins Univ, Baltimore, MD.

Genome wide analysis of gene expression has been made possible by current microarray technologies allowing investigators to have a global view of the consequences of disease on gene expressio, or possibly the consequences of gene dysregulation on disease risk. Alzheimer's disease (AD) is a dementia with rare, Mendelian, earlier onset cases and more common, later onset cases with significant genetic contribution. Other than genes, age is one of the strongest risk factors for AD. We have performed a genome wide gene expression study on temporal lobe RNA from 26 brains from donors with confirmed late onset AD and 23 brains from donors without brain pathology. We used the Illumina Sentrix HumanRef-8 Expression BeadChips to interrogate 24,000 transcripts from genes across the genome. We examined the effects of age and sex in the expression of genes in the absence of pathology, as well as the expression differences in AD patients after controlling for these and other variables. We find that many genes have lower expression with increasing age and among them there is significant enrichment of genes involved in RNA processing and protein biosynthesis. Alzheimer's disease is also accompanied by significant expression changes for many genes independent of age. The set of genes with reduced expression in AD is enriched for those involved in neuronal activities including synaptic transmission. Genes expressed at higher levels in AD include an excess of transcription factors. Interestingly we find a highly significant overlap between genes whose expression changes with age in normal brains and genes whose expression changes in AD after controlling for age. This might reflect an accelerated aging process in the brain of people who develop AD or it might suggest that acceleration of age related changes in the expression of some genes increases the risk to develop AD.

## 2222/T/Poster Board #771

**Exploring gene regulation by antidepressant drugs.** *M.A. Kennedy[1], X.Y. Deng[1], K. Doudney[1], P.C. McHugh[1], P.R. Joyce[2].* 1) Dept Pathology, University of Otago, Christchurch, New Zealand; 2) Dept Psychological Medicine, University of Otago, Christchurch, New Zealand.

Antidepressant drugs are widely used but there is much about these drugs that is not well understood. It is now clear that antidepressants affect brain function in many ways beyond their immediate pharmacological actions, including stimulation of many cellular and molecular changes and promotion of neuronal survival and growth. As one approach to explore these diverse antidepressant actions, we used transient luciferase reporter gene assays with selected transcriptional promoters, in the neural cell line RN46A, to examine the chronic effects of antidepressant drugs (paroxetine, citalopram, nortriptyline) and control non-antidepressant CNS drugs (haloperidol and valproic acid). Among the 19 promoter constructs initially tested, six showed significantly increased gene expression following 14 days exposure to 0.5 uM paroxetine (ADM, GCHFR, PDIA3, GCH1, CREB1, and PHB). ADM and GCHFR promoter constructs were chosen for subsequent drug exposure experiments due to their most significant expression changes. Time-course analysis showed that paroxetine treatment led to an increase in gene expression within 48 hrs of exposure, peaking after 72 hrs drug exposure. A dose-response test confirmed 0.5uM as an appropriate concentration to use for drug exposure experiments. Both ADM and GCHFR promoters showed up-regulation by paroxetine, haloperidol and sodium valproate. This effect was specific to ADM and GCHFR, and did not occur with control promoters not thought relevant to antidepressant function. However, with extended experimentation we found that observed differences in expression of reporter constructs between treated and untreated RN46A cells were not consistently reproducible, with significant day to day variability in outcomes despite considerable care in experimental design and execution. Therefore, we are now applying real-time quantitative PCR (qPCR) as an alternative approach to evaluate antidepressant-induced gene expression changes in this cell line. Among the five candidate reference genes tested so far, ACTB, RNF4 and DAZAP1 are expressed relatively stably across all the treatments. We are now assessing the relative expression level of a wide range of candidate genes drawn from our prior work, and from the literature, in this cell culture model. It is our goal to identify a robust and easily manipulated model system for exploring regulation of genes by antidepressant drugs, to further our understanding of the molecular action of these substances.

## 2223/T/Poster Board #772

**A screen for genetic suppressors of Rett syndrome in the mouse.** *M. Justice, J. Shaw, F. Probst.* Dept Molec & Human Gen, Baylor Col Med, Houston, TX.

Mutations in the methyl CpG binding protein 2 (*Mecp2*) cause Rett Syndrome, an X-linked neurological syndrome that affects primarily girls. A large degree of variation in the severity of the disease is observed, which is often not correlated with the extent of X-inactivation, suggesting that modifier genes may affect the symptoms. Mouse mutants that eliminate Mecp2 function provide a good animal model for Rett Syndrome. Studies of the mouse model have shown that the mutant symptoms in male mice can be rescued by re-introduction of the Mecp2 gene and partially rescued by introduction of Brain derived neurotrophic factor (*Bdnf*), providing substantial evidence that the alleviation of symptoms is possible. Genetic modifier screens have been valuable in organisms such as the fruit fly, worms, yeast, and bacteria to reveal genes that act in a biological pathway. In the mouse, such screens are now feasible because of the availability of the complete genome sequence, which aids in the rapid identification of lesions induced by the supermutagen N-ethyl-N-nitrosourea (ENU). We are carrying out a genetic modifier screen of *Mecp2*-/Y males to uncover loci that may ameliorate or cure the symptoms of Mecp2 mutation. In a screen of over 1400 male mice, we have identified four mouse lines that carry suppressors of the disease symptoms. Our preliminary data show that some suppressors may be more effective than others, and reveal a map location for the first modifier. Identifying the suppressors at a molecular level will allow for the development of small molecules that may mimic the natural suppressor. Our ultimate goal is to discover and validate drug targets for the treatment of Rett Syndrome.

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## 2224/T/Poster Board #773

**Polymorphisms in Interleukin 4 (IL4), Interleukin 13 (IL13) and IL 4 receptor (IL4R) are associated with Asthma Exacerbations in African Americans.** *S. Pillai, S. Yancey, L. Edwards, K. Sutton, S. Stinnett, H. Ortega, W. Anderson.* GlaxoSmithKline, RTP, NC.

Polymorphisms in IL4, IL13 and IL4R have been shown to be associated with asthma related phenotypes in African Americans. We assessed whether the polymorphisms in these genes have an effect on asthma exacerbations in an African American population. We conducted a retrospective pharmacogenetic analysis (N=322) of subjects in a 52-wk trial comparing exacerbation rates between fluticasone propionate (FP) plus salmeterol (FSC 100/50mcg BID) and FP 100mcg BID via Diskus®. At screening, while of steady state low dose ICS and after withholding bronchodilators, the $FEV_1$ was between 60-90% of predicted and subjects demonstrated ≥12% reversibility. Subjects received FP250 for 4 wks prior to randomization. Fifteen SNPs in the IL4, IL13 and IL4R genes were genotyped. Exacerbations were defined as worsening asthma requiring oral steroids, hospitalization, unscheduled urgent care, ≥30% decrease in $FEV_1$from baseline or ≥30% decrease in AM PEF on any 2 consecutive days. Analysis of exacerbations was conducted by three methods: logistic regression for occurrence of exacerbations, Poisson regression for the rate of exacerbations and Cox proportional hazards models for time to first exacerbation. Genotype, treatment, and treatment-by-genotype interaction were assessed adjusting for age, BMI, asthma duration, baseline $FEV_1$, reversibility, sex, and investigator region. A polymorphism in IL4R (rs1805010) was associated with occurrence (p=0.04) of exacerbations during the 52 week treatment period. Polymorphisms in IL4 (rs2243263, p =0.032) and IL13 (rs1295686, p=0.046) were associated with rate of exacerbations. A non-synonymous coding polymorphism, rs20541 (Arg144Gln) in IL13, which is in tight LD with rs1295686 was associated with time to first exacerbation (p=0.02) with significantly higher exacerbation rate and earlier time to first exacerbations in subjects homozygous for the variant allele. No genotype-by-treatment interactions were observed. These results suggest that polymorphisms in IL4, IL13 and IL4R, reported to be associated with asthma related phenotypes are also associated with asthma exacerbations in African Americans. (SFA103153).

## 2225/T/Poster Board #774

**Glycogen synthase kinase 3β polymorphisms and weight change during clozapine treatment.** *R.P. Souza[1,2], D.V. Rosa[1,3], M.A. Romano-Silva[3], H.Y. Meltzer[4], J.A. Lieberman[5], J.L. Kennedy[1,2].* 1) Neurogenetics Section, Centre for Addiction and Mental Health, Ontario, Canada; 2) Department of Psychiatry, University of Toronto, Ontario, Canada; 3) Laboratorio de Neurociencias, Universidade Federal de MInas Gerais, Minas Gerais, Brazil; 4) Departments of Psychiatry and Pharmacology, Vanderbilt University, Tennessee, United States; 5) Department of Psychiatry, Columbia University, New York, United States.

Antipsychotic medications are an important component in the medical management of many psychotic conditions. Although clozapine, the prototype atypical antipsychotic, has superior efficacy and fewer extrapyramidal effects compared with typical analogs, it is associated with increased prevalence of adverse, weight-gain effects. Search for predictors of drug-related morbidity is becoming increasingly important in persons with major mental illness. Weight gain, glucose and lipid abnormalities are observed more frequently in some novel antipsychotics. The mechanism underlying clozapine-induced body weight changes remain unknown. We examined 13 GSK3β markers (rs6805251, rs4688043, rs7624540, rs13319151, rs6438552, rs4072520, rs9878473, rs4491944, rs6772172, rs11919783, rs11923196, rs6779828, rs9846422, rs3755557) and two markers after the 3'-untranslated region of the gene (rs9846422, rs3755557). Genotyping was performed by GoldenGate assay (Illumina, San Diego, CA, USA) at The Centre for Applied Genomics at the Hospital for Sick Children in Toronto, Ontario, Canada. For the analyses on clozapine-induced weight gain, a total number of 67 subjects were recruited from Case Western Reserve (n=56; 38 males and 18 females, mean age 35±8) and from Hillside Hospital (n=11, 4 males and 7 females, mean age 33±6). Weight gain was expressed as a dichotomous variable in the whole sample at six weeks using as criteria a weight increase greater than seven percent from baseline at enrolment. The Food and Drugs Administration has established this threshold as producing a clinically meaningful and significant metabolic outcome. Individual SNP analyses of weight gain greater than seven percent (case) and absence of weight gain greater than seven percent (control) data and Hardy-Weinberg equilibrium assessment were performed using $\chi 2$ tests. Statistical Package for the Social Sciences, version 10.0.7, was used to access genotypic association and Haploview 4.0 for allelic association. Linkage disequilibrium was assessed using Haploview version 4.0. Haplotype analyses were performed using UNPHASED 3.0.10 and Haploview version 4.0. No allelic, genotypic or haplotypic significant association with clozapine-induced weight gain was found in our sample. Our results suggest that the GSK3β that may not affect susceptibility to gain weight induced by clozapine. Further analyses in this region are required to strengthen this negative finding.

## 2226/T/Poster Board #775

**Association of polymorphisms in transcription factor genes with variable bronchodilator response among asthmatics.** *Q.L. Duan[1], R. Du[1], J. Lasky-Su[1], A.J. Murphy[1], A.B. Partch[1], S.B. Liggett[2], A.A. Litonjua[1], K.G. Tantisira[1], S.T. Weiss[1,3].* 1) Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 2) Cardiopulmonary Genomics Program, University of Maryland School of Medicine, Baltimore, MD; 3) Partners Center for Personalized Genetic Medicine, Partners Health Care, Boston, MA.

Variability in transcription factor (TF) regulation has been associated with numerous human diseases and differential response to drug therapies including asthma medications such as leukotriene modifiers and corticosteroids. Recently, TFs have been shown to be regulated by proasthmatic cytokines (e.g. Interleukins 4 and 13, transforming growth factor-β, Leukotriene $D_4$) and exposure to a bronchodilator (isoproterenol) in human airway epithelial and smooth muscle cells. We hypothesized that variants in the genes encoding these TFs regulate their expression within these cells, which could contribute to asthma and variability in bronchodilator response (BDR). We identified 98 genes coding for 59 TFs that were differentially expressed (≥ 50% up- or down-regulated) in response to isoproterenol within these proasthmatic cells. A total of 1155 single nucleotide polymorphisms (SNPs) located across these genes and up to 20 kb of non-coding, flanking regions were genotyped in Caucasians participating in the Childhood Asthma Management Program (403 parent-child trios) by the Illumina HumanHap550v3 BeadChip. We tested each SNP for association with BDR, measured as the percent change in $FEV_1$ as result of bronchodilator administration, using family-based analysis (FBAT) of the trios and population-based analysis of the probands only, incorporated into generalized linear models in SAS (version 9.0; SAS Institute, Cary, NC). Analyses were adjusted for age, sex and baseline lung function. Under the additive model, 104 SNPs and 133 SNPs provided p-values < 0.1 using FBAT and population based analyses, respectively. Forty-six of these SNPs overlapped, covering 19 candidate genes, which are represented by 38 tag SNPs due to linkage disequilibrium (correlation coefficient ($r^2$) ≥ 0.8). Replication genotyping of these SNPs are ongoing in three asthma cohorts. Identification of genetic variants regulating BDR in this fashion could be used to predict asthma therapy outcomes and may represent a novel way to translate *in vitro* cellular drug response to bedside clinical management.

## 2227/T/Poster Board #776

**Effects of *UGT1A1* polymorphism on irinotecan-induced drug reaction and serum bilirubin levels.** *A. HIRASAWA[1], T. AKAHANE[1], T. TSURUTA[1], Y. KOBAYASHI[1], H. NOMURA[1], K. BANNO[1], H. TSUDA[1], K. SAITO[2], T. ZAMA[2], Y. TANIGAWARA[3], N. SUSUMU[1], D. AOKI[1].* 1) Dept. Gyne/Obst, Sch. of Med, Keio Univ., Tokyo, Japan; 2) Dept. Otolaryngol-Head/Neck Surg, Sch. of Med, Keio Univ; 3) Dept. Hospital Pharmacy, Sch. of Med, Keio Univ.

Irinotecan is metabolized to active form which is further conjugated and detoxified by the UGT1A1 enzyme. The severe toxicities in patients who receive irinotecan are related to its genetic variants, and the serum total bilirubin (BIL) levels are useful for the prediction of adverse reactions to irinotecan. However, the relationship between the specific *UGT1A1* genotype and BIL levels has not yet been confirmed. Then, we studied 443 cases with no history of liver dysfunction to explore their relationship. Genomic DNAs were extracted from peripheral leukocytes after the informed consent was obtained. The assays for genotyping the polymorphisms in the *UGT1A1* (*6, *27, *28, *60) were based on either Invader assay or direct sequencing. The frequencies of *28 and *60 variants were much lower than those published results for whites, while the frequencies of *6 and *27 variants were much higher. Furthermore, 89% (24/27) subjects with hyperbilirubinemia had *6, *28 or *60 variants. This study showed that several *UGT1A1* genotypes were significantly associated with the increased BIL levels. These findings will be useful for further pharmacogenomical studies on adverse reactions to irinotecan.

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## 2228/T/Poster Board #777

**Discovery, replication and clinical validation of genetic variants associated with carboplatin-induced cytotoxicity in Caucasians.** *R. Huang[1], D. Ziliak[1], E. Gamazon[1], S. Duan[1], W. Zhang[1], E. Kistner[2], P. Chen[3], S. Mi[1], G. Chenevix-Trench[4], J. Beesley[4], S. Johnatty[4], A. DeFazio[5], S. Das[3], N. Cox[1], M. Dolan[1], Australian Ovarian Cancer Study.* 1) Dept Med, Univ Chicago, Chicago, IL; 2) Dept Health Studies, Univ Chicago, Chicago, IL; 3) Dept Human Genetics, Univ Chicago, Chicago, IL; 4) Queensland Institue of Medical Research, Australia; 5) Westmead Hospital, Australia.

Carboplatin is commonly used in treating cancers; however, a portion of patients do not respond to therapy while others develop severe treatment-induced toxicity. To gain a better understanding of the genetic variants associated with platinum sensitivity, we applied a genome-wide approach integrating genotypes, mRNA expression and cellular sensitivity to carboplatin using HapMap lymphoblastoid cell lines (LCLs). For discovery, 90 HapMap CEU LCLs were utilized while 52 unrelated non-HapMap CEPH LCLs were used as a replication set. Cell growth inhibition was determined in LCLs at increasing concentrations of carboplatin. The percent survival at different treatment concentrations along with carboplatin $IC_{50}$ (concentration required to inhibit 50% cell growth) were used as carboplatin sensitivity phenotypes. Genome-wide mRNA expression was determined using the Affymetrix GeneChip® Human Exon 1.0 ST Array. Genome-wide association studies were performed between the components of the model to identify genetic variants significantly associated with carboplatin sensitivity through their effects on mRNA expression. We identified 65 SNPs that are associated with at least one carboplatin sensitivity phenotype through the expression of 61 genes. Five of these SNPs were replicated in separate set of LCLs. In addition, we constructed a multi-SNP prediction model, utilizing risk allele counting and weighted risk allele counting, which take into account the effect of risk allele for each SNP, to predict carboplatin $IC_{50}$. A set of SNPs appear to consistently predict carboplatin $IC_{50}$ in the discovery and replication dataset. Furthermore, the replicated SNPs were genotyped in 543 Australian Ovarian Cancer Study patients treated with carboplatin and paclitaxel. A SNP was significantly associated with progression free survival ($P_{adjust}<0.05$) in all patients with a more pronounced effect in a subset of optimally debulked cases ($P_{adjust}=0.007$). This SNP is located in *NRG3* gene locus and is significantly associated with the expression of several other genes. We demonstrated the potential of utilizing a genome-wide approach in a cell-based model to identify SNPs predicting for survival in a clinical study. This work has important implications in the field of oncology where using cell-based models would be highly beneficial for pharmacogenomic discovery.

## 2229/T/Poster Board #778

**Pharmacogenetics of Pharmacotherapies for Smoking Cessation: Analysis of Placebo-Controlled Trials for Varenicline and Bupropion Efficacy and Side Effects.** *D. King[1], S. Paciga[1], E. Pickering[1], N. Benowitz[2], L. Bierut[3], D. Conti[4], J. Kaprio[5], C. Lerman[6], P. Park[7].* 1) Pfizer Global Research and Development, Groton/New London, CT; 2) Division of Clinical Pharmacology and Experimental Therapeutics, University of California at San Francisco, San Francisco, CA; 3) Department of Psychiatry, Washington University, St. Louis, MO; 4) Department of Preventive Medicine, Keck School of Medicine, Zilkha Neurogenetics Institute, University of Southern California, Los Angeles, CA; 5) Department of Public Health, University of Helsinki, Helsinki, Finland; 6) Department of Psychiatry, University of Pennsylvania, Philadelphia, PA; 7) Pfizer Global Pharmaceuticals, New York, NY.

**Background** Despite the availability of effective therapies to aid smoking cessation, most smokers find quitting difficult and the majority of successful quitters relapse. While there is considerable evidence to support a genetic risk for nicotine dependence, much less is known about the pharmacogenetics of successful smoking cessation. In the first pharmacogenetic investigation of the relative efficacy of varenicline and bupropion, we examined whether variation in genes important in the pharmacodynamic and pharmacokinetic effects of nicotine or drug treatment predicts medication efficacy and side effects. **Methods** Subjects participated in randomized, double-blind, placebo-controlled smoking cessation clinical trials, comparing varenicline, a nicotinic acetylcholine receptor (nAChR) partial agonist, with bupropion, a norepinephrine and dopamine reuptake inhibitor. DNA was obtained from 1476 consenting individuals in these trials, and primary analysis was performed on 1175 smokers of European ancestry (additional subjects were included in robustness analyses). We analyzed 785 SNPs representing 24 gene loci, which resolved into 245 LD blocks. Candidate genes of interest included nAChR subunits, varenicline transporter and additional targets, and genes involved in nicotine or bupropion metabolism. **Results** Analysis of baseline smoking (cigarettes/day) and nicotine dependence (Fagerstrom test) validated an association with a locus on chr15 that includes three nAChR subunit genes (*CHRNA5*, *CHRNA3*, *CHRNB4*) ($p<0.01$). Within the varenicline arm, continuous abstinence (weeks 9-12) was associated with *LOC123688* and nAChR subunit genes (*CHRNA4*, *CHRNA7*, *CHRNB2*) (OR=1.76; 95%CI=[1.23,2.52]) ($p<0.005$); within the bupropion arm, abstinence was associated with *CYP2B6* (OR=1.78;[1.27,2.50]) ($p<10^{-3}$). Incidence of nausea on treatment was predicted by SNPs in several nAChR subunit genes (OR=1.99;[1.43,2.78]) ($p<10^{-4}$) and time to relapse to smoking following therapy was associated with SNPs in *HTR3B* (HR= 1.97;[1.45,2.68]) ($p<10^{-4}$). **Conclusion** These data provide both novel and supporting evidence for genetic loci contributing to smoking cessation and therapeutic response. Importantly, different genetic signals are associated with varenicline vs. bupropion treatment response, suggesting that additional research may lead to clinically useful markers to guide treatment decisions, resulting in improved smoking cessation rates overall, and a reduction in smoking prevalence.

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## 2230/T/Poster Board #779

**A Pharmacogenetic Study of Novel SNPs in *VKORC1* and *CYP2C9* in an African American Cohort: Implications in Warfarin Maintenance Dose.** *M.A. Perera[1], L.H Cavallari[2], S. Patel[2], T. Ramikie[1], M. Flynn[1], N.J. Cox[1]*. 1) Medicine, University of Chicago, Chicago, IL; 2) Pharmacy, University of Illinois, Chicago, IL.

Genetic factors have appeared as important markers in determining warfarin dose requirements. Common functional variants in *CYP2C9*, named *CYP2C9*2* and *CYP2C9*3*, significantly lower maintenance doses, and have higher risk of serious bleeds. Both of these alleles have a low allele frequency (~ 10%) in Caucasians; have a lower frequency in African Americans (2 - 5%). In addition to *CYP2C9*, variation in the *VKORC1* gene accounts for 20 - 30% of the observed population variation in warfarin dose. Warfarin dosing algorithms incorporating the variation in both these genes have been very successful in explaining large portions of the variability of warfarin dose in both Caucasians and Asian populations. However these SNPs do not fully incorporate the variation seen in African Americans, and therefore are not as predictive of dose in this population ($r^2 = 0.19$ in African Americans as compared to $r^2 = 0.40$ in Caucasians). To provide a comprehensive evaluation of variation that may contribute to warfarin dose in African Americans we used both comparative genomics and putative transcriptional binding factor prediction to target resequencing efforts around regions of highest interest in both *VKORC1* and *CYP2C9*. We then used a tagging SNPs approach to genotype novel variation as well as functional variation in both genes to determine association with warfarin maintenance dose in 300 African American anticoagulation subjects. Upon testing the functional variants in *CYP2C9*, only *CYP2C9*5* and **11* were shown to be significant, along with several novel SNPs All previously known *VKORC1* variants were found to be significant. However, we also identified several novel SNPs that contribute to warfarin dose, many of which are more highly associated than the known *VKORC1* variants (p = 0.0003). These SNPs were found upstream of *VKORC1*. From these initial associations we developed an African American specific dosing algorithm, which incorporated all variation that influenced warfarin maintenance dose in this population. We validated the predictive power of this algorithm in an independent African American cohort of 50 patients. This study is the first to look at novel variation in these genes in an African American population, which may lead to more comprehensive and predictive dosing in this population.

## 2231/T/Poster Board #780

**Polymorphisms in the UGT1A locus are associated with hyperbilirubinemia in pazopanib treated patients.** *B. Reck, Z. Xue, L. Huang, K. Baker, M. Chen, V. Mooser, L. Cardon, C. Spraggs, L. Pandite, CF. Xu.* GlaxoSmithKline Research and Develolpment; Harlow, UK, North Carolina, and Philadelphia, USA.

Pazopanib is an oral multikinase angiogenesis inhibitor which has demonstrated antitumor activity in several tumour types. While the safety profile of pazopanib was generally acceptable and tolerable, elevations in total bilirubin (TBL) have been observed in some patients treated with pazopanib. Although such elevations are generally asymptomatic, the aetiology and mechanism of TBL elevation in pazopanib treated patients remain unknown. To evaluate the potential genetic effect for the observed increases of TBL, pharmacogenetic analyses of markers from 280 candidate genes were performed on samples from Caucasian patients collected from two renal cell carcinoma clinical studies. Maximum on-treatment TBL was found to be associated (P<0.01) with 94 markers from 35 genes in the first study (N= 112). Of these, ten markers were found to also be associated (P<0.01) with maximum on-treatment TBL in the second dataset (N=124). All 10 markers were located on chromosome 2 in a region containing the gene encoding uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1). Linkage disequilibrium analysis showed that these markers are strongly correlated with each other ($0.7 \leq r2 \leq 1$). Among them, the TA repeat polymorphism in the promoter of UGT1A1 (rs8175347) is a known valid biomarker that has been associated with plasma bilirubin concentration and drug induced hyperbilirubinemia. After adjusting for the effect of this polymorphism on TBL, no additional independent genetic associations were found between any of the other markers and TBL. It follows that the associations observed at this locus are likely to be a reflection of the same causative variant in the region. Accordingly, some cases of isolated hyperbilirubinemia in pazopanib treated patients are a manifestation of Gilbert's syndrome which is associated with the TA repeat polymorphism of the UGT1A1 gene.

## 2232/T/Poster Board #781

**Pharmacogenetic markers for cholinergic effects on nicotine addiction and smoking cessation.** *J. Sarginson[1], J. Killen[2], L. Lazzeroni[1], S. Fortmann[2], A. Schatzberg[1], G. Murphy[1].* 1) Psychiatry, Stanford University School of Medicine, Stanford, CA; 2) Stanford Prevention Research Center, Stanford University School of Medicine, Stanford, CA.

Stopping smoking is difficult even with treatment. Many patients prescribed pharmacologic treatments for smoking cessation experience side effects or lack of efficacy. We are performing a pharmacogenetic study of the efficacy and tolerability of bupropion and transdermal nicotine (TN), two treatments for smoking cessation. Samples are drawn from two smoking cessation studies. In the first study, 301 smokers received bupropion plus TN for 11 weeks, followed by 14 weeks of placebo or bupropion (MT1). In the second, 276 smokers received bupropion and TN for 8 weeks (MT2). The two studies used comparable selection criteria and recruited from the same community. We focused on 8 SNPs in the 15q24 region that contain the nicotinic cholinergic receptor subunits CHRNA5, CHRNA3 and CHRNB4. In other studies, this region has been implicated in several aspects of nicotine addiction and smoking cessation. Nicotinic cholinergic receptors are activated directly by TN and are present on neurons that are affected by bupropion. The primary clinical outcome measures in our studies were point-prevalence abstinence and time to relapse, but craving, withdrawal symptoms and adverse events due to the study drugs, including changes in weight and mood, were also considered. Analyses of baseline smoking quantity (SQ) and baseline addiction using the modified Fagerstrom score (mFTQ) were also undertaken. Analyses of baseline SQ and mFTQ were carried out on DNA samples from 577 individuals. The findings provided further support for a strong association between SQ and both the CHRNA5 SNP rs16969968 (D398N) and the CHRNA3 SNP rs1051730 (Y215Y), two SNPs that are in strong linkage disequilibrium (LD) in this cohort. Two further SNPs located in CHRNA3 and CHRNB4 appear to have independent associations with SQ. Pharmacogenetic analysis showed a strong association between rs8192475 (R37H) in CHRNA3, higher craving after quitting and increased withdrawal symptoms over time. This effect was seen in MT2 but not in MT1, suggesting that extended treatment may mitigate the pharmacogenetic effect. Additional markers for point prevalence abstinence in MT2 were also identified in CHRNA5 and CHRNB4. These results provide further support for the role of the CHRNA5/A3/B4 in determining number of cigarettes smoked. We have also identified markers for response to smoking cessation therapy. (Supported by the NIH and the California TRDRP).

## 2233/T/Poster Board #782

**NRXN1 variants are associated with behavioral difficulty in response to levetiracetam.** *T.J. Urban[1], N.M. Walley[1], P. Nicoletti[1], E.L. Heinzen[1], D. Ge[1], K.V. Shianna[1], R.A. Radtke[2], D.B. Goldstein[1].* 1) Center for Human Genome Variation, Institute for Genome Sciences and Policy, Duke Univ Medical Ctr, Durham, NC; 2) Department of Medicine, Division of Neurology, Duke Univ Medical Ctr, Durham, NC.

**Rationale:** The clinical use of levetiracetam (LEV) is limited in a subset of patients by neurobehavioral side effects including irritability and aggression, and more rarely, psychosis. The genetic basis for behavioral difficulty (BD) in response to LEV is entirely unknown.

**Methods:** A genome-wide association study (GWAS) of common single nucleotide polymorphisms (SNPs) and both common and rare copy number variants (CNVs) was conducted on a cohort of chronic epilepsy patients (n= 237) retrospectively assessed for BD following exposure to LEV. Patients were classified as cases if LEV was discontinued specifically due to BD, and controls if there was no mention in the patient history of BD after at least one year of continuous LEV treatment.

**Results:** No common SNPs were significantly associated with LEV-related BD after correction for multiple testing. However, the most strongly associated SNP was located in the NRXN1 gene (OR = 5.22, 95%CI: 2.5 - 10.9, $p = 8.2 \times 10^{-5}$), deletions in which had previously been associated with schizophrenia. Inspection of CNVs in the NRXN1 region revealed that three individuals carried deletions spanning coding exons of NRXN1, and these deletions were exclusive to cases (3/47 *vs.* 0/190, p = 0.007).

**Conclusions:** These results suggest that NRXN1, which is known to have a strong involvement in risk for schizophrenia, is also associated with LEV-related neurobehavioral side effects. These findings, however, require independent replication, which is an ongoing effort. Additional ongoing studies include targeted sequencing to uncover smaller-scale rare variants affecting antiepileptic drug response as well as shared risk factors between epilepsy and schizophrenia.

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## 2234/T/Poster Board #783

**A Pharmacogenetic Test To Enhance Understanding of Molecular And Pharmacological Chaperones By Using START (Sapropterin Therapy Actual Response Test) With Mutation Analysis For BH4 Response Testing In Phenylketonuria (PKU).** *J.R. Utz[1], D. Markowitz[1], C.Pham Lorentz[1], K.D. Rudser[2], B.M. Diethelm-Okita[1], D.C.C. Erickson[1], C.B. Whitley[1].* 1) Department of Pediatrics , Univ Minnesota, Minneapolis, MN; 2) Division of Biostatistics and Office of Clinical Research, Univ Minnesota, Minneapolis, MN.

Background: Sapropterin (SAP), synthetic tetrahydrobiopterin (BH4) for BH4 responsive PKU, works as chemical chaperone of phenylalanine hydroxylase (PAH) to facilitate and stabilize active PAH fold conformation. Only 20-56% of patients responded to SAP in clinical trials. The high cost of response tests and lifelong therapy warrants better understanding of patients likely to benefit from SAP. No standard pharmacogenetic tests exist to identify responsive genotypes. Previous BH4 and SAP pharmacogenetic studies indicate genotypes do not consistently predict response, but are weakened by varied: 1) SAP/BH4 doses; 2) response definitions; 3) duration; 4) phenylalanine (PHE) test times at varied protein catabolic states; 5) methods to control and report dietary PHE. START is a double-blind, placebo-controlled, 4-week test to identify SAP response while avoiding confounders. Hypothesis: START with mutation analysis gives a pharmacogenetic test to find genotypes that consistently predict SAP response and augment understanding of molecular and chemical chaperones. Methods: START results were evaluated for response and genotype correlates and trends in molecular characteristics. Results: To date, 44/56 enrolled patients completed START. Of these 44, 24 (54.5%) are SAP responders, 31 mutations are known, 24 different genotypes are present, 18 genotypes deemed response predictive. Alleles singly associated with response prediction include Y414C (5/5 patients responsive, 3 genotypes) and R408W (12/13 patients, 10/11 genotypes non-responsive). R408W, located at the hinge of an activating fold site, contains bulky aromatic amine tryptophan substituted for aliphatic arginine. START found aromatic amines substituted for aliphatic chains were non-responsive with low residual PAH activity in 12/13 (92%) patients, 10/11 genotypes (91%). Higher molecular weight amino acids substituted by smaller in 14/15 patients (93%) were responsive with high residual PAH activity. Conclusions: START identified 18 genotypes predictive of SAP response and showed response is swayed by mutant locus at activating PAH fold sites and spatial folding changes incurred by substituted amino acid size/configuration. Bulky aromatic amines substituted for aliphatic chains are associated with non-response. Findings warrant ongoing study of BH4 response via START to elucidate genotype response and mutant molecular characteristics' impact on protein folding dynamics in molecular and chemical chaperones.

## 2235/T/Poster Board #784

**Novel genes governing platinum chemotherapy toxicity susceptibility using an unbiased genome-wide approach.** *P.H. O'Donnell, E. Gamazon, W. Zhang, M.E. Dolan.* The University of Chicago, Chicago, IL.

Clinical observations show that Asians experience greater toxicity from platinum-containing chemotherapy regimens compared to other populations, suggesting that Asians may represent a particularly informative population in which to study genetic causes of platinum susceptibility. We utilized well-genotyped, Epstein-Barr virus-transformed lymphoblastoid cell lines (LCLs) from 90 apparently healthy Asian individuals in the International HapMap project to determine in vitro cisplatin and carboplatin-cytotoxicity phenotypes for use in genome-wide association studies. First, drug-specific $IC_{50}$s were employed as dependent variables in a linear regression with genotypic data for each line. Assuming an additive genetic model, analysis was restricted to SNPs (~1.2 million) having a minor allele frequency of ≥ 5% and for which missing data was < 20%. This analysis resulted in a list of 479 SNPs for cisplatin and 199 SNPs for carboplatin that were significantly associated with cytotoxicity ($P < 10^{-4}$). Candidate SNPs were then linearly modeled against baseline gene expression (~50,000 transcripts) to determine SNP-gene associations. This identified SNPs associated with 119 genes for cisplatin and 32 genes for carboplatin which were significant (Bonferroni $P_c < 0.05$). Finally, baseline expression levels for these genes were evaluated for association with the original $IC_{50}$ phenotypes for each drug ($P < 0.05$). A resulting short list of genes, and associated SNPs, defining the "susceptibility signature" for each drug in Asians was identified. For cisplatin, 8 target genes (*MAGEC3, WRNIP1, RPS28, CL640, CAST, SIVA, PAIP1, C11ORF17*) were implicated. For carboplatin, 2 target genes (*WRNIP1, SIVA*) were identified. Notably, *WRNIP1* and *SIVA* were important for cytotoxicity of both drugs, suggesting a possible platinum drug-class effect. Evaluation of expression changes in the identified genes and the impact on platinum susceptibility is currently being evaluated in a replication Asian LCL cohort (HapMap Chinese in Denver) and in other ethnic HapMap populations. In summary, an unbiased genome-wide approach allowed identification of novel cisplatin and carboplatin susceptibility genes which deserve further testing as possible mediators of the unique susceptibility of Asians, and perhaps other individuals, to toxicities from these chemotherapies.

## 2236/T/Poster Board #785

**Genome-wide SNP Association for Clinical Pain Sensitivity in Humans.** *H. Kim, S. Smith, R. Dionne.* NINR/NIH, Bethesda, MD.

Aims of the investigation: Microarray-based genotyping technology allows genome-wide screening for single nucleotide polymorphism (SNP) association studies. The major advantage of this approach is that no specific functional genetic hypotheses are required prior to undertaking analysis. Given the presumably polygenic and complex nature of pain, SNPs genotyped across the whole genome is an appropriate tool in the search for responsible genetic variations for pain. We applied genome wide SNP genotyping to investigate the role of genetics in responses to clinically induced pain in humans. Methods: Normal subjects (112 European Americans and 39 African Americans) were evaluated at the National Institutes of Health following informed consent under a human research protocol approved by the Institutional Review Board. Subjects underwent a standardized minor surgical procedure under local anesthesia and were monitored up to 3 hours after the surgery. The maximum post-operative pain rating, post-operative pain onset time and the analgesic onset time after ketorolac administration were used as measures of clinical pain and the onset of NSAID analgesia. The samples were genotyped at ~500,000 markers using Affymetrix 500K SNP assay. Results: While 4 SNPs (rs2562456, rs7295290, rs10014562 and rs17011183) showed significant association with analgesic onset time (false discovery rate (FDR) range from $1.18 \times 10^{-4}$ to $2.93 \times 10^{-2}$) in European Americans, 2 different SNPs (rs2943024 and rs6725855) were associated with maximum post-operative pain rating (FDR range from $3.35 \times 10^{-3}$ to $1.13 \times 10^{-2}$) in African Americans. Conclusion: Genome wide association study found that genetic variations play a role in the individual variations in responses to analgesic drugs in European Americans but not in African Americans. On the contrary, genetic variations were associated with the amount of pain caused by minor surgery in African Americans only. These results suggest that genetic factors influence pain sensitivity and analgesia in humans in an ethnicity dependent manner. Acknowledgement: This study was supported by the Division of Intramural Research, National Institute of Nursing Research, National Institutes of Health, Bethesda, MD 20892, USA.

## 2237/T/Poster Board #786

**Characterization of genetic variants in the 3' UTR of organic cation transporter 3 (OCT3).** *L. Chen, A. Roomiani, S. Yee, K. Giacomini.* Department of Bioengineering and Therapeutic Sciences, UCSF, San Francisco, CA.

The organic cation transporter 3 (OCT3) is widely distributed in body tissues and mediates the transport of important endogenous and exogenous organic cations including histamine, norepinephrine, serotonin, metformin and oxaliplatin. Several genome-wide association studies (GWAS) indicate that OCT3 plays an important role in the development of prostate cancer and in cardiovascular disease. The goal of this study was to identify and characterize genetic variants in the 3' untranslated region (3'UTR) of OCT3, which is 3926bp in length. We identified 30 novel single nucleotide polymorphisms (SNPs) in DNA samples from 272 individuals from Chinese, European, African and Mexican ancestries. Genetic variants ranged in allele frequencies from singletons, identified one time on one chromosome to 46% in a single population. The OCT3 3' UTR was divided into five segments due to its unusual length and each segment was cloned into a pMIR-REPORT vector system. Genetic variants were generated by site directed mutagenesis and functional analysis using the dual luciferase assay was performed. Reporter plasmids were transfected into four cell lines: HepG2, HCT116 and two prostate cancer cell lines (DU-145, PC-3). Two of the genetic variants (457T/C and 514C/A, allele frequencies of 5%) showed a significantly reduced luciferase activity ($p < 0.01$) in all four cell lines when expressed in the full length 3'UTR. No significant change in luciferase activity was observed when the variants were present in truncated segments of the OCT3 3'UTR. Further studies are underway to determine the mechanism for the reduced activity of the two variants in the 3'UTR of OCT3. These variants may play a role in variation in drug disposition and response and may also be important in risk for human disease.

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## 2238/T/Poster Board #787

***In Vivo* Pharmacogenomic Characterization of Enhancers in Liver Membrane Transporters.** *M.J. Kim[1,2,3], P. Skewes-Cox[4], S. Hesselson[5], P.Y. Kwok[5], D.L. Kroetz[1], K.M. Giacomini[1], N. Ahituv[1,2]*. 1) Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, CA; 2) Institute for Human Genetics, UCSF; 3) Pharmaceutical Sciences and Pharmacogenomics, UCSF; 4) Biological and Medical Informatics Program, Department of Biochemistry, Microbiology and Medicine, Howard Hughes Medical Institute, UCSF; 5) Cardiovascular Research Institute, UCSF.

Liver membrane transporters play an important role in determining individual drug response due to their involvement in hepatic uptake and elimination. Numerous pharmacogenetic assays have demonstrated that nucleotide variation in the coding regions of these transporters and differences in their expression levels have a strong influence on drug response. However, little is known about how genetic variation in enhancers, sequences that regulate gene promoters, influence transporter expression and function and how they contribute to differences in drug response. In this study, we set out to identify enhancers that regulate liver membrane transporters and to investigate whether nucleotide variation in these enhancers can alter their expression levels. Using comparative genomics, we identified evolutionary conserved sequences (ECRs) surrounding nine liver membrane transporters (*SLC22A1, SLCO1B1, SLCO1B3, ABCC2, ABCB11, SLC22A7, SLCO2B1, SLC10A1, SLC47A1*) known to interact with commonly-used pharmaceuticals. These sequences were then ranked for the presence of liver-specific transcription factor binding sites and the top 50 ECRs were analyzed for enhancer activity *in vivo* using the mouse hydrodynamic tail vein assay. This assay utilizes a rapid intravascular injection of DNA into the mouse tail vein, leading to its specific expression in the liver. Eleven out of the 50 ECRs tested exhibited significant liver enhancer activity when compared to the control vector. Several of these positive enhancers were then sequenced in DNA samples from an ethnically diverse human population (N=250). Selected common variants were assayed for differential enhancer activity compared to the reference allele and several variants were found to affect activity. Assays to associate these variants with liver transporter expression levels in human liver tissues are currently on-going. Combined, these studies provide a model for elucidating how nucleotide variation in enhancer sequences can contribute to inter-individual differences in drug response. Furthermore, understanding how genetic variability in membrane transporters influences drug response and adverse drug effects will ultimately lead to more efficacious and efficient treatments.

## 2239/T/Poster Board #788

**Pharmacogenetics: In-vitro drug toxicity screening.** *T. Wiltshire[1], N. Butz[1], O. Suzuki[1], B. Steffy[1], D. Scoville[1], R. Thomas[2]*. 1) Sch Pharmacy, Univ North Carolina, Chapel Hill, NC; 2) The Hamner Institute for Health Sciences, Research Triangle Park, NC.

Pharmacogenetic studies have successfully identified genetic variants that contribute to variation in susceptibility to drug responses, but it is still a complex and challenging task to evaluate broadly across the human genome to identify the genetic components of response to drugs. Despite limitations there is a pressing need to identify genetic components that contribute to the efficacy and toxicity of drugs, across a wide spectrum of agents. We have proposed that using an alternative model population approach will provide many underlying mechanisms and pathways that are implicated in drug activity and responses. We have developed a platform of both in-vitro and in-vivo assays from genetically well defined mouse strains which will enable us to assess effects of toxicity and efficacy of current and novel agents in drug therapies. Here, we present a new strategy to identify genes and gene pathways that underlie susceptibility only to cellular-level adverse drug reaction. We have the ability to pinpoint genetic components that contribute to, or underlie, the toxicity phenotypes. We have screened 25 drugs (mostly anti-cancer agents) in whole well viability assays across 32 genetically well-defined primary cell lines. We have also screened the same cell lines with 50 toxicants/drugs in a high-content imaging screen that determines changes in specific cell-health status phenotypes (nuclear changes, membrane permeability, mitochondrial membrane potential and apoptosis). We can assess a high level of heritability (60-80%) for many of these phenotypes and have performed genome-wide association analysis to identify loci that underlie the toxicity traits. Although no targets have been fully validated yet we identify a number of potential candidate genes for specific drug toxicity phenotypes.

## 2240/T/Poster Board #789

**ABCC2 Genetic Variations and Carbamazepine Drug Response.** *S. Kim[1], W. Kim[2], J. Lee[1], J. Yi[1], Y. Cho[2], K. Heo[2], S. Lee[1], K. Kim[1], B. Lee[2], M. Lee[1]*. 1) Pharmacology, Yonsei University College of Medicine, Seoul, Korea; 2) Neurology, Yonsei University College of Medicine, Seoul, Korea.

ABCC2 (MRP2; multidrug resistance protein 2) is a primary active transporter that exports many chemical agents. Genetic variations in the ABCC2 located in brain endotheilium may affect individual drug responses to antiepileptic drugs such as carbamazepine. We investigated the association between ABCC2 polymorphisms and the drug response, including CNS side effects, of carbamazepine in Korean epileptic patients. Five tag SNPs(single nucleotide polymorphisms) in the ABCC2 gene were analyzed in 146 epilepsy patients. Patients were divided into two groups: those who experienced side effects of the CNS(central nervous system) (n=47) and those who did not (n=99). A nonsynonymous polymorphism, V417I (c.1249G>A), showed a strong association with CNS side effects caused by carbamazepine (P= 0.009). In the logistic regression analysis using multiple variables, the odd ratio of CNS side effects caused by carbamazepine was 5.32-fold higher in patients with the c.1249A allele (P=0.002). The functional study using a FACScan flow cytometer revealed that the V417I variation selectively reduced carbamazepine transport across the cell membrane. These results strongly suggest that the c.1247G>A polymorphism in the human MRP2 gene is associated with CNS side effects to carbamazepine. (This study was supported by grants 05132KFDA173 from the Korea Food & Drug Administration and A030001 from the Korea Health 21 R&D Project, Ministry for Health, Welfare and Family Affairs, Korea.).

## 2241/T/Poster Board #790

**ES cell derived hepatocytes are a useful model of drug toxicity.** *D. Dimmock[1], A. Buchaklian[1], K. Si-Tayeb[2], D. Helbling[1], S. Duncan[2]*. 1) Pediatrics, Medical College of Wisconsin, Milwaukee, WI; 2) Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI.

Several recent studies have demonstrated that hepatocyte cultures of human cancer origin may be a valuable model for predicating human hepatotoxicity and adverse drug reactions. However, the human hepatocellular carcinoma tissues do not reflect true hepatocyte drug response as they are lacking several key drug transport proteins. Hence, primary hepatocyte cultures are becoming a preferred vehicle for drug testing. These primary hepatocyte cultures have to be established from freshly harvested human livers, necessitating a biopsy or cadaveric sampling. Such harvesting is rarely an option in children with rare metabolic diseases as they seldom die at an equipped center with appropriate parental consent obtained. Since these cells can only undergo a very limited number of passages, the experiments that can be performed are limited by cell availability. It would be very valuable to have hepatocyte cultures which can be recurrently derived from the same individual, for instance from a stem cell, hence reducing intra-experimental variability to the condition being tested. These hepatocytes could be used to test for the therapeutic or toxic effects of pharmaceutical agents. Published reports in mammalian, including human primary hepatocytes demonstrate that, after exposure to acetaminophen, there is a dose dependent increase in apoptosis and cell death compared with vehicle treated cell lines. To evaluate ES cell derived hepatocytes as a model of such toxicity; we differentiated primary human ES cells using specific factors. Cultures were then incubated for 24 hours in media containing a final concentration of 5 or 10umol of acetaminophen or PBS vehicle. An alamar blue based dye (Resazurin) and a "live-cell" protease were used to measure resultant viability. The results demonstrate dose dependent toxicity of acetaminophen, consistent with the response of published human primary hepatocytes. This suggests ES derived hepatocytes have an equivalent pharmacodynamic response to toxins as primary hepatocytes and may serve as a useful model.

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## 2242/T/Poster Board #791

**The Influence of SLCO1B1 Variants on Cerivastatin Drug Transport.**
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Drug metabolizing enzymes and membrane transporters work in concert to regulate the absorption, distribution, metabolism and excretion of drugs. Genetic variation in drug metabolizing enzymes and membrane transporters can modulate the beneficial as well as the deleterious effects of drugs. In a study of patients who developed rhabdomyolysis while taking the HMG-CoA reductase inhibitor, cerivastatin, we sought to identify genetic variants that might explain the high incidence of rhabdomyolysis associated with this medication. We re-sequenced several genes involved in the uptake and metabolism of cerivastatin and identified seven non-synonymous coding variants in SLCO1B1 which encodes the liver specific membrane drug transporter OATP1B1. Cerivastatin and other HMG-CoA reductase inhibitors are substrates of OATP1B1, leading to the hypothesis that SLCO1B1 variants may alter cerivastatin transport. To test this hypothesis, we stably transfected HEK293-FRT and MDCK-FRT cells with plasmids containing the variant cDNAs created by site-directed mutagenesis and measured the function of the transporter in an in vitro assay. To date a novel frameshift mutation in exon 9 of the SLCO1B1 gene discovered in this specific population group is determined to have a complete loss of function. The study of the remaining six nonsynonymous mutations on cerivastatin uptake is currently underway. The functional data characterizing the effect of all identified SLCO1B1 variants on cerivastatin uptake in stably transfected HEK293-FRT and MDCK-FRT cell lines will be presented. These results may shed light on risk factors for rhabdomyolysis, a serious side effect of statin drugs.

## 2243/T/Poster Board #792

**ABCB1 Polymorphism is Associated with Side Effects of Methylphenidate in Attention Deficit Hyperactive Disorder.**
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P-glycoprotein (ABCB1; ATP binding cassette, subfamily B, member 1, MDR1; multidrug resistance protein 1) located at the luminal side of brain endothelial cell is a potent candidate to alter the pharmacokinetic values of psychotropic medications. We investigated the association between ABCB1 polymorphisms and side effects of methylphenidate, a brain stimulant known as a ABCB1 substrate mainly used in attention-deficit hyperactive disorder (ADHD). One hundred ninety two Korean patients who had been administered methylphenidate for their ADHD were divided into two groups according to the level of experiencing drug side effects at four weeks and genotyped with five tag SNPs (single nucleotide polymorphisms) of ABCB1. Our results showed that patients with T homozygote at G2677A/T have more side effects of methylphenidate at 4 weeks than patients with other alleles. The functional study using ATPase assay revealed that ABCB1 with 2677T allele had diminished methylphenidate transporting activity than 2677G or 2677A alleles. The side effects of methylphenidate have been regarded as one of major factors associated with drug compliance and treatment response in ADHD. Therefore, our results implied that the polymorphism of ABCB1 would be an important factor in prescribing methylphenidate in patients with ADHD. (This study was supported by grant A030001 from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Korea).

## 2244/T/Poster Board #793

**Azathioprine induced severe Pancytopenia in one patient with Crohn's disease: identification of a novel Thiopurine S-Methyltransferase allelic variant IVS8-1G>A.**
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INTRODUCTION Thiopurine S-methyltransferase (TPMT) polymorphisms are a major factor responsible for large individual variations in thiopurine toxicity due to excessive accumulation of cytotoxic metabolites. This present clinical observation describes a 14-year-old girl with Crohn's disease who developed severe pancytopenia during her Azathioprine (AZA) treatment. METHODS Red blood cell thioguanine nucleotides (RBC 6-TGN) and 6-methylmercaptopurine ribonucleotides (6-MMP) concentrations were measured by high-performance liquid chromatography. The open reading frame of TPMT gene (exons 3 to 10) and their consensus flanking sequences were sequenced (Applied Biosystems 3130 XL). RESULTS A 14-year-old girl with Crohn's disease treated by AZA was hospitalized for a wide pancytopenia highlighted by a routine blood analysis. Viral infections were excluded. Genotyping for the three predominant TPMT mutations (TPMT*2, TPMT*3B, TPMT*3C) showed a heterozygous TPMT*2 genotype but 6-TGN levels were unexpectedly elevated (3414 pmol/8*$10^8$RBC) and the concentration of 6-MMP was inferior to 20 pmol/8*$10^8$ RBC. Familial genetic analysis showed that the patient was carrier of a heterozygous composite TPMT genotype for two non-functional mutations. We identified a novel TPMT variant allele IVS8-1G>A corresponding to a G>A transition at the splice acceptor site of the intron 8 probably responsible of TPMT deficiency. CONCLUSION In cases of TPMT phenotype/genotype discordance concomitant with the occurrence of a severe adverse effect, sequence analysis of the complete open reading frame of the gene is interesting to identify rare inactivating variants. This case illustrates that TPMT genotype determination before treatment and measurement of 6-TGN and 6-MMP concentrations at steady-state remain important tools for thiopurines monitoring and clinical decision making.

## 2245/T/Poster Board #794

**CYP2C19 and CYP2D6 variation and response to citalopram treatment.**
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Subjects in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study were treated for major depressive disorder with citalopram. We used the STAR*D data to assess the association of genetic variation in two cytochrome P450 genes with drug response. Associations of remission and tolerance with CYP2C19 and CYP2D6 alleles were evaluated in a subset of 1074 white non-Hispanic patients using logistic regression models. In addition, CYP2C19 and CYP2D6 genotypes were used to classify subjects into categories based on known activity properties of the alleles (e.g. poor, intermediate, extensive and ultrarapid metabolizers), and association of these categories with treatment outcomes was assessed. Tolerance was defined based on study exit data. Patients who continued citalopram treatment in Level 2 of STAR*D were considered tolerant, while patients who refused to continue citalopram or left the study due to side effects were considered intolerant. Remaining subjects were classified as probably tolerant, probably intolerant, or intolerant based on the level of side effects at study exit as measured by the Global Rating of Side Effect Burden scale. The inactive CYP2C19 *2 allele was found to be associated with lower odds of tolerance (p-value = 0.02; OR = 0.67, 95%CI = (0.48,0.94). Similarly, CYP2C19 activity quantified from 1=poor metabolism to 6=ultrarapid metabolism demonstrated a trend of association with tolerance (p-value=0.06, OR = 1.15, 95%CI = (1.00, 1.32)). Furthermore, a significant association of remission with 2C19 activity classes was detected in the subgroup of tolerant subjects (p=0.03). Without consideration of the 2C19 genotype, there was no detectable association between 2D6 genotype and tolerance or remission. However, there was a significant interaction between the effects of CYP2C19 and 2D6 genotypes on remission (p=0.03). These findings suggest that variations of the CYP2C19 gene are associated with remission in white non-Hispanic depressed adults treated with citalopram. As the 2D6 enzyme does not play a primary role in the metabolism of citalopram, it is not surprising that CYP2D6 genotype is not as strongly associated with remission. However, the detected interaction between the effects of these two genes on tolerance and remission demonstrates that both enzymes appear to be playing a role, and the combination of a patient's genotypes at these two genes contributes to their response to citalopram treatment.

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## 2246/T/Poster Board #795

**Genome-wide association study of response to warfarin in a UK prospective cohort.** *S. Bourgeois[1], A. Jorgensen[2], N. Soranzo[1], P. Whittaker[1], O. McCann[1], N. Hammond[1], S. Bumpstead[1], p. Williamson[2], P. Deloukas[1], M. Pirmohamed[2].* 1) Wellcome Trust Sanger Institute, Hinxton Cambridge, United Kingdom CB10 1SA; 2) Department of Pharmacology & Therapeutics, University of Liverpool, Sherrington Building, Ashton Street,Liverpool UK L69 3GE.

Warfarin is a widely prescribed anticoagulant drug to treat thromboembolic events that often give rise to stroke, deep vein thrombosis, and pulmonary embolism. Studies aimed at discovering the genetic and environmental determinants of warfarin dose requirements have been replicated in many cohorts, leading to the development of algorithms that can predict warfarin dose to within 20% of the actual dose in 55% of patients. Beyond CYP2C9 and VKORC1, in a recent genome-wide association study (GWAS) of 1000 Swedish patients we indentified CYP4F2 which accounts for just over 1% of the observed dose variation. In anticipation of no further common variants with large effects we set to explore clinically relevant outcome measures, such as dose stability, high INR during the first week of treatment, and time to bleeding event analyses. We genotyped a sample of 752 warfarin patients enrolled in a prospective study in Liverpool using Illumina's 610K QUAD chip. Post QC, data were first analyzed by linear regression; not surprisingly, only VKORC1, CYP2C9*2 and *3 were significant determinants of dose requirements, whereas we lacked power to detect a CYP4F2 effect. Interestingly, Cox regressions for time to stable INR, INR over 4 during first week of treatment, and bleeding events (classified according to several criteria) identified novel loci which may be implicated in patients' response to warfarin. A meta-analysis of the UK and Swedish scans for bleeding events is underway. The detailed phenotypic data allowed us to further dissect the influence of known and novel determinants of the inter-variability in response to warfarin, highlighting the great potential gains that can accrue from studying such phenotypes in addition to the dose. Such novel information is expected to further improve the utility of warfarin pharmacogenetics in the clinic. We acknowledge funding from the UK Department of Health.

## 2247/T/Poster Board #796

**Validation and Application of a Broad-Based ADME Genotyping Assay in Research and Clinical Trials.** *A.M.K. Brown[1,2], Y. Renaud[1], C. Ross[3], M. Hansen[4], U. Zanger[5], J-C. Tardif[1,2], M.S. Phillips[1,2].* 1) Genome Quebec & Montreal Heart Institute Pharmacogenomics Centre, Montreal, QC, Canada; 2) Université de Montréal, Montreal, QC, Canada; 3) University of British Columbia, Vancouver, BC, Canada; 4) Illumina, Inc. San Diego, CA; 5) Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany.

Many medications show clear patient variability in terms of efficacy and undesired adverse side effects. Genetic variation in a well understood set of genes encoding enzymes and transporters involved in pathways affecting the Absorption, Distribution, Metabolism and Excretion (ADME) of drugs may directly contribute to the observed variation in patient response. To identify how ADME pathways affect drug response and safety, we have developed a broad-based ADME genotyping panel that encompasses the majority of the known variation present in the top ~180 ADME genes as ranked by several pharmaceutical companies. The genotyping panel, which consists both functional and haplotype tagged SNPs, is composed of ~3,000 SNPs that covers most of key functional variants that comprise the ADME "Core List" of genes. The assay was developed using Illumina's GoldedGate chemistry and the design has been optimized using novel strategies to overcome 3 identified failure modes; i) the presence of variants underlying oligo sequences, ii) assayed SNPs in close proximity, and iii) regions of high homology (including CNV and low complexity DNA). To validate the accuracy of the genotyping assays, we have screened samples that have known functional rare variants, HapMap samples to validate the haplotype tagging SNPs and a set of 150 liver DNA's (Institute of Clinical Pharmacology, Stuttgart) that have been extensively genotyped for many ADME variants and haplotype tags. Data from the Illumina broad-based panel has also been cross validated by comparing data from different technological platforms. Since the Stuttgart liver samples have been extensively characterized for ADME gene expression and enzymatic activity of many ADME proteins, these samples will be used to assess additional genotype/phenotype correlations that can be made using the broad-based panel over existing ADME data. To date, the assay has been used to screen patients involved in two clinical trials of drug response, as well as a research project on adverse drug reactions in children with promising results. We hope to continue to use this panel to screen subjects in clinical trials and research projects with the goal of identifying genetic variation in ADME genes responsible for variable drug responses.

## 2248/T/Poster Board #797

**Tacrolimus pharmacogenomics in kidney transplant: preliminary results from a 7 center consortium.** *P.A. Jacobson[1], A. Israni[2], S. Basu[3], T. Bergemann[3], R. Leduc[3], B. Julian[4], A. Matas[5], W. Oetting[1], DeKAF Investigators.* 1) Clinical Pharmacology, University of Minnesota, Minneapolis, MN; 2) Hennepin County Medical Center, Nephrology; 3) Biostatisics, University of Minnesota; 4) Unviversity of Alabama, Birmingham, AL; 5) Transplant Surgery, University of Minnesota.

There are conflicting data on the influence of single nucleotide polymorphisms (SNPs) on the pharmacokinetics of tacrolimus (TAC), a calcineurin inhibitor, which is the mainstay immunosuppressant in solid organ transplantation (tx). Methods: TAC pharmacogenetics were studied through a 7-center consortium in the U.S. and Canada in 680 kidney transplantation subjects. TAC trough concentrations were determined in each patient as part of clinical care twice in weeks 1-8 posttx and then once in each of months 3, 4, 5 and 6 posttx. Trough TAC concentrations were adjusted for TAC dose and weight. TAC doses were adjusted to maintain blood concentrations in the therapeutic range as defined by center. Recipient DNA was genotyped using a customized Affymetrix SNPChip containing 3500 SNPs of which 1000 were drug absorption, distribution, metabolism and excretion SNPs. Results: Mean±SD age and weight of the recipients were 49.5±12.7 yrs and 82.2±19.6 kg, respectively. 545 recipients were nonAA (African American) and 135 were AA. TAC mean daily dose requirements were higher in AA (7.6±3.4 mg) than nonAA (5.6±3.3 mg). Mean TAC troughs were lower in AA (6.7±3.4) than nonAA (8.6±3.4 ng/mL). Repeated measures analyses were performed to examine the effect of individual SNPs on adjusted troughs. In univariate analyses when adjusted for race, 30 SNPs were highly significant ($p \leq 0.0002$) towards TAC troughs. The top SNP was cytochrome P450 (CYP) 3A5*1 (rs776746) with a p=4.2x10-37. Troughs (adjusted for daily dose and weight) were 177.8±53.4 for individuals with 3A5*3/*3, 93.4±72.7 for 3A5*3/*1 and 72.9±71.9 for 3A5*1/*1. After adjustment for CYP3A5*1, five other SNPs (2 from CYP3A4 and 1 each from MDM2, ATF6 and COMT genes) remained significant ($p<0.0005$). The CYP3A4, MDM2 and ATF6 increased TAC troughs whereas the COMT SNP was associated with lower TAC concentrations. Conclusion: We found that the CYP 3A5*1 allele was significantly associated with lower TAC concentrations in a large, ethnically diverse population. Multiple previously unexplored polymorphisms were identified. These variants may further explain the substantial interpatient variability in TAC concentrations and will be validated with intensive pharmacokinetic evaluation in patients specifically recruited with these genotypes.

## 2249/T/Poster Board #798

**Association of polymorphisms of CYP2D6 and other PK-related genes on recurrence-free survival in Japanese breast cancer patients receiving adjuvant tamoxifen therapy.** *K. Kiyotani[1], T. Mushiroda[1], M. Sasa[2], T. Yamakawa[3], K. Hirata[4], M. Okazaki[5], S. Ohsumi[6], Y. Takatsuka[7], Y. Bando[8], I. Sumitomo[2], T. Tsunoda[9], N. Hosono[10], M. Kubo[10], Y. Nakamura[1,11], H. Zembutsu[11].* 1) Lab for Pharmacogenetics, RIKEN Center for Genomic Med, Yokohama, Japan; 2) Department of Surgery, Tokushima Breast Care Clinic, Tokushima, Japan; 3) Yamakawa Clinic, Kochi, Japan; 4) First Department of Surgery, Sapporo Medical University School of Medicine, Sapporo, Japan; 5) Department of Surgery, Sapporo Nyusen Clinic, Sapporo, Japan; 6) Department of Breast Oncology, National Hospital Organization Shikoku Cancer Center, Ehime, Japan; 7) Department of Surgery, Kansai Rosai Hospital, Hyogo, Japan; 8) Department of Molecular and Environmental Pathology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan; 9) Lab for Medical Informatics, RIKEN Center for Genomic Medicine, Yokohama, Japan; 10) Lab for Genotyping Development, RIKEN Center for Genomic Medicine, Yokohama, Japan; 11) Lab of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

The clinical efficacy of tamoxifen has been suggested to be influenced by the activity of metabolizing enzymes and transporters that involved in the formation, detoxification and elimination of tamoxifen and its active forms, 4-hydroxytamoxifen and endoxifen. We investigated the effects of polymorphisms in the genes possibly associated with pharmacokinetics (PK) of tamoxifen and its metabolites in 283 breast cancer patients who were received adjuvant tamoxifen monotherapy. Kaplan-Meier estimates showed that CYP2D6*10 were significantly associated with recurrence-free survival (log-rank P = 0.00029). Multivariate Cox proportional hazard analysis demonstrated that the CYP2D6 genotype was one of the independent factors affecting recurrence-free survival (P = 0.00055; adjusted hazard ratio (HR), 9.36; 95% CI, 2.63-33.29 for CYP2D6*10/*10, and P = 0.037; adjusted HR, 3.79; 95% CI, 1.08-13.22 for CYP2D6*1/*10 compared to patients with CYP2D6*1/*1). We then investigated the association of polymorphisms in tamoxifen PK-related genes, including other P450 isoforms, UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs). However, no polymorphisms in these genes tested in this study were significantly associated with recurrence-free survival of patients receiving tamoxifen monotherapy (P > 0.05). The present study suggests that the CYP2D6 genotype should be considered when selecting adjuvant hormonal therapy for breast cancer patients.

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## 2250/T/Poster Board #799

**Warfarin dose variation and VKORC1/CYP polymorphisms.** *M.K. Kringen[1], K.B.F. Haug[1], R.M. Grimholt[1], C. Stormo[1], A.P. Piehler[1], I. Seljeflot[2], J.P. Berg[1], O. Børs[1].* 1) Department of Medical Biochemistry and Clinical Pharmacology, Oslo University Hospital, Ullevål, Oslo, Norway; 2) Center for Clinical Heart Research, Department of Cardiology, Oslo University Hospital, Ullevål, Oslo, Norway.

Background: Warfarin dosage has traditionally been a challenge to clinicians because of its narrow therapeutic range and patient variability in clinical response. Polymorphisms affecting both warfarin pharmacokinetics and pharmacodynamics have been in focus, especially the impact of CYP2C9, VKORC1 and now recently, the CYP4F2 gene. Previously we have found VKORC1*2 (rs9934438) to account for 24.5%, and CYP2C9*2 and CYP2C9*3 combined (rs1799853 and rs1057910), to account for 7.2% of the warfarin dose variation in patients from the Warfarin Aspirin Reinfarction Study (WARIS-II). In this study we investigated whether VKORC1*3 (rs7294), VKORC1*4 (rs17708472) and CYP4F2 (rs2108622) were associated with warfarin dose requirements in the same patient group. Methods: The patients (n=105) in this study were a subgroup of the WARIS-II study who had been assigned to either warfarin alone or warfarin in combination with aspirin after acute myocardial infarction. DNA from patients was genotyped for VKORC1*3, VKORC1*4 and CYP4F2 (rs2108622) by real-time PCR. Genotyping of VKORC1*2, CYP2C9*2 and CYP2C9*3 were done previously. Results: None of the SNPs deviated from the Hardy-Weinberg equilibrium (exact test). Multivariate regression of CYP4F2 (rs2108622), adjusted for age and treatment group, showed a weak association on warfarin dose for the heterozygous CYP4F2 variant (P=0.05). However, adding this polymorphism into our main regression model together with our previously reported data on the VKORC1*2, CYP2C9*2 and CYP2C9*3 genotypes, age and treatment group, gave no significant associations for neither of the CYP4F2 genotypes and reduced the $R^2$. There was a significant association between warfarin dose and VKORC1*3 and VKORC1*4 when adjusted for age and treatment group only (P<0.001 and P=0.003, respectively). They did not, however, add more information when included into the main regression model, and the associations between warfarin dose and VKORC1*3 and VKORC1*4 were lost. Conclusion: In our study of patients with myocardial infarction, up to 32% of the variability in warfarin dose requirements could be explained by VKORC1*2, CYP2C9*2 and CYP2C9*3. The addition of VKORC1*3, VKORC1*4 or CYP4F2 (rs2108622) into the main regression model did not explain more of the variability in warfarin dose requirements, indicating that genotyping of CYP2C9*2, CYP2C9*3 and VKORC1*2 is sufficient for warfarin dose prediction.

## 2251/T/Poster Board #800

**Teststrip-based genotyping to assist in the prediction of anticoagulant dose requirement.** *H. Puehringer[1], G. Klose[2], B. Schreyer[2], W. Krugluger[3], R.M. Loreth[2], C. Oberkanins[1].* 1) ViennaLab Diagnostics, Vienna, Austria; 2) Clinical Haemostaseology, Westpfalz-Klinikum GmbH, Kaiserslautern, Germany; 3) Institute of Laboratory Medicine, Donauspital, Vienna, Austria.

Background: Coumarin derivatives (warfarin, phenprocoumon) are the most widespread oral anticoagulant drugs for the prevention and treatment of thromboembolic disorders. However, these vitamin K antagonists have a narrow therapeutic range and a wide interindividual variability in dose requirement. Despite adjustment for clinical variables, adverse events are frequently encountered during the initial phase of therapy. Genetic polymorphisms in the drug-targeted vitamin K epoxide reductase complex 1 (VKORC1) and in the drug metabolizing enzyme CYP2C9 have been reported to account for the majority of variations in the therapeutic response to warfarin. Aims and Methods: A genetic test (StripAssay) for the simultaneous detection of two VKORC1 polymorphisms (-1639G>A, 3730G>A) and the functionally defective CYP2C9 variants *2 (430C>T) and *3 (1075A>C) was developed. The protocol is based on multiplex PCR and reverse-hybridization of biotinylated amplification products to allele-specific probes on membrane teststrips. The new StripAssay is currently being used in an ongoing clinical study to classify patients into high, intermediate and low dose responders to coumarin anticoagulants. Results: Preliminary data based on more than 170 patients treated with phenprocoumon (Marcumar) indicated a considerably lower stable dosage required for therapeutic anticoagulation in carriers of a combined VKORC1 -1639A and CYP2C9 *2 or *3 genotype compared to carriers of a single variation or wildtype alleles. The VKORC1 3730G>A polymorphism seemed to have no additional predictive power for phenprocoumon dose variability. Summary and Conclusions: The new diagnostic assay and the results obtained during our study will assist clinicians to achieve a safer and more individualized anticoagulant therapy. (oberkanins@viennalab.co.at).

## 2252/T/Poster Board #801

**Validating a novel hepatocyte culture platform for *in vitro* pharmacogenomics.** *M. Saito[1,2], C. Cotsapas[1,2], S. Khetani[3], S. Katz[3], E. Liu[3], J. Yeh[3], S. Gupta[2], M. Mino-Kenudson[4], R. Gould[5], S. Bhatia[3,5], M. Daly[1,2].* 1) CHGR, Massachusetts General Hospital, Boston, MA; 2) Medical and Population Genetics, Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge MA, 02142; 3) Laboratory for Multiscale Regenerative Technologies, MIT, 77 Massachusetts Ave. Building E19-502d, Cambridge MA, 02139; 4) Department of Pathology, Harvard Medical School and Pathology, Massachusetts General Hospital, 55 Fruit St. Boston MA, 02114; 5) Novel Therapeutics Platform, Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge MA, 02142.

Population variation in drug metabolism leading to adverse toxic events is a major medical burden, resulting in the withdrawal of several widely prescribed pharmaceuticals. Understanding the genetic underpinnings of this variation is crucial to prevention efforts and to incorporating variation response assays earlier in the drug development process. Such efforts have been hampered by the absence of suitable *in vitro* models of human liver, as hepatocytes lose the ability to induce metabolic enzyme production in response to chemical stimulus within hours of isolation from tissue. We have developed an *in vitro* hepatocyte culture system which preserves this ability for several weeks [Khetani and Bhatia, Nat Biotech 2008]. This provides the first realistic platform in which to quantitatively screen metabolic response to therapeutics in humans, potentially enabling us to perform large-scale pharmacogenomic assays. Here, we describe a comparison of *in vivo* and *in vitro* responses to two drugs in mice, in which we calibrate the ability of our platform to recapitulate *in vivo* biology and perform pharmacogenomics experiments in humans. We administered therapeutically equivalent doses of acetaminophen (APAP) and 5`-fluorouracil (5FU) to pools of four C57Bl/6J male mice. We measured gross response with serum chemistry and microscopic liver histopathology after 24 hours of drug administration; to look for more subtle responses, we also performed gene expression profiling of RNA prepared from the liver. In parallel, we exposed hepatocyte co-cultures from drug-naïve mice to equivalent doses of APAP and 5FU for 24 hours. We performed serum chemistry tests on the culture media and expression profiling on RNA prepared from the cells. We detected dose-dependent elevations in alanine and aspartate aminotransferases (ALT and AST) both *in vivo* and *in vitro*, with elevations occuring at the same dosage levels. We have also been able to determine dose-dependent *in vivo* gene expression signatures unique to each drug compared to control samples. We are currently exploring the overlap between these signatures and those obtained *in vitro*. Our initial results indicate substantial correlation, suggesting our hepatocyte culture system is able to recapitulate *in vivo* events. We are therefore hopeful that our platform will allow us to execute pharmacogenomic experiments on human hepatocytes to uncover the molecular processes underlying response to therapeutics.

## 2253/T/Poster Board #802

**A Pharmacogenetic Approach to Pharmacological Chaperone Therapy for Fabry Disease.** *X. Wu[1], E. Katz[1], K. Mascioli[1], K. Chang[1], R. Khanna[1], S. Sitaraman[1], D. Palling[1], R. Schiffmann[2], D. Lockhart[1], K. Valenzano[1], E. Benjamin[1].* 1) Pharmacology, Amicus Therapeutics, Cranbury, NJ; 2) Baylor Research Institute, Dallas, TX.

Fabry disease is an X-linked lysosomal storage disorder caused by mutations in the gene that encodes $\alpha$-galactosidase A ($\alpha$-Gal A), and is characterized by pathological accumulation of globotriaosylceramide (GL-3). More than 600 mutations have been reported of which more than 60% are missense. The iminosugar AT1001 (migalastat hydrochloride) is a pharmacological chaperone that selectively binds $\alpha$-Gal A, increasing physical stability, lysosomal trafficking, and total cellular amount and activity. To identify AT1001-responsive $\alpha$-Gal A mutant forms, each of 394 known Fabry disease-causing missense mutations and small in-frame insertion and deletion mutations were engineered and expressed in HEK-293 cells. Incubation with AT1001 for 4-5 days increased $\alpha$-Gal A levels for 60% of the mutant forms as measured by using a 4-MU-$\alpha$-D-galactopyranoside hydrolysis enzyme activity assay and by Western blot. The HEK-293 cell assay results were consistent with those obtained in an *ex vivo* Fabry patient-derived T-cell assay and the *in vivo* white blood cell response dataset from the AT1001 Phase 2 clinical trials. Based on the HEK-293 cell assay results, criteria for the selection of Fabry patients most likely to respond to AT1001 were developed. Based on these criteria, the responsive mutant forms evaluated in the Phase 2 clinical trials were generally representative of the larger set predicted to be responsive. Concomitantly, $\alpha$-Gal A-deficient mice that express a responsive human mutant form (R301Q) showed significant increases in $\alpha$-Gal A and decreases in GL-3 levels in multiple tissues and plasma after daily or less frequent (4 days on/3 days off) oral administration of AT1001 for 28 days. Collectively, these results indicate that genotype is predictive of cellular $\alpha$-Gal A response to AT1001, and may be used to identify the patients most likely to benefit in further clinical evaluation of AT1001 as a treatment for Fabry disease.

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## 2254/T/Poster Board #803

**TIMP2 REGULATES EXPRESSION OF TGF- AND IL-4 IN HEPATIC STELLATE CELLS CULTURE.** *Y. Sánchez-Toscano[1], M.R. Bueno-Topete[1], A.B. Martínez-Rizo[1], L. Sánchez-Orozco[1], J. Armendáriz-Borunda[1,2], R. Troyo-Sanromán[3].* 1) Instituto de Biología Molecular en Medicina y Terapia Génica, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico; 2) OPD Hospital Civil de Guadalajara, Universidad de Guadalajara, Jalisco, México; 3) Coordinación de Investigacion, Universidad de Guadalajara, Guadalajara, Jalisco, México.

Background: TGF-β is the most relevant pro-fibrogenic cytokine during the process of liver fibrosis. Nevertheless, novel studies shown an induction in the expression of collagen type I, III and IV in response to the signaling route IL-4/STAT6, almost with the same intensity as TGF-β. Knock-down of TIMP-2 expression decreases liver fibrosis in experimental models, but the interrelationship between TIMP-2 and the signaling route of TGF-β e IL-4 is unknown. Aim: To decrease the gene expression of TIMP-2 using small interfering RNAs (siRNA) and to evaluate the key molecules participating in the signaling route of TGF-β and IL-4, in culture hepatic stellate cells Methods: Human (HSC180) and rat (8B) hepatic stellate cells were cultured. Transfection studies were performed using siRNA/TIMP-2(100nM)/lipofectamine once the stellate cells reached a confluency of 70%. After 48 hr of transfection, total RNA and proteins were collected to evaluate the gene expression of TIMP-2 by real time RT-PCR and the protein levels of TGF-β, Smad2P, Smad7 and IL-4 were determined by Western blot. Results: Knock-down of TIMP-2 expression significantly decreased 95% compared with the control, using siRNA 100nM. Interestingly, the protein levels of TGF-β, Smad2P, Smad7 and IL-4 were decreased (>70%) compared with the controls. Conclusion: TIMP-2 obliteration drastically decreases the over expression of pro-fibrogenic molecules involved in the progression of liver fibrosis.

## 2255/T/Poster Board #804

**Legal Liability and Incidental Findings in Clinical Pharmacogenetic Testing.** *P.C. Kuszler[1], W. Burke[2], K.B. Shutske[3], S.B. Haga[4].* 1) Law, University of Washington, Seattle, WA; 2) Bioethics and Humanities, University of Washington, Seattle, WA; 3) Institute for Public Health Genetics, University of Washington, Seattle WA; 4) Institute for Genome Sciences and Policy, Duke University, Durham, NC.

Pharmacogenetics offers the promise of tailored drug therapy that will improve safety and efficacy. However, many pharmacogenetic tests reveal information beyond that relevant to the therapeutic question for which testing is done. These incidental findings present challenges to informed consent and raise questions about when disclosure is appropriate and necessary. A provider may face liability either from the failure to disclose the risk of incidental findings in the process of informed consent or from failure to adhere to the standard of care in ordering, interpreting or acting on test results that present as incidental risk findings. A systematic search of electronic case law databases (Westlaw, Lexis; date range 1999-2009) reveals no case law on pharmacogenetic testing. There is however, case law evolving from analogous clinical technologies, notably imaging technologies. Several scholars, notably Wolf, et al., (J Law Med Ethics. 2008) have posited a decision tree addressing incidental findings in genetic research. Drawing upon this work and our study of case law, we propose a decision-making framework for disclosure in pharmacogenetic testing in clinical practice. This framework provides for a tiered approach to disclosure of incidental findings and posits a high level of duty when the information has the potential to reduce morbidity or mortality and a diminished duty as the utility of the information becomes increasingly clinically ambiguous. Patient factors are also relevant: While the duty to disclose incidental information seems clear when the information will offer a patient the opportunity to prevent future illness, it may be of less moment if co-morbidities would prevent the patient from pursuing or benefiting from preventive measures. On the other end of the spectrum, information that has no known utility may, with growing knowledge of gene-disease associations, become significant, clinically useful information. This dynamic may challenge the traditional legal parameters of duty in the provider/patient relationship, particularly if usual practice includes electronic recording of pharmacogenetic test information for future use. These issues raised by pharmacogenetics are likely to represent the first wave of growing obligations for clinicians related to the incidental information deriving from genome-based testing in health care.

## 2256/T/Poster Board #805

**Who Are Adopters of Pharmacogenomics among U.S. Physicians?** *E.J. Stanek[1], C.L. Sanders[1], J.R. Teagarden[1], K.A. Johansen[2], R.E. Aubert[1], B.C. Agatep[1], M. Khalid[1], A. Patel[1], F.W. Frueh[1], R.S. Epstein[1].* 1) Medco Health Solutions, Inc., Franklin Lakes, NJ; 2) American Medical Association, Chicago, IL.

Background: Information about pharmacogenomics (PGx) is evolving rapidly, but little is known about how US physicians perceive and use PGx testing. Methods: We conducted an anonymous, cross-sectional survey of US physicians in late 2008. The fax-based survey solicited demographic and professional profile elements, as well as PGx education, beliefs, practices, and preferred information sources. Respondent characteristics were compared to the American Medical Association MasterFile to assess generalizability. Factors associated with early and future adoption of PGx were tested for statistical significance with chi-square and multivariate logistic regression analyses. Results: We surveyed 397,832 physicians and 10,303 (3%) completed surveys were returned. Respondent characteristics were similar to the overall US physician population. Overall, 98% of respondents agree that patient genetic profiles may influence drug therapy, and 26% have prior PGx education. However, only 10% feel adequately informed about PGx testing. Early adopters (those ordering a PGx test in the previous 6 months) accounted for 13% of respondents, and future adopters (those anticipating ordering a PGx test in the next 6 months) accounted for 26%. Ten percent of respondents reported that PGx tests had benefited their patients by improving drug effectiveness and 10% reported their patients benefited from reduced toxicity. Early adopters are more likely to be oncologists, 15-29 years from medical school, and recipients of formal PGx education. They are also more likely to feel adequately informed about the availability of genetic testing, and believe that tests have benefited their patients by improving drug effectiveness, reducing drug toxicity, or increasing patients' understanding of therapy. Physicians who had not yet adopted PGx testing, but anticipated doing so, were more likely to be male, older, and educated about PGx. They also believe that a patient's genetic profile influences drug therapy and that the use of PGx tests benefit patients by improving drug effectiveness or reducing toxicity. Conclusions: An estimated 13% of US physicians have adopted PGx testing, while 26% anticipate adoption. Surprisingly, almost all believe that a patient's genetic makeup influences drug therapy. The contrast between acceptance of PGx as a therapeutic modifier and actual adoption rate highlights the need to clearly demonstrate patient benefit and the importance of physician education.

## 2257/T/Poster Board #806

**A GENETIC VARIANT SIGNIFICANTLY ASSOCIATED WITH RESPONSE TO ANTIEPILEPTIC MEDICATION IN TEMPORAL LOBE EPILEPSY.** *M.S. Silva[1], E. Bilevicius[2], R. Secolin[1], F. Cendes[2], I. Lopes-Cendes[1].* 1) Dept Medical Genetics, FCM - Unicamp, Campinas, Sao Paulo, Brazil; 2) Dept Neurology, FCM-Unicamp, Campinas, São Paulo, Brazil.

Rationale: Mesial temporal lobe epilepsy (MTLE) is associated with a high proportion of patients who do not respond well to treatment with antiepileptic drugs (AEDs). There is functional evidence that genetic variation, such as single nucleotide polymorphisms (SNPs), can affect the expression of drug transporter genes. In addition, there is a previous report of a significant association between an SNP in the ABCB1 gene and pharmacoresistance in a group of patients with different types of epilepsy; however, this study was not further replicated. The purpose of our study was to evaluate whether SNPs in drug-transporter genes could be associated with pharmacoresistance to AEDs in a large group of patients with MTLE. Methods: Consecutive patients with clinical-EEG diagnosis of MTLE were ascertained at the epilepsy clinic of our University Hospital. We included a total of 138 drug-resistant patients and compared with 88 drug-responsive patients who were seizure free on AEDs. We genotyped 30 dbSNPs in 4 different genes (RALBP1, ABCB1, ABCC2, ABCC4). Genotyping was carried out using the TaqMan system (Applied Biosystems). The significance of allelic association was assessed using logistic regression (logistf function in R environment). P-values were corrected by Bonferroni. In addition, we analyzed the genetic structure of both population groups using Fst and AMOVA with Arlequin v.3.11. Results: Genotypic frequencies for all SNPs genotyped were in Hard-Weinberg equilibrium in both groups. Sample power calculation showed a statistical power of 0.98 for the sample studied. The mean Fst was 0.00111 between drug-responsive and drug-resistant patients. Genetic variance between groups was 0.11 and within groups was 99.88. Logistic regression showed no significant association between any of the SNPs studied in RALBP1, ABCB1 and ABCC4 and pharmacoresistance. However, we found a significant association between an exonic SNP rs3740066 (Ile1324Ile), at the ABCC2 gene and pharmacoresistance (p=0.0368); OR = 1.51, IC (1.01:2.29). Conclusion: We found that both populations, drug-resistant and drug-responsive patients with MTLE, are very similar in their genetic structure. In addition, they show a large amount of genetic variability within the groups. We exclude the association between pharmacoresistance to AEDs in patients with MTLE and SNPs in the ABCB1 gene; by contrast, we found a significant associating between a SNP in the ABCC2 gene and this phenotype.

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## 2258/T/Poster Board #807

**Identification of novel variants in the *CYP2D6* gene in Mestizo and Amerindian populations of Mexico.** *A. Contreras, I. Silva-Zolezzi, T. Monge, L. Alfaro, S. Hernandez, H. Miranda, K. Carrillo, G. Jimenez-Sanchez.* National Institute of Genomic Medicine (INMEGEN), Mexico City, Mexico.

Genetic variants of drug metabolizing enzymes play a critical role on interindividual differences in drug response and adverse reactions. The *CYP2D6* gene, a member of Cytochrome P-450 family, has a central importance in the metabolism of a large number of commonly prescribed drugs, including antidepressants, antipsychotics and antihypertensives. *CYP2D6* shows a high degree of interindividual genetic variability that influences its expression and function. In addition, population studies have shown a wide range in the frequency of *CYP2D6* polymorphisms. For example, frequency of the *CYP2D6*10* allele ranges from 1.5% in Caucasian population to 52.5% in Chinese population. Mexicans have a specific ancestry which includes Amerindian, European, and in a lesser extent, African populations. To characterize genetic variation in the *CYP2D6* gene in Mexican population, we resequenced *CYP2D6* including 1.6 kb 5' flanking region, and its 9 exons and 8 introns in 96 Mexican Mestizos; 50 from Guerrero (GUE) and 46 from Sonora (SON). We observed 55 different genetic polymorphisms, including 14 novel: 13 SNPs and a *CYP2D6/2D7* gene conversion. The wild-type *CYP2D6*1* allele was to the most frequent (50.0%) and included a new version of it which contains rs1081004 (3.12%). The second most frequent (25.5%) was allele *CYP2D6*2*, included 3 novel haplotypes (18.22%). Allele *CYP2D6*4* (14.15%), characterized by a splicing defect (1846G>A), included a version with a novel non-synonymous SNP in exon 9 (C4160A; Ala449Asp). In addition, another non-synonymous SNP (G3251C; Arg330-Pro) was identified in exon 7. A novel *CYP2D6/2D7* gene conversion in exon 2 was identified in GUE (4%), and was confirmed in additional Mestizo: Yucatan (3.4%) and Zacatecas (1.67%), and Amerindian populations: Mixtecos (5%), Tepehuanos (3.3%) and Mayas (1.67%). Our results contribute to the characterization of *CYP2D6*, which will facilitate the development of pharmacogenetics for the Mexican population.

## 2259/T/Poster Board #808

**Prediction of irinotecan toxicity and response in patients with advanced colorectal cancer.** *M. Wadelius[1], H. Garmo[2, 3], A. Berglund[4], L. Fredriksson[1], H. Kohnke[1], H. Sørbye[5], B. Glimelius[4, 6].* 1) Dept Medical Sciences, Clinical Pharmacology, Uppsala University, Sweden; 2) Regional Oncologic Centre, Uppsala University Hospital, Sweden; 3) Division of Cancer Studies, School of Medicine, King's College London, UK; 4) Dept Oncology, Radiology and Clinical Immunology, Uppsala University, Sweden; 5) Dept Oncology, Haukeland University Hospital, Bergen, Norway; 6) Dept Oncology and Pathology, Karolinska Institutet, Stockholm, Sweden.

Introduction: Irinotecan and 5-fluorouracil (5-FU) are frequently used to treat metastatic colorectal cancer. Irinotecan's active metabolite is metabolised by UDP-glucuronosyl transferase 1A1 (UGT1A1), which is deficient in Gilbert's syndrome. Irinotecan and metabolites are transported by P-glycoprotein, encoded by ABCB1. 5-FU targets folate metabolism through inhibition of thymidylate synthase (TYMS). Methylentetrahydrofolate reductase (MTHFR) generates active folate necessary for normal haematopoiesis. Methods: We collected blood or normal colon biopsies from 140 Swedish and Norwegian colorectal cancer patients treated with irinotecan and 5-FU in a randomised clinical trial. We genotyped them for UGT1A1*28, UGT1A1*60, ABCB1 1236C>T, 2677G>T/A and 3435C>T, TYMS*2 and *3, and MTHFR 677C>T and 1298A>C using capillary DNA sequencing, real-time PCR and PCR with electrophoresis. Results: 25% of the patients experienced serious toxicity after one or two treatment cycles. Patients homozygous for UGT1A1*28 had an increased risk of early toxicity, OR 4.4 (95% C1 1.3 - 15.2), especially neutropenia, OR 21.3 (95% CI 2.0-225). The ABCB1 3435 variant genotype T/T increased the risk of early toxicity, OR 3.8 (95% CI 1.1-13.2). Compared with wild type patients, those homozygous for ABCB1 variant haplotypes were less likely to respond to treatment, OR 0.31 (95% CI 0.11-0.87) and survived shorter time, HR 1.97 (95% CI 1.06 - 3.65). Conclusion: We verified an increased risk of early toxicity, particularly neutropenia, in patients with Gilbert's syndrome. ABCB1 variant genotypes were associated with early toxicity, less response and shorter survival, but these findings need to be confirmed in future studies.

## 2260/T/Poster Board #809

**Admixture Analysis for Dissecting Pharmacogenetics Phenotypes in Psychiatry.** *V. De Luca, F. Panariello, J.L. Kennedy.* Dept Neurogenetics, Univ Toronto, Toronto, ON, Canada.

Despite the considerable amount of data from several studies in psychiatric pharmacogenetics, there is no agreement regarding the ideal cut-off for the quantitative phenotypes to determine people at risk for side effect and non-response to treatment. The aim of this study is to define the ideal cut-off for three common psychiatric phenotypes (antipsychotic response, weight gain and tardive diskinesia) by using admixture analysis, a common statistical procedure that has been applied to determine the cut-off for early onset of many psychiatric disorders. Admixture analysis was applied to identify model(s) of separate normal distributions of AOO characterized by different means, variances and population proportions in our sample of 204 patients with schizophrenia assessed for response (BPRS), tardive diskinesia (AIMS) and percentage weight change after antipsychotic treatment. Once the total sample was divided in different severity groups, we have also evaluated the demographic features of each subgroup. Regarding the weight gain, the model that best fits the observed distribution was a mixture of two gaussian distributions with three one-off points. The mean weight chnages estimated in this model were 2.61% (SD=3.73) and 8.65% (SD=8.75). The cut-off point was 8% weight change from baseline that is very close to the proposed 7% based on clinical evidence. When we analyzed the distribution of BPRS and AIMS in our sample, the admixture analysis program was unable to identify a cut-off that can be applied for analyzing genetic data. Our conclusion is that the admixture analysis can be employed to dissect certain pharmacogenetics phenotype such as antipsychotic-induced weight gain, however its application to other phenotypes should be further assessed in independent samples.

## 2261/T/Poster Board #810

**Variant discovery by targeted resequencing of "high-value" regions in drug pathways.** *E.T. Lam[1], K.M. Long[2], D.T. Okou[3], M.E. Zwick[3], P.-Y. Kwok[1], H.L. McLeod[2], M. Wagner[2].* 1) Institute of Human Genetics, University of California, San Francisco, San Francisco, CA; 2) Institute for Pharmacogenomics and Individualized Therapy, University of North Carolina at Chapel Hill, Chapel Hill, NC; 3) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA.

In order to realize the goal of personalizing medicine, markers associated with drug response need to be identified and validated. Recognizing that drug response, much like other complex phenotypes, is influenced by multiple genetic factors, we set out to identify novel variants in networks of genes that may contribute to the patient's overall response phenotype. The advent of massively parallel sequencing or next-generation sequencing (NGS) technologies promises to enable sequencing in an ultra high-throughput fashion and at a much lower cost. We aim to take advantage of these NGS technologies for targeted resequencing of candidate loci. Currently, target capture technologies that facilitate high-throughput and robust enrichment of regions of interest are being developed. In our pharmacogenetics study, we have used a microarray-based approach with NimbleGen capture arrays to capture loci involved in drug metabolism and activity of 5-fluorouracil (5FU), a compound commonly used in chemotherapy. We are particularly interested in regions with a high *a priori* probability of being functional. We have designed microarrays to target "high-value" regions that are either exonic or highly conserved across vertebrates. Our arrays target over 4200 regions from 95 genes, encompassing approximately 0.8 Mb. We have exploited CEPH lymphoblastoid cell lines for drug response measurements for chemotherapy agents otherwise not suitable for testing in normal subjects. Toxic response to 5FU for these cell lines was measured, and much variation in response was observed. DNA extracted from these cell lines were used in our capture experiments, and the captured DNA was sequenced on the Illumina GAII. Preliminary data analyses show high degree of enrichment of sequences of interest. We will present our framework of prioritizing the variants found for validation based on coverage and predicted functional significance and correlating genotype to phenotype.

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## 2262/T/Poster Board #811

**CYP2D6 genotyping for functional gene dosage analysis by allele copy number detection.** *N. Hosono[1], M. Kato[1], K. Kiyotani[1], T. Mushiroda[1], S. Takata[1], H. Sato[1], H. Amitani[1], Y. Tsuchiya[1], K. Yamazaki[1], T. Tsunoda[1], H. Zembutsu[2], Y. Nakamura[1,2], M. Kubo[1].* 1) Center for Genomic Medicine,Riken, Yokohama, Kanagawa, Japan; 2) The Institute of Medical Science, University of Tokyo,Tokyo, Japan.

Cytochrome P450 2D6 (CYP2D6) is one of the most important drug-metabolizing enzymes and has been reported to have genetic variations that affect its enzymatic activity. For the pharmacogenetic study of CYP2D6, accurate measurement of its functional gene dosage is essential. However, current genotyping techniques are insufficient due to their inability to provide the exact copy number of functional CYP2D6 alleles. We developed three quantitative real-time PCR (qPCR) assays for estimating the total copy number of the CYP2D6 gene, as well as multiplex PCR-based real-time Invader assays (mPCR-RETINAs) for estimating the allele ratios at 24 variation-loci which were reported to have functional significance in vitro and/or in vivo. After determining the allele copy number at each locus, we estimated the frequencies of CYP2D6 alleles in a population and the diplotype in each individual by a CNV phaser. Among 455 Japanese individuals were examined, 42 (9.2%) had one CYP2D6 gene copy, 207 (45.5%) had two copies, 161 (35.4%) had three copies, 40 (8.8%) had four copies, and five (1.1%) had five copies of the CYP2D6 gene. We found 14 reported CYP2D6 alleles and their frequencies were similar to previous reports. Additionally, we found two novel CYP2D6 alleles (CYP2D6*10-*36-*36 and *10-*10-*36) and confirmed them by Southern-RFLP. In the diplotype analysis, we observed that CYP2D6*1/*1 and *1/*10-*36 were the most common diplotypes and we could precisely estimate the functional gene dosage in each individual. Our method is the first to determine the exact functional CYP2D6 gene copy number. We believe our method will facilitate the detailed pharmacogenetic analysis of CYP2D6.

## 2263/T/Poster Board #812

**Modeling the impact of improved prediction of type 2 diabetes using SNP-based score on the design of type 2 diabetes prevention trials.** *K. Song[1], x. Lin[2], D. Waterworth[1], M. Nelson[1], V. Mooser[1], J. Whittaker[1].* 1) Statistical Genetics Capability Development, GlaxoSmithKline; 2) Discovery Analytics, GlaxoSmithKline.

Several recent studies have shown that incorporation of genetic variants only offers a slight improvement in the prediction of T2D at the individual level over clinical predictors alone. Still, the benefits may be larger for groups, and in particular for reducing the duration and size of prevention T2D trials, which are driven by the number of recruited participants expected to develop clinically defined T2D throughout the clinical trial. Here, we proposed a targeted T2D design to evaluate the potential benefits and costs using genetic variants and clinical factors for patient selection. For comparison between the proposed targeted design and the traditional clinical trial design, we calculated the sample size required to detect 90% power. The result showed that a modest reduction in the number of subjects on trial can be achieved at the expense of screening larger numbers prior to enrollment compared to the traditional design. Assuming we wish to power the study to compare a treatment effect of 33% conversion rate to a control effect of 100% conversion rate, sample sizes required by two designs differed by 19% when the probability of developing T2D during the course of the trial (positive predictive value or PPV) was improved from 15% without genetic selection criteria to 25% with it and the negative predictive value (NPV) decreased from 85% to 80%. The difference in the number of patients to be enrolled increased by 64% with PPV = 35% and NPV = 90%. Although only modest improvements in our ability to predict T2D risk is needed to benefit prevention trials, we conclude that further studies are needed to validate these benefits and implementation for a selection strategy.

## 2264/T/Poster Board #813

**Genetical Basis of Variation in Induction of CYP3A4 Enzyme.** *N. Rahmioglu[1], J. Heaton[2], G. Surdulescu[1], N. Smith[2], T. Spector[1], K.R. Ahmadi[1].* 1) Department of Twin Research and Genetic Epidemiology, King's College London, London, UK; 2) Pharmaceutical Science Division, King's College London, London, UK.

Response to medication is highly variable and the current "one size fits all" approach to therapy is costly (adverse drug responses cost the NHS £466 million a year) and dangerous. Personalized treatment has the potential to increase efficacy and decrease toxicity if "response" can be predicted accurately. Formal studies are needed but anecdotal evidence suggests that (i) all drugs are more effective in some groups of the population whilst showing non-or minimal benefit to other groups; and (ii) there are myriad genetic and non-genetic influences affecting drug response. One family of genes known to be implicated are those encoding the Cytochrome P450s (CYPs) family of enzymes. Among the P450s, CYP3A4 is the most important enzyme metabolising more than 50% of all prescribed medications. Many ADRs or poor therapeutic responses are due to genetically and environmentally "induced" CYP3A4 activity which shows large inter-individual variation. As part of a systems biology investigation into one of the major causes of adverse drug reactions, namely induction and inhibition of the drug metabolising enzyme CYP3A4, we have setup a large cohort of 400 healthy identical and non-identical twin pairs (100MZ, 300DZ) phenotyped for baseline and induced CYP3A4 activity. Briefly, the study involved administering a standard dose of St John's Wort (SJW) a mild, herbal antidepressant but also a potent inducer of CYP3A4 to each twin 3 times a day for 14 days. On day 14, each twin was also administered one 300mg tablet of quinine, a probe drug metabolized exclusively by CYP3A4. Fasting blood and urine was collected on day 0 and 15 at visits to St. Thomas' Hospital from each twin. We used baseline and post-dose urine and plasma from each participant in conjunction with Ultra Performance Liquid Chromatography (UPLC) to obtain the CYP3A4 activity measurement, which is the ratio of the primary metabolite of quinine (3-Hydroxyquinine) to quinine. We are currently processing the data. We will present results from our heritability analysis, genome-wide association study, and candidate gene study and highlight the most important genetic variants responsible for variability in the induced CYP3A4 activity.

## 2265/T/Poster Board #814

**Genetic variations associated with warfarin resistance in the Japanese population identified through genome-wide association study.** *PC. Cha[1], T. Mushiroda[2], K. Nakazono[3], A. Takahashi[3], S. Saito[4], H. Shimomura[4], T. Suzuki[4], S. Minami[5], H. Zembutsu[1], N. Kamatani[3], Y. Nakamura[1,2].* 1) Lab. of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Shirokanedai, Tokyo, Japan; 2) Research Group for Pharmacogenomics, RIKEN Center for Genomic Medicine, Yokohama, Japan; 3) Laboratory for Statistical Analysis, RIKEN Center for Genomic Medicine, Tokyo, Japan; 4) Tokushukai Hospital Group, Tokyo, Japan; 5) Laboratory of Endocrinology and Metabolism, Department of Bioregulation, Nippon Medical School, Kawasaki, Japan.

Warfarin is a commonly-used anticoagulant that is hard to dose owing to its narrow therapeutic index and wide inter-individual variations in its therapeutic dose. Although age, body surface area (BSA), and genetic polymorphisms in the *CYP2C9* and *VKORC1* genes have been found to explain a part of these inter-individual variations, identification of additional factors influencing the dose is critically important for safer and more appropriate use of this drug. In this study, we successfully identified through genome-wide approach, two novel Asian population-specific SNPs that are likely to be associated with warfarin resistance in a Japanese population. We evaluated associations of these SNPs by using an independent Japanese population sample. Furthermore, we found that in the combined study, these SNPs achieved linear regression $P$ = 3.76E-10 and $P$ = 9.23E-06, respectively. Integrating genotype information of these SNPs into the Warfarin-responsive index (WFRI) model previously developed based on genotypes of only SNPs in the *VKORC1* and *CYP2C9* genes clearly distinguished subjects who confer warfarin resistance that may therefore have higher risk of cerebral infarction ($P$ = 1.44E-16). Additionally, incorporating genotype information of these SNPs had improved predictability of the warfarin dosing algorithm that considered only age, BSA, and genotypes of the *VKORC1* and *CYP2C9* genes. Although the biological significance of these two SNPs is yet unclear, our results may provide insights to improvement of warfarin treatment, particularly to patients who need a higher dose of warfarin. In addition to that, our study may also imply that common SNPs other than those in *CYP2C9* and *VKORC1* genes that show strong effect on therapeutic warfarin dose might not exist; yet, there might be other SNPs, most possibly rare SNPs or population-specific SNPs, that are influencing therapeutic warfarin dose with moderate or small effects.

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## 2266/T/Poster Board #815

**A genome-wide association study on opioid analgesic sensitivity in patients undergoing painful cosmetic surgery.** 
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**Objectives**: Opioids are commonly used as effective analgesics for the treatment of acute and chronic pain. However, considerable individual differences have been widely observed in sensitivity to opioid analgesics, which can lead to wide variability in the dose of opioid analgesics required to achieve adequate pain control. This study aimed at comprehensively identifying genetic polymorphisms that could contribute to individual differences in sensitivity to an opioid analgesic, fentanyl, by genome-wide association study (GWAS). **Methods**: Subjects were 121 healthy patients who were scheduled to undergo cosmetic orthognathic surgery for mandibular prognathism at Tokyo Dental College Suidoubashi Hospital and provided informed, written consent for the genetics studies. Patients with chronic pain, those taking pain medication, and those who had experienced Raynaud's phenomenon were excluded. Postoperative pain was managed with a bolus dose of 40 μg fentanyl on demand using a patient-controlled analgesia (PCA) pump when patients felt pain, with a lockout time set at 10 min. Postoperative PCA fentanyl use during the first 24-h postoperative period was recorded, whereas venous blood (10 ml) of the subjects was sampled for preparation of DNA specimens for genotyping. Whole-genome genotyping was performed with iScan System (Illumina K.K.) and the BeadChip Human1M v1.0 or Human1M-Duo v3. **Results and Discussion**: For quality control, markers with genotype call frequency for samles ≥ 0.95 and minor allele frequencies ≥ 0.15 were selected for the study. In association study for 527,753 markers that passed the criteria, several single nucleotide polymorphisms (SNPs) were identified to be significantly associated with postoperative PCA fentanyl use (μg/kg) during the first 24-h postoperative period. Association was also significant between some SNPs and perioperative fentanyl use (μg/kg), which was calculated as the sum of intraoperative fentanyl use and postoperative fentanyl use. These SNPs could serve as markers for predicting the analgesic requirements in patients. Although future replication studies with independent samples will be required to confirm the association identified in this study, this is the first GWAS to explore candidate polymorphisms affecting sensitivity to an opioid analgesic. Our findings will lead to satisfactory pain control for patients suffering intolerable pain and open a path for personalized pain treatment in the future.

## 2267/T/Poster Board #816

**Comparison of multiple instrument platforms with qPCR and Hi-Res Melting(R) capability for CYP2C9 and VKORC1 SNP genotyping performance.** 
*J.T. McKinney, C.N. Gundry, R. Lems, M. Wall, M. Poulson, D.H-F Teng.* Idaho Technology, SLC, US.

One immediate application of personalized medicine is reducing adverse drug reactions genetically-predicted dosage prediction. Idaho Technology has developed rapid genotyping assays using the single-labeled SimpleProbe chemistry to genotype the CYP2C9 *2 and *3 SNPs, as well as the VKORC1 c.1 G-1639A SNPs. The assays allow PCR amplification and produce melting curves characteristic of each genotype. The SimpleProbes match the expected variant and create a single base mismatch when the wildtype sequence is present. This results in a low temperature melting peak from the probe when the wildtype allele is detected, and a relatively higher probe melting peak when the variant allele is present. A single SimpleProbe detects both alleles, and because the probe is a perfect match to the variant allele, other rare sequence variants in close proximity to the *2 and *3 alleles can produce false positives using other probe technologies. Due to the design of the Idaho Technology probes, if these rare variants are present, a 2nd base-pairing mismatch is created under the probe. This additional mismatch results in even lower probe melting peaks than observed from the single-mismatched homozygous wildtype-to-probe hybrid. Ninety human genomic samples were extracted according to the same protocol and used at 5-15 ng per reaction. The LightCycler® 480, LightScanner®, LightScanner 32 (LS32), and Rotor-Gene® 6200 instruments produced equivalent genotyping results for the VKORC1 c.1 G-1639A and CYP2C9 *2 and *3 variants. Rare variants near both the CYP2C9 *2 and *3 loci were observed and create the aforementioned 2-bp mismatch within the probe-target hybridization region. These rare variants were observed on all instrument platforms, however, because the LS32 Hi-Res Melting fluorimeter does not detect the FAM fluorophore on the SimpleProbes, the melting data from the standard air chamber melt required higher analysis sensitivity settings to detect the rare variants. All other instruments generated Hi-Res Melting data and performed well. Despite reductions in the price per sequencing reactions, other technologies such as this probe technique are currently the most cost-effective way to genotype simple SNPs.

## 2268/T/Poster Board #817

**Analysing variation in *CYP1A2* in the peoples of Ethiopia and identifying evidence of purifying selection acting on the gene.** 
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Cytochrome P450 *1A2* metabolizes a wide range of therapeutic drugs, including several used to treat diseases common in sub-Saharan Africa. Variation in the gene (*CYP1A2*) has been reported to be associated with differential efficacy of therapeutic drugs and adverse drug reactions. To gain a better understanding of the extent of variation in the coding and exon-flanking non-coding regions of *CYP1A2* we re-sequenced 762 chromosomes from members of five ethnic groups (Afar, Amhara, Anuak, Maale and Oromo) distributed in a rough north east to south west transect across Ethiopia. Ethiopian populations were expected to display substantial variation because: a) human genetic diversity is known to be greatest in sub-Saharan Africa and is reported to decrease with distance from Addis Ababa and b) there has been substantial migration of Semitic speaking peoples from the Arabian Peninsula into Ethiopia over the past few thousand years. We found 49 variable sites of different types overall, 30 of which are novel. Nine non-synonymous changes (seven of which are novel) and one synonymous change were found in the coding region. Haplotype analysis of the entire gene revealed 55 different haplotypes, only three of which were previously reported. When haplotypes were constructed using only non-synonymous polymorphisms, so as to restrict the haplotype set to those most likely to affect enzyme structure/function, ten haplotypes were identified, eight of which have not previously been reported. Comparing these data with those from publically available databases it appears that Ethiopian groups display much greater variation than do other populations (gene diversity using complete coding region haplotypes (non-synonymous variants only): Ethiopia = 0.17 ± 0.02; Rest of the World combined = 0.08 ± 0.03). Many haplotypes can be predicted to be of importance in the planning and provision of healthcare. In addition Ethiopian populations exhibit most of the common variation observed elsewhere. Analysis of these data, comparing gene diversities of the non-coding region and synonymous SNPs with those of non-synonymous SNPs, while controlling for variation due to differential demographic histories, supports the hypothesis that purifying selection has acted on the gene. This, in turn, supports the hypothesis that mutations affecting the CYP1A2 enzyme may be suitable candidates for complex disease association studies (e.g. several cancer types and coronary heart disease).

## 2269/T/Poster Board #818

**Pharmacogenetics of Risperidone Therapy in Autism: association analysis of eight candidate genes with drug efficacy and adverse drug reactions.** 
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The atypical antipsychotic risperidone is used to control disruptive behaviors associated with autism. Little has been reported on the factors, genetic or other, that underlie the variability in individual response to risperidone, particularly for autism. In this study we simultaneously explored the effects of multiple candidate genes on clinical improvement and occurrence of adverse drug reactions (ADRs), in autism. Forty five autistic patients receiving monotherapy with risperidone up to one year were studied. Selected candidate genes were those involved in the pharmacokinetics (*CYP2D6* and *ABCB1*) and pharmacodynamics (*HTR2A*, *HTR2C*, *DRD2*, *DRD3*, *HTR6*) of the drug, and the *BDNF* gene, found to be relevant in other studies. Using the generalized estimating equation method these genes were tested for association with drug efficacy, assessed using the Autism Treatment Evaluation Checklist, and with safety and tolerability measures, including prolactin levels, body mass index, waist circumference and neurological adverse effects. Our results confirm that risperidone therapy was very effective in reducing some autism symptoms and caused few serious adverse effects. After adjusting for confounding factors, our results show that the *HTR2A* c.-1438G>A, *DRD3* c.25T>C (p.S9G), *HTR2C* c.-995G>A and *ABCB1* c.1236C>T polymorphisms were predictors for clinical improvement with risperidone therapy. The *HTR2A* c.-1438G>A, *HTR2C* c.68G>C (p.C33S), *HTR6* c.7154-2542C>T and *BDNF* c.196G>A (p.V66M) polymorphisms influenced prolactin elevation. The *HTR2C* c.68G>C and *CYP2D6* polymorphisms were associated with risperidone induced increase in BMI or waist circumference. Although association results should be taken as suggestive, given the small sample size, we identified for the first time several genes implicated in risperidone efficacy and safety in autism patients, therefore making a preliminary contribution to the personalized therapy of risperidone in autism.

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## 2270/T/Poster Board #819

**Human_CVD 50K SNP array analysis in the Drug-Induced Arrhythmia Risk Evaluation (DARE) Study.** *C. Dalageorgou[1], D. Zheng[1], V. Marshall[3], S. Shakir[3], A.J. Camm[2], S. Jeffery[1], E.R. Behr[2], Y. Jamshidi[1].* 1) Clinical Developmental Sci, St George's Medical Sch, London, United Kingdom; 2) Cardiac and Vascular Sciences, St George's Hospital Medical Sch, London, United Kingdom; 3) Drug Safety Research Unit, Southampton, United Kingdom.

The risk of pathologic prolongation of the QT interval (> 550 ms) and *Torsade de Pointes* (TdP) is the most common cause of withdrawal of commercially used drugs. TdP can be self-limited or can degenerate to fatal arrhythmias, such as ventricular fibrillation. Genes encoding cardiac ion channels have identified potential contributory variants in only <20% of patients with Drug-Induced Long QT Syndrome (dLQTS). As part of the Drug-Induced Arrhythmia Risk Evaluation Study (DARE), we analysed DNA samples from 89 Caucasian Subjects, experiencing drug-induced adverse proarrhythmic events, and 90 matched population controls, using the Illumina Infinium Human_CVD 50K Bead Array. The resulting association analysis, adjusted for -age and -sex, identified significant association with single nucleotide polymorphisms (SNPs) in a number of genes. The most highly associated SNPs were located in the Dopamine Receptor D3 (*DRD3*) gene, (most significant $P = 6 \times 10^{-5}$). Dopamine receptors are important in the regulation of cardiovascular function and interestingly anti-arrhythmic drugs such as amiodarone and verapamil have been shown to increase intracellular levels of both dopamine and L-DOPA. Our study identifies *DRD3* as a potentially novel gene which is significantly associated with the risk of drug-induced QT prolongation and consequent arrhythmias.

## 2271/T/Poster Board #820

**Location of lipid metabolism genes via oligogenic analysis in a pharmacogenetic familial clinical trial.** *E.W. Daw[1], I.B. Borecki[1], S. Ketkar[1], J.M. Ordovas[2], M.Y. Tsai[3], P.N. Hopkins[4], J. Hixson[5], M.A. Province[1], D.K. Arnett[6].* 1) Division of Statistical Genomics, Washington University, St. Louis, MO; 2) JM-USDA-HNRCA, Tufts University, Boston, MA; 3) Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN; 4) Department of Internal Medicine, University of Utah, Salt Lake City, UT; 5) Human Genetics Center, University of Texas Health Science Center at Houston, Houston, TX; 6) Department of Epidemiology, University of Alabama at Birmingham, School of Public Health, Birmingham, AL.

The GOLDN study is a clinical trial to examine the Genetics of lipid metabolism and response to treatment in 201 families, ranging in size from 53 to 3. This data is unusual in providing controlled gene by environment interaction data in families. 1027 subjects completed the initial Post-prandial Lipemia (PPL) arm of the study: after fasting, subjects were asked to drink a fatty shake and a variety of lipid levels (hdl, ldl, cholesterol, triglycerides, chylomicrons, particle sizes by nmr, etc.) were measured just prior to drinking the shake, at 3.5 hours, and at 6 hours after drinking the shake. Subjects were then placed on a 3-week fenofibrate trial and the PPL study was repeated at the end. To identify genes in the lipid metabolism network that influence PPL response, we analyzed each of the lipid measures at each time point, both pre and post fenofibrate trial, as well as the slopes between time points and the area under the curves. Growth curve (GC) models were applied to help control for the potentially noisy nature of lipid measurements. Since the GC models assume normality, all measures were tested for normality and those failing were log transformed. If a measure was still not close to normal after a log transform, a Box-Cox power transformation was considered. Microsatellite markers were typed in over 2000 family members, including markers typed in additional family members in the predecessor study, used here to improve meiotic inference. We conducted an oligogenic simultaneous segregation and linkage analysis with Monte Carlo Markov chain (MCMC) methods for each lipid measurement, and identified a number of linked regions. Chromosomes with regions of particular interest include 4 (near GGAA19H07), 7 (between GGAA3F06 and GATA13G11), 11 (between GATA23E06 and GATA64D03), 16 (near GATA71H05), and 17 (between GATA49C09 and ATA43A10). In some regions there are low-level linkage signals across a number of measures, possibly indicating genes that have a broad but small effect. In other regions there are stronger signals, but sometimes only in one of two measures. Both types of results offer a window into this complex network.

## 2272/T/Poster Board #821

**Coverage of the PharmaADME Consortium Core Marker List by TaqMan® DME and Copy Number Assays.** *T. Hartshorne, S. Desai, K. Li, T. Ceccardi.* Molecular Biology Systems, Applied Biosystems, Foster City, CA.

The PharmaADME Consortium (PharmaADME.org) is composed of individuals from academia and pharmaceutical and genomic technology industries, who together developed a consensus list of key genes involved in the absorption, distribution, metabolism and excretion (ADME) of drugs. The list includes phase 1 and 2 drug metabolizing enzymes and transporter genes. A core list of 184 putative functional genetic variants in 33 significant ADME genes, which likely impact the pharmacokinetics of drug metabolism, was also created. By providing this information to technology providers, products could be developed to support pharmacogenetic research, with the objective of improving the quality of pharmaceuticals available to consumers. We report here on our progress towards covering the core ADME marker list with TaqMan® Genotyping Assays. The ADME marker list includes 172 SNP and InDel polymorphisms, 11 copy number variants, and one microsatellite polymorphism. We first reviewed our available list of TaqMan® Drug Metabolism (DME) Genotyping Assays, which detect polymorphisms in coding and regulatory regions in 221 DME genes. We then focused on developing allelic discrimination assays to any SNP or InDel ADME marker for which we did not have an assay. Challenges for assay design included: assay specificity for targets with high homology to pseudogenes or gene family members, triallelic SNP targets, other SNPs in close proximity to the target, and repetitive target context sequences. All assays were tested on up to 180 gDNAs from four populations. For some challenging targets, several rounds of assay design and testing were required to obtain a robust TaqMan® DME Assay. At this time, 155 DME assays covering SNP and InDel ADME targets are available, including 8 assays to genotype 4 triallelic SNPs. Efforts are underway to develop assays for the remainder of the SNP and InDel targets. For the 11 copy number variants on the core ADME list, which include deletions and duplications of 6 total genes, TaqMan® Copy Number Assays were developed and tested on 180 gDNAs. In total, over 90% of the core ADME markers are covered by TaqMan® DME or Copy Number Assays, which offer a simple and scalable workflow for genotyping these important alleles in pharmacogenetic research.

## 2273/T/Poster Board #822

**Natural Variation in the 3'UTRs of 37 Human Membrane Transporter Genes.** *S. Hesselson[1], J. Eshragh[1], D. Stryke[2], D. Kroetz[3], K.M. Giacomini[3], P.Y. Kwok[1].* 1) Cardiovascular Research, UCSF, San Francisco, CA; 2) Department of Pharmaceutical Chemistry, UCSF, San Francisco, CA; 3) Department of Bioengineering and Therapeutic Sciences, UCSF, San Francisco, CA.

Genetic variation in membrane transporter genes can contribute to interindividual variation in drug absorption, distribution, and elimination. Most of the interest in membrane transporter pharmacogenetics has focused on the coding and promoter regions. In contrast, few studies have focused on genetic variation in the 3'UTRs of membrane transporters, which may also influence transporter expression and function. We sequenced 32,378 bp of the 3'UTRs of 15 ATP-binding cassette (ABC) and 22 solute carrier (SLC) membrane transporters in order to identify and estimate the minor allele frequency (MAF) of polymorphisms in healthy individuals. We sequenced DNA samples from 272 people from the SOPHIE cohort (68 African Americans, 68 Caucasians, 68 Chinese Americans and 68 Mexican Americans). We observed 433 polymorphisms in the 3'UTRs of these 37 transporter genes. The majority of these polymorphisms were SNPs, however, 30 were indels. More polymorphisms were observed in the 3'UTR (1/75 bp) than were observed in the exons of 24 previously sequenced membrane transporters (1/166 bp) and 107 previously sequenced ABC and SLC proximal promoters (1/85 bp). The average number of polymorphisms per base pair in the 3'UTRs and proximal promoters overall was similar, however in the 3'UTRs more polymorphisms were observed in ABC genes than SLC genes (ABC 1/52 bp, SLC 1/107 bp), while the opposite is true for proximal promoters (ABC 1/106 bp, SLC 1/75 bp). Of the polymorphisms observed in the 3'UTRs, 36% are population specific. The majority of these are singletons (62%). African American samples have the highest number of rare and common population specific alleles (46%). Asians have a similar number of singletons as African Americans, however the number of SNPs with a MAF higher than 1% was much higher in African American samples than the other 3 populations. Many 3'UTR polymorphisms with high frequency minor alleles have been observed: 74% have a MAF greater than 1% in at least one population, and 18% have a MAF greater than 10% in at least one population. In the future, polymorphisms observed in this sequencing effort will be functionally characterized to determine if they alter membrane transporter expression and function. The polymorphisms identified in this study will lead to a better understanding of how natural genetic variation effects drug transport and may help to identify individuals who will have an adverse reaction to commonly used medications.

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## 2274/T/Poster Board #823

**The VKORC1 1173C/T polymorphism and warfarin maintenance dose in Asians and Caucasians: a meta-analysis.** *K. Huang.* Epidemiology, Pfizer Inc, New York, NY.

Background: Warfarin sodium (Coumadin) is the most frequently used oral anticoagulant. Warfarin inhibits vitamin K epoxide reductase that prevents vitamin K recycling and the subsequent gamma-carboxylation of clotting factors. Variants in the gene encoding Vitamin K epoxide reductase complex subunite 1 (VKORC1) may affect the response to warfarin. Objectives: To conduct a meta-analysis to determinate associations between VKORC1 1173 C/T polymorphism and mean weekly maintenance dose of warfarin in Asians and Caucasians. Methods: A search from the PubMed database (from inception to May 2009) was conducted and references identified from bibliographies were sought. Studies were considered if they evaluated an association between the VKORC1 1173 C/T polymorphism and mean weekly (daily) maintenance dosing of warfarin. Only studies published in the English language were included. 12 studies were identified and 8 studies meeting inclusion criteria were selected. Data was extracted from selected studies and additional information was obtained by contacting study authors. Hardy Weinberg equilibrium was tested across studies using the Wald-type test. Statistical heterogeneity was measured by means of Cochran's Q test. Pooled tests for the means weekly dose by the VORKC1 1173 C/T polymorphism in subgroups representing different races were performed by weighted linear meta-regression using a random-effects model. All statistical tests were two-sided. Results: This analysis comprised a total of 1,036 subjects, including 328 Asians and 708 Caucasians. Genotype frequency for CC was higher in Caucasians compared to Asians (36.3% vs. 1.8% respectively) while Asians had a higher TT genotype frequency compared to Caucasians (81.1% vs. 17.7%). Subjects with the CC genotype had the highest unadjusted mean weekly maintenance dose (46.9mg, 95%CI: 32.3-61.4 in Asians and 43.9mg, 95%CI, 38.8-49.0 in Caucasians). Subjects with the CT genotype and subjects with TT genotype required a lower unadjusted mean weekly maintenance dose that was 31mg, 95%CI: 25.9-36.2 in Asians and 22.8mg, 95%CI: 19.9-25.8 in Asians vs. 32.1mg, 95%CI: 27.1-37.2 in Caucasians and 21.4mg, 95%CI: 16.1-26.7 in Caucasians respectively. Conclusions: Results of this meta-analysis indcate that the VORKC1 1173 C/T polymorphism is associated with maintenance dose of warfarin in both Asians and Caucasians.

## 2275/T/Poster Board #824

**Association between common genetic variants in the *NOS1AP* (*CAPON*) gene in patients experiencing amiodarone-induced ventricular arrhythmias.** *Y. Jamshidi[1], D. Zheng[1], I.M. Nolte[3], C. Dalageorgou[1], H. Snieder[3], V. Marshall[4], S. Shakir[4], A.J. Camm[2], S. Jeffery[1], E.R. Behr[2].* 1) Clinical Developmental Sciences, St Georges Hospital Medical School, London, United Kingdom; 2) Cardiac and Vascular Sciences, St Georges Hospital Medical School, London, United Kingdom; 3) Department of Epidemiology, University Medical Center Groningen, University of Groningen, the Netherlands; 4) Drug Safety Research Unit, Southampton, United Kingdom.

Amiodarone is a potent anti-arrhythmic agent used to treat various types of tachyarrhythmias. It has multiple electrophysiological effects and is associated with a high incidence of side-effects, such as QT prolongation and bradycardia. Uncommonly QT interval prolongation can lead to the polymorphic ventricular tachycardia Torsade de Pointes which may be self-limited or degenerate to fatal arrhythmias such as ventricular fibrillation. Recent studies have shown consistent association between resting heart rate corrected QT interval (QTc) and common variants of the NOS1 regulator, *NOS1AP*. As part of the Drug-Induced Arrhythmia Risk Evaluation (DARE) Study, we have systematically collected a unique national cohort of patients experiencing drug-induced ventricular arrhythmias and severe QT prolongation as well as matched population controls. We postulated that variation within the *NOS1AP* gene differed between cases and controls. In order to test this hypothesis, we sequenced the *NOS1AP* gene in 30 Caucasian subjects treated with amiodarone who experienced a drug-induced arrhythmic event and 90 Caucasian control subjects. The sequencing assays included all coding regions of the gene. Additionally we have genotyped 198 single nucleotide polymorphisms (SNPs) with minor allele frequencies > 1% across the *NOS1AP* gene (500b upstream and 1 kb downstream) using Infinium Technology (Illumina). We found no novel coding mutations / polymorphisms. The resulting association analysis showed that thirty polymorphisms within the NOS1AP gene were associated with amiodarone-induced QT prolongation at a significance level of 0.05 (most significant P = $5 \times 10^{-5}$, OR=5.6 using an additive model). These did not include either of the SNPs rs10494366 and rs16857031 known to be associated with resting QTc nor SNPs that were in strong linkage disequilibrium with them. Our results suggest that common variations in the *NOS1AP* gene play a role in the pathogenesis of amiodarone-induced QT prolongation and consequent arrhythmias and these might be independent of the known association with resting QTc. Further studies are underway to confirm this finding in additional replication cohorts.

## 2276/T/Poster Board #825

**Genetic determinants of warfarin dosing and VKORC1 haplotype analysis.** *M.T.M. Lee, C.H. Chen, C.H. Chou, L.S. Lu, J.Y. Wu, Y.T. Chen.* Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

Background: Warfarin, a widely prescribed oral anticoagulant, is used for the prevention of thromboembolism. Polymorphisms in CYP2C9 and VKORC1 have been shown to associate with warfarin dose requirements. However, it is likely that other genes could also affect warfarin dose. In this study, we aimed to identify additional genes influencing warfarin dosing in the Han-Chinese population. VKORC1 haplotype also exhibit population differences, we also examined VKORC1 haplotype in six Asian populations. Methods: In this study, we screened for SNPs in 13 genes (VKORC1, CYP2C9, CYP2C18, PROC, APOE, EPHX1, CALU, GGCX, ORM1, ORM2, factor II, factor VII, and CYP4F2) and tested their associations with warfarin dosing with univariate and multiple regression analysis. In addition, frequencies and haplotype structure of six VKORC1 SNPs (-1639 G>A, 497 T>G, 1173 C>T, 1542 T>G, 2255 C>T, and 3730 G>A) were analyzed in six Asian populations (Han-Chinese, India, Indonesia, Philippine, Thailand, and Vietnam). Results: Results showed that CYP2C18, PROC and EPHX1 have small but significant association with warfarin dose. In multiple regression analysis, PROC and EPHX1 explained 3% of the dose variation. In VKORC1 haplotype analysis, rs2884737 (497 T>G) is only polymorphic in the Indian population. Remaining SNPs demonstrated high LD and have similar frequencies and haplotype structure in all but the Indian population. The Indian population is made up mostly the H7 haplotype (76%) while the rest of the recruited populations are consisted of the H1 haplotype (>80%).

## 2277/T/Poster Board #826

**Azorean pharmacogenetic study: the variability of 13 genes implicated in drug response.** *T. Pereirinha[1], S. Bulhões[1], P.R. Pacheco[1,2], M.J. Brilhante[1], R. Cabral[1,2], C.C. Branco[1,2], L. Mota-Vieira[1,2].* 1) Mol Gen & Pathol Unit , Hosp Divino Espirito Santo PD, EPE, Azores Islands, Portugal; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Genetic polymorphisms in genes encoding for drug metabolizing enzymes, transporters and therapeutic targets have been recognized as one of the factors responsible for inter-individual differences in drug response. As incorrect dosages contribute to a high rate of adverse effects, there is clinical interest in improving strategies for the determination of the appropriate dose. In this study, we analyzed a total of 28 variants, in 13 genes (*MDR1*, *5-HT2C*, *5-HT2A*, *PDE6A*, *ADCY9*, *ADRB2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *VKORC1*, *TPMT*, *NAT2* and *UGT1A1*) in 170 healthy blood donors from the anonymized Azorean DNA bank. Polymorphisms genotyping was performed by real-time PCR and fragment analysis by capillary electrophoresis. Results demonstrated that in Azoreans the TPMT*3C and NAT2*14, two common variants in Africans, have relatively high frequencies (2.9% and 0.3%, respectively) when compared with Caucasians, and reveals statistically significant difference with mainland Portugal ($\chi^2$, p<0.001). This African trace corroborates previous studies of the Azoreans genetic background. The allelic frequencies of the remaining 26 pharmacogenetics variants are similar to those found in other Caucasian populations. Currently, we are analyzing the Azorean pharmacogenetic profiles of complex diseases, in order to help the drug therapy prescription and, consequently, to improve the establishment of personalized medicine. Funded by Azorean Government (M 1.2.1./I/002/2008).

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## 2278/T/Poster Board #827

**Statin-Induced Myotoxicity Adverse Drug Reactions; Pharmacogenomic Evaluation of SLCO1B1 and Atrogin-1 in Statin Myotoxic Cohorts.**
*M.S. Phillips[1,2], A.M.K. Brown[1,2], M-P. Dubé[1,2], C. Johansen[3], R.A. Hegele[3], J-C. Tardif[1,2].* 1) Génome Québec & Montreal Heart Institute Pharmacogenomics Centre, Montreal, Quebec; 2) Université de Montréal; 3) University of Western Ontario, London, Ontario.

Pharmacogenomics is a powerful technique that has the potential to enhance drug safety and efficacy. HMG-CoA reductase inhibitors (statins) are widely prescribed and are an effective therapy for the lowering of plasma LDL-cholesterol. However, statin treatment has also been associated with a wide range of muscular side effects, from non-specific or atypical myalgias to the extreme rhabdomyolysis syndrome. We have recruited a statin myotoxicity cohort consisting of ~2,500 dylipidemic patients presenting clear rhabdomyolysis/muscular intolerance phenotypes along with ~2,000 dyslipidemic controls (~4,500 patients in total). In recent publications, the SEARCH collaborative group have identified variants in the solute carrier organic anion transporter 1B1 gene (SLCO1B1) that are associated with high dose simvastatin myotoxicity. Furthermore the atrogin-1 gene (FBXO32), a subunit of the ubiquitin protein complex, has been associated with increased muscle atrophy and implicated in statin-induced muscle toxicity. In order to test if these observations are present in our large myotoxic cohort, we have selected ~260 cases along with ~400 matched controls for genetic screening. We have genotyped them for the key functional and haplotype-tagged SNPs within the SLCO1B1 gene to see if the same association is observed with other statins and at lower doses. In our preliminary analysis, we have not observed significant association in our myotoxic patients with the SLCO1B1 variants when taking atorvastatin or simvastatin at lower doses. This could be due to several factors, including our statin intolerance phenotype definition, the type of statins being taken by the patient, and potential ascertainment or selection biases. We are replicating these observations in an independent cohort from a tertiary referral lipid clinic that consists of 126 patients with symptomatic statin-induced myopathy and 144 case-matched controls (London, Ontario). Furthermore, to evaluate if the atrogin-1 gene is involved with statin-induced myotoxicity, we have sequenced the gene in ~100 statin-induced phenotypic cases to identify any novel functional variants in this population. To date, we have identified >70 novel variants that we are screening in the larger cohort to access their involvement with statin-induced myotoxicity. With studies like these, we hope to identify potentially interesting biomarkers for the statin-induced clinical phenotype that can be developed into predictive tests.

## 2279/T/Poster Board #828

**Pharmacogenomics of the gastrointestinal safety of cyclooxygenase inhibitors.**
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Traditional nonsteroidal anti-inflammatory drugs (NSAIDs) can cause gastrointestinal (GI) side effects. Selective inhibitors of COX-2 have a more favorable profile of GI tolerability. We hypothesize that genetic factors may contribute to individual susceptibility to GI side effects in response to either the NSAID diclofenac or the purpose developed selective COX-2 inhibitor etoricoxib. We genotyped over 6,000 subjects enrolled in the MEDAL program, a large drug trial comparing the GI safety of diclofenac and etoricoxib, using a custom 50,000 SNP genotyping array. Subjects were followed up for an average of over 18 months. After very stringent quality control, including removing all subjects of non-white ethnicity, we retained 4,441 subjects and 33,938 SNPs. We ran survival analyses to test association with predicted time to complicated and uncomplicated upper GI clinical events, stratified by drug. No analyses reached genomewide levels of significance.

## 2280/T/Poster Board #829

**Pharmacogenomic investigation in hypertensive patients using candidate genes approach and Genome Wide Association Study.**
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Pharmacogenomic association studies help to identify DNA variants which impact on the individual response to drugs. The knowledge of sample variability in drug response can allow to personalize drug dosing and treatment regimes. The fundamental question concerns whether it is possible to differentiate the patients with potentially responses to the treatment (R) from those with the greatest risk of no response (NR). Our approach to these questions for the disease of interest, Hypertension, is to identify SNPs in hypertensive patients that segregate with drug efficacy either in candidate genes (CG) that relate to the mechanism of action of the specific drug or in other DNA regions detected with a Genome Wide Association Study (GWAS). SNPs identified from CG or GWAS could allow NR to be excluded from subsequent clinical trial studies, therefore allowing enriched, smaller, faster, less expensive clinical studies on patients with a better chance of responding favorably. Here, we present a real pharmacogenomic example: we adopted a genetic association design, where the phenotype of interest was measured as a quantitative trait (decrease of systolic blood pressure office after drug treatment) and the variables affecting the distribution of the phenotype are the SNPs, the therapy (drug or placebo) and the SNP*therapy interaction. Once we identified those SNPs, we developed an algorithm to detect the genotypic profiles that best discriminate R from the NR. In a final step, we merged the best predictive SNPs found from our GWAS strategy with those from a CG approach. Both categories of SNPs convey a specific predictive power that is magnified by their joint integration into a unified model.

## 2281/T/Poster Board #830

**Effects of Cellular Confounding Variables in Epstein Barr Virus (EBV) Transformed Lymphoblastoid Cell Lines on Pharmacological Phenotypes.**
*A.L. Stark[1], W. Zhang[2], S. Mi[2], S. Duan[2], P.H. O'Donnell[2], R.S. Huang[2], CM. Beiswanger[3], M.E. Dolan[2].* 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Section of Hematology/Oncology, Dept Medicine, Univ Chicago, Chicago, IL; 3) Coriell Institute for Medical Research, Camden, NJ.

Publicly available genetic and expression data on lymphoblastoid cell lines (LCLs) comprising the International HapMap collection make them a unique resource for understanding the genetic underpinnings of cellular phenotypes. One use of these cell lines is for pharmacogenomic discovery and validation of clinical findings associated with drugs. However, variable cellular growth rate and EBV copy number can be confounders in such studies. Our objective is to examine the degree to which confounding variables affect pharmacological endpoints in LCLs. To this end, we evaluated the relationships between cellular growth rate/baseline EBV copy number and drug-induced cytotoxicity in LCLs exposed to daunorubicin, etoposide, carboplatin, cisplatin, cytarabine, pemetrexed, 5-deoxyfluorouridine, vorinostat, methotrexate, 6-mercaptopurine, and 5-fluorouracil. There was no significant correlation between baseline EBV copy number with drug-induced cytotoxicity or cellular growth rate. However, cellular growth rate and drug-induced cytotoxicity were significantly correlated for all drugs except vorinostat. We found that cellular growth rate is under appreciable genetic influence ($h2 = 0.30$-$0.39$) with five suggestive linkage regions having LOD scores above 1.5 across the genome. Not surprisingly, a portion of SNPs that significantly associate with drug cytotoxicity are also associated with cellular growth rate ($p \leq 0.0001$). We are currently also evaluating whether several independently-transformed LCLs from the same individual vary between each other with respect to cellular growth rate, EBV copy number, and drug-induced cytotoxicity. Studies using LCLs should consider that cellular growth rate is an important component of cytotoxicity phenotypes, and a portion of the genetic determination of cytotoxicity may be explained through heritable growth rate polymorphisms.

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## 2282/T/Poster Board #831

**Factors accounting for the response variability in serum 25(OH)D in postmenopausal women.** *L.J. Zhao, J.P. Zhou, F.X. Bu, R.R. Recker, J.M. Lappe.* Osteoporosis Res Ctr, Creighton Univ Med Ctr, Omaha, NE.

Vitamin D deficiency is a common health problem. Serum 25-hydroxyvitmain D [25(OH)D] is the functional indicator of vitamin D status. Low circulating serum 25(OH)D values is associated with a higher risk of common cancers and other disorders. Vitamin D supplementation is the best approach for achieving adequate serum 25(OH)D levels. A wide range of response variation in serum 25 (OH)D was observed in previous vitamin D intervention trials. However, factors contributing to the response variation are unknown. In order to identify these unknown factors, we conducted retrospective data analyses in 318 unrelated postmenopausal women with age >55 years from eastern Nebraska (~41° N). These subjects were treated with vitamin D (1100 IU/day) for four years and their vitamin D intake compliance rates were >80%. Values of serum 25(OH)D were measured at baseline and again at each annual visit. The treatment-induced pair-wise serum 25(OH)D in 1-, 2-, 3-, and 4-year were highly correlated (r>0.6 for all pairs, p<10-6). The average serum (25OH)D increment is 25.59±16.5 nmol/L (mean±SD), but varied widely, with increments ranging from -29.99 nmol/L to +72.87 nmol/L. The percentage of subjects who did not raise their 25(OH)D to optimal serum level (80nmol/L) were 17%, 18%, 17%, and 26% in year-1, 2, 3, and 4, respectively. We estimated factors contributing to the response variability in serum 25(OH)D. Among the tested factors, baseline serum 25(OH)D was the most significant one, explaining 16% of total response variance. Other significant factors included total body fat, amount of trial vitamin D intake, concentration of serum bone specific alkaline phosphatase (BSAP), serum parathyroid hormone (PTH), and serum creatinine. Collectively, they explained 29.9%, 24.7%, 22.9%, and 32.2% of 1-, 2-, 3-, 4-year total serum 25(OH)D response variation, respectively. We further compared differences between responders and non-responders. Thirty responders and 30 non-responders were selected from the 318 subjects based on their baseline serum 25(OH)D (<80 nmol/L), increment of serum 25(OH)D, and vitamin D status after treatment. Serum PTH, BSAP, and calcium concentrations were significantly different between responders vs non-responders (P<0.05). Responders generally had lower serum PTH, higher serum calcium, and lower BSAP levels. Conclusion: Baseline serum 25(OH)D, PTH, and BASP levels were important for the change of serum 25(OH)D in response to vitamin D supplementation.

## 2283/T/Poster Board #832

**Reaction of human pulp cells to DNA damage induced by urethane dimethacrylate (UDMA), a component of dental restorative materials.** *J. Blasiak[1], E. Pawlowska[2], T. Poplawski[1], K. Loba[1], J. Szczepanska[2].* 1) Dept Molecular Genetics, Univ Lodz, Lodz, Poland; 2) Department of Pediatric Dentistry, Medical University of Lodz, Poland.

The use of new dental restorative materials with new chemistry, the etching the dentin, and the need to ensure complete polymerization and sealing of the restoration to the tooth have raised questions about the biological safety of new materials and techniques. The essential components of such materials are methacrylate resins, which, after polymerization, can release monomers into the oral cavity and pulp, from where they can migrate into the bloodstream reaching virtually all organs. The local concentration of the released monomers can be in the millimolar range, high enough to induce adverse biological effects. Genotoxicity of methacrylate monomers is of a special significance due to potential serious phenotypic consequences, including cancer, and long latency period. In the present work, we investigated cytotoxicity and genotoxicity of urethane dimethacrylate (UDMA) in the human pulp cells. UDMA at concentrations up to 1 mM evoked a concentration-dependent decrease in the viability of the cells up to about 80%, as assessed by flow cytometry. This agent did not induce strand breaks in the isolated plasmid DNA, but evoked concentration-dependent DNA damage in the human pulp cells evaluated by the alkaline and neutral comet assay. This damage included oxidative modifications to the DNA bases, as checked by DNA repair enzymes Endo III and Fpg as well as single DNA strand breaks. UDMA did not evoke double strand breaks. The cells exposed to UDMA at 750 μM were able to remove about 90% of damage to their DNA in 120 min. The drug evoked apoptosis and induced an increase in the G2/M cell population, accompanied by a decrease in the S cell population and an increase in G0/G1 cell population. Due to broad spectrum of UDMA genotoxicity and a potential long-lasting exposure to this compound, its use should be accompanied by precautions, reducing the chance of its release into blood stream and the possibility to induce adverse biological effects. This work was supported by the grant N N403 188134 from the Ministry of Science and Higher Education.

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## 2284/T/Poster Board #833

**Confounding factors in ARVC caused by mutations in *TMEM43*.** *A.F.M. Haywood, K.A. Hodgkinson, N.D. Merner, J. Houston, A.D. Greenslade, S. Conners, P.S. Parfrey, T.L. Young.* Discipline of Genetics, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland and Labrador, Canada.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiac disorder characterized by ventricular tachycardia, sudden cardiac death and fibrofatty replacement of cardiomyocytes. Predominantly mutations in desmosomal genes for example, *desmoplakin, plakophilin-2, desmoglein-2, desmocollin-2* and *plakoglobin* have been identified to cause ARVC, where cells are predicted to succumb to mechanical stress. We recently identified the latest ARVC gene, a non desmosomal gene *TMEM43/LUMA*, in which a S358L missence mutation causes a particularly lethal form of ARVC (*ARVD5*) in the Newfoundland population. The importance of this gene discovery has been validated by the identification of S358L and two new missense *TMEM43* mutations, R28W and R312W, which are deemed deleterious through bioinformatics and both segregate with affected patients from Ontario, Nova Scotia and the UK. Interestingly, *ARVD5* patients identified with the founder mutation (*TMEM43*; S358L) have clinical features that overlap with dilated cardiomyopathy. To see if mutations in *TMEM43* play a role in other cardiomyopathies, we screened 54 probands from Newfoundland diagnosed with dilated cardiomyopathy, hypertrophic cardiomyopathy, ARVC or sudden cardiac death (otherwise unspecified), for mutations in *TMEM43*. None of the 54 probands had the founder S358L mutation. However two probands were heterozygous for R312W mutation, one also carries a pathogenic *LMNA* mutation, and the other carries a separate *LMNA* mutation along with a large deletion in dystrophin. Several other *TMEM43* mutations were identified among the 54 probands including two other missense variants and a potential splice site mutation of unknown significance. These findings suggest that cardiomyopathies other than ARVC may be caused by mutations in *TMEM43* or the pathogencity of the previous variants indentifies in LMNA questionable or the multiple mutations underlie the disease phenotype. Interestingly, *TMEM43/LUMA* interacts with A and B type-lamins, some of which are produced from *LMNA* in the inner membrane of the nucleus. It may prove to be important when making diagnoses to look at the series of related genes together. This data suggests the need to expand the definition of cardiac disease caused by variations in *TMEM43*, and the need for this gene to be added to the genetic screening panel for ARVC.

## 2285/T/Poster Board #834

**Functional Analysis of 5-Lipoxygenase Promoter Variants.** *H. Allayee[1], S. Vikman[1], P. Armstrong[2], R.M. Brena[3], J. Hartiala[1], C.B. Stephensen[2].* 1) Dept Preventive Med, Univ Southern California, Los Angeles, CA; 2) Program in International and Community Nutrition, Univ Calif Davis, Davis, CA; 3) Dept Biochemistry and Molecular Biology, Univ Southern California, Los Angeles, CA.

Variants of a hexanucleotide repeat polymorphism in the promoter of the 5-Lipoxygenase (5-LO) gene have been associated with cardiovascular disease (CVD) traits in humans, which may be due, at least in part, to differential expression of the at-risk alleles. To more fully characterize these variants, we carried out gene expression and methylation studies in primary leukocytes from healthy individuals carrying various 5-LO promoter alleles. Regardless of genotype, 5-LO and 5-LO activating protein (FLAP) gene expression was highest in granulocytes compared to monocytes and lymphocytes whereas leukotriene A4 hydrolase (LTA4H) expression was highest in monocytes. In all three leukocyte populations, 5-LO mRNA levels were positively correlated with those of FLAP and LTA4H, with the highest correlation observed in granulocytes. In lymphocytes, individuals homozygous for the shorter 3 and 4 repeat alleles had between 20-35% higher 5-LO, FLAP, and LTA4H expression compared to homozygous carriers of the wildtype 5 repeat allele ($p = 0.03 - 0.0001$). Methylation analysis of four CpG islands in a 1500bp region encompassing the 5-LO promoter and the first ~100bp of intron 1 revealed relatively low overall DNA methylation across all genotypes and leukocyte populations. However, analysis of the promoter repeats themselves demonstrated that, regardless of cell population, the 4 allele was methylated approximately twice as much as the 3 allele ($p < 0.0001$). Our results demonstrate that, in lymphocytes, the shorter repeat alleles of the 5-LO promoter lead to higher gene expression, which may be regulated through differential methylation of the CpGs located within these repeats.

## 2286/T/Poster Board #835

**Melting Curve Analysis for Fine Mapping of Variants in 6 lipid-related genes: Implications for Improving Coronary Risk Classification.** *J.F. Carlquist[1,2], B.D. Horne[1,2], J. McKinney[3], N.J. Camp[2], J.B. Muhlestein[1,2], C.P. Mower[1], J.J. Park[1], Z.P. Nicholas[1], J.L. Anderson[1,2].* 1) LDS Hosp, SLC, UT; 2) Univ Utah, SLC, UT; 3) Idaho Technology Inc., SLC, UT.

Among the traditional risk factors for coronary artery disease (CAD), total cholesterol emerged as one of the earliest and bears some of the most important risk for CAD. Among 17,000 consenting subjects undergoing angiography and prospectively enrolled in the Intermountain Healthcare Collaborative Heart Study (IHCS), we sought to determine whether genetic variants in the principal lipid metabolic genes contribute to risk for CHD. Scanning was performed by melting curve analysis (MCA) for all exons including 20 bp 5' and 50 bp 3' (to capture coding and donor and acceptor splice variants) and untranslated regulatory regions for the following genes: LCAT, CETP, LIPC, LPL, SCARB1, and ApoF. Presumptive variants identified by MCA were confirmed by cycle sequencing. The multi-stage study design was comprised of a SNP discovery phase (62 healthy subjects), a haplotype and tagging SNP discovery phase (339 healthy subjects), and a CAD association phase (1,003 angiographic CAD cases and 510 angiographic controls). In all, 115 target sequences were scanned for each of the 62 individuals in the SNP discovery cohort for a total of 23,939 bases of interrogated sequence. Among the 6 genes, a total of 92 SNPs were identified; 19 (21%) were previously unreported variants. Thirty six of the variants were identified in exons; 18 of these (50%) were synonymous and 18 (50%), non-synonymous. Twenty nine (32%) of the identified SNPs were in intron-exon boundaries containing splice sequences and 27 (29%), in the 5' and 3' untranslated regions. In the haplotype discovery phase, 163 of 169 MCA presumptive variants and 419 of 420 MCA presumptive non-variants were confirmed by cycle sequencing (MCA sensitivity: 98.6%, specificity: 99.4%). Of the 91 SNPs, 38 (42%) were chosen as tagging SNPs and explained >90% of the intra-genic variation in the 6 genes. When applied to the disease association cohort, 10 SNPs were found to have associations of $p<0.20$ with lipid parameters and, thus, were carried forward for evaluation of CAD event phenotypes in subsequent studies. These findings demonstrate the feasibility, sensitivity, and specificity of MCA in SNP discovery and expand and complete the discovery effort for common (>5% MAF) SNPs and haplotypes for these 6 genes, excluding deep introns. Further, the genetic variants in these genes may be used to derive a genetic risk score for the potential reclassification of risk determined by traditional (Framingham) methods.

## 2287/T/Poster Board #836

**Searching for mutation casuing mitral valve prolapse in *MMVP3*.** *R. Durst, L. Maire, J. Null, R. Levine, S. Slaugenhaupt.* CHGR, MGH, Boston, MA.

Mitral valve prolapse (MVP) is a common cardiac disorder that exhibits a strong hereditary component. Its complications include congestive heart failure, endocarditis and atrial arrhythmias. Although MVP is associated with a wide array of genetic disorders, specific genes involved in its pathogenesis have eluded detection. Recently one X-linked and three autosomal MVP loci have been described. The first loci was reported on chromosome 16 (MMVP1) and we identified the remaining two: MMVP2 on chromosome 11p15.4 and MMVP3 on chromosome 13q31.3-32.1. The MMVP3 locus is defined as an 8.2 Mb region between the markers D13S794 and D13S786. Despite the fact that the MMVP3 region is so large, it contains only 17 single-copy genes. Five of these genes are particularly good biological candidates (UGCGL2, GPC5, GPC6, ITR and HS6ST3). Given the fundamental importance of the extracellular matrix components in MVP, UGCGL2 is a compelling candidate. UGCGL2 is a UDP-glucose ceramide glucosyltransferase which is likely to play a role in GAG biosynthesis.GPC5 and GPC6 are heparan sulfate proteoglycans that are anchored to the cell-surface via a covalent linkage to glycosylphospatidylinositol. Their main function is to regulate the signaling of Wnts, Hedgehogs, fibroblast growth factors, and bone morphogenetic proteins making these genes good candidates for a role in MVP. ITR (intimal thickness-related receptor) may play a role in vascular smooth muscle remodeling. HS6ST3 (heparan sulfate 6-O-sulfotransferase 3) is a 6-O-sulfation enzyme is part of the sulfation pathway of N-sulfoglucosamine. Knockout mice for HS6ST2, which is yet another enzyme in the same pathway, develop mitral valve prolapsed, making HS6ST3 a strong candicate in the MMVP3 region.Our search for the MMVP3 gene continues as we examine the SNPs identified in our initial gene screening and as we sequence the remaining candidate genes. Determination of the genetic basis of MVP is important because the disease often manifests clinically in the 5th or 6th decade of life through presentation as a severe cardiac event. Earlier intervention in genetically susceptible individuals, for example by reducing leaflet stresses, could potentially arrest or prevent progression to a clinically severe stage. Further, discovering the genetic basis of MVP is the first step towards understanding the cascade of cellular events that result in MVP.

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## 2288/T/Poster Board #837

**Myocyte-specific enhancer factor-2A gene polymorphism and susceptibility to coronary artery disease.** *S. Elhawari[1], O. Al-Boudari[1], E. Andres[1], P. Muiya[1], B.F. Meyer[1], F. Al-Mohanna[2], M. Alshaid[3], N. Dzimiri[1].* 1) Genetics, King Faisal Spec. Hospital & Res. Ctr, Riyadh, Saudi Arabia; 2) Biological and Medical Research, King Faisal Spec. Hospital & Res. Ctr, Riyadh, Saudi Arabia; 3) King Faisal Heart Institute, King Faisal Spec. Hospital & Res. Ctr, Riyadh, Saudi Arabia.

The role of the myocyte-specific enhancer factor-2A (MEF2A) gene in coronary artery disease remains unclear. We first screened the exon and exon-intron junctions of the gene in 200 Saudi individuals for single nucleotide polymorphisms (SNPs), and then evaluated their role in 1156 CAD patients compared with 859 angiographed controls. More than 40 SNPs were detected in these regions of the gene. Of these, exon 11 displayed a very high degree of heterogeneity resulting from deletions of various (1-6) sets of tri-nucleotide (CAG)n chains encoding a polyglutamate (424QQQQQQQ430) sequence leading to absence of different sets of amino acids, as well as other single/multiple base deletions immediately following this chain. No significant difference was observed between patients and controls in the various sets of (CAG)n deletions. In contrast, one SNP, rs1059759G>C [Odds ratio (95% Confidence Interval) = 1.21(1.02-1.34); p =0.029] was associated, and another, rs34851361A>G [1.22(0.9-1.54); p=0.088] was only weakly associated with CAD. Three other SNPs rs325408 [0.74(0.61-0.91); p=0.004], rs3730059 [0.15(0.03-0.68); p=0.014] and rs12442844 [0.78(0.68-0.91); p=0.001] appeared to show protective properties. Additionally, in the region following the CAGs, we observed two types of changes, one comprising an nt146550:AGCAGC/- (deletion) in 8% of the population and another involving 7 sets of different stretches of deletions in the subsequent region nt146556CCGCCGA146562. The single and double nt deletions led to premature STOP codons at nt146635 and nt146645, respectively. However, none of these changes displayed noticeable difference between the two groups, possibly due to their low prevalence in our study population. Our results suggest that the polymorphic changes in exon 11 of the MEF2A may predispose individuals to CAD.

## 2289/T/Poster Board #838

**The role of Zhx2 in atherosclerosis, plasma lipid metabolism, and developmental regulation.** *A. Erbilgin[1], P.S. Gargalovic[1,2], O. Kohannim[1,2], J. Pagnon[1,2], X. Wang[1,2], L. Castellani[1,2], R. LeBeouf[3], A.J. Lusis[1,2].* 1) Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA 90095; 2) Department of Human Genetics, University of California, Los Angeles, CA 90095; 3) Department of Pathobiology, University of Washington, Seattle, WA.

We have recently identified a transcription factor, Zhx2, as a novel regulator of plasma lipid metabolism. An endogenous retroviral insertion within the coding region of this gene in BALB/cJ mice leads to severely reduced levels of transcript and protein, and corresponds to lower levels of total cholesterol and triglycerides. BALB/cJ mice carrying Zhx2 transgenes exhibit a correction of these plasma lipid phenotypes. We have now further characterized the role of Zhx2 by observing the contribution of this gene to atherosclerosis. BALB/cBy mice with the wild-type Zhx2 allele and BALB/cJ mice were placed on a low-density lipoprotein receptor null background. Significant differences in lipid profiles and atherosclerosis were observed when these mice were maintained on an atherogenic diet. Male BALB/cJ mice exhibited a 1.9-fold decrease in triglycerides and a 2-fold decrease in total cholesterol levels compared to BALB/cBy mice, accompanied by a nearly 13-fold decrease in atherosclerotic lesions. There is evidence that Zhx2 is involved in developmental regulation, and it is unknown whether this observed increase in atherosclerosis is due to the differences in lipid levels or the perturbation of another mechanism regulated by this transcription factor. Zhx2 is responsible for the post-natal repression of the $\alpha$-fetoprotein (AFP) and H19 genes in mice. In most strains of inbred mice, AFP is expressed at high levels in the mouse fetal liver but rapidly repressed at birth. In contrast, BALB/cJ mice maintain relatively high levels of AFP into adulthood due to the non-functional Zhx2 allele, which is normally highly expressed in the adult liver. In order to further explore the role of Zhx2 in this context, we are conducting a developmental curve study that compares the gene expression in the hearts and livers of BALB/cJ mice and congenic mice containing the wild-type Zhx2 allele. Samples from the heart and liver were collected at birth, 5 days, 14 days, and 25 days of age and gene expression differences were assessed using real-time quantitative PCR. These data show remarkable differential expression of lipoprotein lipase (Lpl) and Ear11 between the two strains at all time-points, in addition to other significant gene expression differences. We are currently assaying global expression through the use of microarrays on these samples in order to identify additional regulatory targets.

## 2290/T/Poster Board #839

***SCN5A* variation is associated with electrocardiogram traits in the Jackson Heart Study.** *J. Jeff[1], K. Brown-Gentry[1], S. Buxbaum[6], D. Sarpong[6], H. Taylor[6], J. Wilson[6], J. Payne[6], J. Maher[7], A. George[3,4], D. Crawford[1,2], D. Roden[3,5].* 1) Human Gen, Vanderbilt Univ, Nashville, TN; 2) Molecular Physiology & Biophysics, Vanderbilt Univ, Nashville, TN; 3) Pharmacology and Medicine, Vanderbilt Univ, Nashville, TN; 4) Institute for Integrative Genomics, Vanderbilt Univ, Nashville, TN; 5) Oates Institute for Experimental Therapeutics, Vanderbilt Univ, Nashville, TN; 6) Jackson Heart Study , Jackson State University, Jackson, MS 39213; 7) McDermott Center for Human Growth and Development, UT Southwestern, Dallas, TX 75309.

Understanding variation in the normal electrical activity of the heart, assessed by the electrocardiogram (ECG), may provide a starting point for studies of susceptibility to serious arrhythmias, such as sudden cardiac death during myocardial infarction or drug therapy. Recent genetic association studies of one ECG trait, the QT interval, have identified common variation in European-descent populations, but little is known about the genetic determinants of ECG traits in populations of African-descent. To identify such factors, we have undertaken a candidate gene study of ECG traits in collaboration with the Jackson Heart Study (JHS, n=5621). JHS is the largest longitudinal study to date that investigates the role genetic factors play in disease in African Americans ascertained from the Jackson, Mississippi area. Nine quantitative ECG traits were evaluated: the P,QT, QTc, T and QRS durations, V-rate, and the P, QRS and T axes. We genotyped 72 variations in the sodium channel, voltage-gated, type V, alpha subunit *(SCN5A)* gene, a member of a gene family that forms sodium channels abundant in the cardiac muscle. Three SNPs deviated from Hardy-Weinberg Equilibrium expectations and four had minor allele frequencies <0.05; these 7 SNPs were removed from further analysis. Using linear regression, we performed tests of association for 65 SNPs assuming an additive genetic model in unrelated individuals (n=3,334). Adjusting for age, sex, and European ancestry, significant associations (p<6.0x10-4) were observed for QRS and P-wave durations. Intronic rs3922844 ($\beta$=1.3; p=2.7x10-7) was associated with increased QRS duration while intronic rs7374138 ($\beta$=-1.2; p= 7.1x10-5) and rs7627552 ($\beta$=-1.1; p=4.8x10-4) were both associated with decreased QRS duration. Nonsynonymous rs7626962 (S1103Y) ($\beta$=-3.5; p=6.5x10-5), a variation observed in African-descent populations but absent or rare in other populations, is associated with decreased P-wave duration, and trends toward significance for the QRS duration ($\beta$=-1.30; p=.005). Intronic rs7692965 ($\beta$=-3.1; p=6.5x10-5), rs7637849 ($\beta$=-1.9; p=1.9x10-4), rs7627552 ($\beta$=-1.9; p=4.9x10-4), rs7624535 ($\beta$=-1.8; p= 2.0x10-4), and rs6763048 ($\beta$=-1.6; p=7.3x10-4) were all associated with decreased P-wave duration. These results suggest that the sodium channel candidate gene *SCN5A* contributes to ECG trait distributions in African Americans, and these same variations may be risk or protective factors associated with susceptibility to arrhythmias.

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## 2291/T/Poster Board #840

**Sequence variation from the 5' region upstream of CETP associated with the tails of HDL and CETP concentration levels.** *T.J. Maxwell[1], X. Lui[1], L.C. Shimmin[1], A.G. Clark[2], T.J. Rea[3], A.R. Templeton[4], J.E. Hixson[1], E. Boerwinkle[1].* 1) Human Genetics Center, UT Houston Health Science Center, Houston, TX; 2) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 3) Department of Human Genetics, University of Michigan School of Medicine, Ann Arbor, MI; 4) Department of Biology, Washington University, St. Louis, MO.

Multiple studies have shown that genetic variation in the promoter region of the Cholesteryl Ester Transfer Protein gene (CETP) is associated with plasma high-density lipoprotein cholesterol concentrations (HDL-C) and CETP protein concentrations. To test the relationships between CETP promoter variants and trait variation, we sequenced a 6.3 kb region 5' upstream through intron 1 of CETP in individuals chosen separately from the upper and lower tails of HDL-C and CETP concentration distributions within race-gender strata. Individuals were chosen and sequenced (76 African Americans (AA) and 84 European Americans (EA) for HDL-C and 78 EA and 92 EA for CETP concentration) from the top and bottom 4% within each stratum. Thirteen other SNPs from the CETP gene region were available from previous studies. The upper and lower tail of each trait was used to create discrete phenotypes of interest. Logistic regression was used to test for differences in genotypic counts between tails. A permutation analog of the sequential step-down Bonferroni (Westfall and Young 1993) was used to obtain significance levels that incorporated the multiple tests and the correlations between them. The data show that, for 47 SNPs in AA and 37 SNPs in EA), over half of the SNPs in each group had a minor allele frequency (MAF) greater than 0.10 and about 10% of the SNPs had a MAF less than 0.01. The level of significance for SNPs in association with CETP concentration was much greater than that for HDL (nominal p-values of 10-8 vs 10-4). Seven SNPs were significant for EA HDL-C and no SNPs were significant for AA HDL-C. Seven SNPs were significant for EA CETP concentration and eight were significant for AA CETP concentration. Six of the SNPs significant for EA HDL-C were also significant for EA CETP concentration, including the four significant SNPs that were significant for CETP concentration in both the EA and AA populations (rs711752; rs183130; rs708272; rs17231506). Many of the SNPs that are associated with CETP concentration in both populations were associated with HDL-C levels in the EA populations but had no significant effect on HDL-C in the AA population. Only one SNP (rs183130) significant for CETP concentration in AA displayed evidence for an association with HDL-C concentration (nominal p-value of 0.0543).

## 2292/T/Poster Board #841

**Variants in NOTCH Pathway Genes Among Individuals with Left-Sided Cardiovascular Defects.** *K.L. McBride[1], G.A. Zender[1], S.M. Fitzgerald-Butt[1], J.W. Belmont[2].* 1) Molecular and Human Gen, Res Inst Nationwide Child Hosp, Columbus, OH; 2) Molecular and Human Gen Baylor Coll Med, Houston, TX.

Cardiovascular malformations (CVMs) are common birth defects that contribute to a large proportion of infant mortality. Evidence suggests that subgroups of CVMs share common genetic etiologies. Our previously published epidemiology and genetic data support this concept for one subgroup, the left-sided cardiac defects of aortic stenosis (AS) coarctation of the aorta (CoA) and hypoplastic left heart syndrome (HLHS). The Notch pathway is important in cardiac development. Ligand(Jagged, Delta) binding causes cleavage of the receptor(Notch1-4), releasing the Notch intracellular domain. It translocates to the nucleus and activates HES transcription factors. We previously screened *NOTCH1* in this subgroup, and discovered variants causing a functional disturbance of the Notch signaling pathway in 4/91 AS, CoA and HLHS individuals. The purpose of the present study was to expand the investigation to include downstream targets of NOTCH1 in a larger cohort of individuals than originally reported. A total of 455 individuals of north European ancestry affected with either AS, CoA, or HLHS, were screened for mutations in the genes *NOTCH1, HEY2* and *HEYL*. Initial screening was performed by dHPLC followed by Sanger sequencing to identify the exact change. Potentially harmful missense variants were defined as pedicted damaging by POLYPHEN and having a PANTHER deleterious probability of ≥0.50. We identified 2 potentially harmful variants in *HEY2* (S172P, n=1; P179L n=2), and 4 in *HEYL* (R141C, n=1; P173S, n=1; W177C, n=2; F179L, n=2). In addition to the previously noted *NOTCH1* variants, we found 2 more potentially harmful variants (V565M, n=1; R621H, n=1). Rare variants causing a synonymous substitution or causing a missense change predicted to be benign were also found in *HEY2* (7 variants in 11 people), *HEYL* (2 variants in 5 people) and *NOTCH1* (9 variants in 10 people); none are previously described SNPs. Combined with prior results we found rare variants in 5 /207 (2.4%) controls vs 32/455 (7.0%) cases for *NOTCH1* ($\chi^2$ p =0.017). Comparison of cases and controls for *HEY2* and *HEYL* awaits completion of the coding region sequencing in controls. This study demonstrates that rare variants in genes of the NOTCH signaling pathway are found among individuals with AS, CoA, and HLHS at a much higher rate than the general population. Combined with previous functional data for NOTCH1, the data suggests this pathway is involved in the pathogenesis of left sided cardiovascular defects.

## 2293/T/Poster Board #842

**The role of the BRAP gene for carotid atherosclerosis.** *Y. Pan[1], Y. Liao[2,3], S. Juo[1,2,4,5].* 1) Inst Med Gen, Kaohsiung Med Univ, Kaohsuing, Taiwan; 2) Dept Medicine, Kaohsiung Med Univ, Kaohsiung, Taiwan; 3) Dept Neurology, Taichung Veterans General Hospital, Taichung, Taiwan; 4) Dept Medical Research, Kaohsiung Med Univ Hospital, Kaohsiung, Taiwan; 5) Center of Excellence for Environmental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

Background and Purpose The BRCA-1 associated protein (BRAP) gene was recently identified as a susceptible gene for myocardial infarction (MI) in Asian population. A functional variant at the BRAP gene was able to enhance BRAP expression, which in turns, modulate the NF-κB-dependent inflammatory pathway on the vascular walls. The purpose of present study was to test the BRAP genetic effect on stroke and subclinical carotid atherosclerosis. Methods Segment-specific intimal-medial thickness (IMT) and plaque index were obtained by carotid ultrasonography in 816 stroke- and MI- free healthy volunteers. Another 262 incident stroke patients were enrolled, including 51 large-artery, 21 cardioembolic, 85 small-vessel, and 105 cryptogenic strokes. Single nucleotide polymorphisms (SNPs) rs11066001 and rs3782886 were genotyped by TagMan method. Results These two SNPs were in strong linkage disequilibrium (D' = 0.99) and both were significantly associated with IMT values at the bifurcation (Bif). The rare allele of rs11066001 appeared to exert a detrimental effect on IMT and the average Bif IMT were 0.68 ± 0.15, 0.65 ± 0.13, 0.63 ± 0.12 in the rare homozygotes, heterozygotes and common homozygotes respectively (p = 0.015). Although the rare allele appeared to be over-represented in subjects with plaques (index ≥ 1), the difference did not reach statistical significance. We did not find any association between BRAP genotypes and stroke risks. Subgroup analyses on the stroke subtypes did not yield better result. Conclusions Our results supported the regulatory role of BRAP gene on carotid atherosclerosis. However, this gene may not be an important determinant of stroke susceptibility.

## 2294/T/Poster Board #843

**Population-based re-sequencing of endothelial lipase and zinc finger protein 202 genes in subjects at the extreme ends of HDL-C Distribution.** *H. Razzaghi[1], S. Santorico[2], J. Hokanson[3].* 1) Dept Med, Univ Colorado Denver, Aurora, CO; 2) Dept Mathematical and Statistical Sciences, Univ Colorado Denver, Denver, CO; 3) Dept Epidemiology, Uni Colorado Denver, Aurora, CO.

Multiple epidemiological studies have constantly shown an inverse relationship between elevated levels of high-density lipoprotein cholesterol (HDL-C) and the risk of CHD. Endothelial lipase (Gene: LIPG, Protein: EL) and zinc finger protein 202 (ZNF202) are two prime candidate genes in HDL metabolism. We sought to determine the genetic contribution of LIPG and ZNF202 to variation in HDL-C levels by re-sequencing both genes including the 2000 bp 5', all exons and introns, and 2000 bp 3' in selected individuals expressing the extreme phenotype of high (x=75.90 mg/dl, n=114) and low (x=31.24 mg/dl, n=121) HDL-C levels. 235 subjects were selected from a biethnic population of Whites and Hispanics. A total of 185 and 122 sequence variants were identified in LIPG and ZNF202 genes, respectively, which all are now available in the NCBI databases. In both genes, only two of the sequence variants were missense (T111I and N396S in LIPG; A154V and K259E in ZNF202) with the remaining in noncoding regions of the gene. This striking finding suggests the variation in HDL-C levels may be due to mutations affecting the gene regulation as opposed to the protein structure. We found several rare variants unique to either the low or high HDL groups in both genes. In Whites, there were 23 SNPs and 39 SNPs that had a rare variant seen only in the high and low HDL groups, respectively. In Hispanics, there were 14 SNPs and 28 SNPs that had a rare variant seen only in the high and low HDL groups. For LIPG, the proportion of unique variants differed between the high and low HDL groups in Whites (p=0.022) and Hispanics (p=0.017). Furthermore, the location of rare variants in Hispanics differed between the high and low HDL groups (p=0.018) with the low HDL group having more unique SNPs present in the introns and UTR regions than in exons. For ZNF202, the proportion of unique variants in the low and high HDL groups differed only in the Hispanics (p=0.021), and no relationship was found with SNP location. A comparison of haplotype frequencies based on tagSNPs as well as the missense SNPs revealed an association for LIPG in Hispanics (p=0.0073, $p_{adj}$=0.0582). Associations were also detected for ZNF202 for both ethnic groups [p(Whites)=0.0121, $p_{adj}$(Whites)=0.0129; p(Hispanics)=0.0246, $p_{adj}$(Hispanics)=0.0585]. In summary, our re-sequencing project identified many additional SNPs in both LIPG and ZNF202 genes and our analyses support the association of these two genes with HDL levels.

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## 2295/T/Poster Board #844

**Screening of mutations in the sarcomere genes in a cohort of 205 cardiomyopathy probands of Japanese origin.** *M.A. Razzaque[1], T. Nishizawa[1], M. Furutani[1,2], M. Kurabayashi[3], K. Joh-o[4], J. Fukushige[5], Y. Ohta[6], S. Arai[1], N. Hagiwara[7], R. Matsuoka[1,2].* 1) IREIIMS, Tokyo Women`s Medical University 8-1, Tokyo, Tokyo, Japan; 2) Division of Pediatric Cardiology, Tokyo Women`s Medical University 8-1, Tokyo, Tokyo, Japan; 3) Department of Medicine and Biological Science Gunma University School of Medicine, Gunma,; 4) Department of Pediatrics, Kyushu Welfare Pension Hospital, Fukuoka, Japan; 5) Pediatric Cardiology, Fukuoka Children's Hospital, Fukuoka, Japan; 6) Ohta Children's Clinic, Hokkaido, Japan; 7) Department of Cardiology, Tokyo Women's Medical University, Tokyo, Japan.

Over the last two decades, a large number of mutations have been identified in sarcomeric proteins as a cause of hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). Mutations associated with cardiomyopathy have been identified in sporadic as well as familial cases in the proteins of sarcomere, cytoskeleton, and the sarcolemma. Mutations in $\beta$-myosin heavy chain (MYH7), cardiac myosin binding protein C (MYBPC3), cardiac troponin T (TNNT2), cardiac troponin I (TNNI3), $\alpha$-tropomyosin (TPM1), myosin regulatory light chain (MYL2), myosin essential light chain (MYL3), cardiac $\alpha$-actin (ACTC) were reported as the major genes causing HCM and DCM. To report the frequency of single and multiple gene mutations in a Japanese cohort of patients with cardiomyopathy, we screened for mutation by direct sequencing in the MYH7, MYBPC3, TNNT2, TNNI3, TPM1, and ACTC in our cohort of 173 probands with HCM, 25 probands with DCM, and 7 probands with restrictive cardiomyopathy (RCM). We found 68 (39%) mutations in the probands diagnosed with HCM, 5 (20%) mutations in the probands with DCM and 4 (57%) mutations in the probands with RCM. In familial cardiomyopathy patients, 3 compound mutations in which 2 are novel and 4 double mutations in which 3 are novel were found in 7 HCM families (10%) out of 71 families, and a novel compound mutation was found in a DCM patient. Probands carrying either compound mutations or double mutations were shown to express a more severe clinical phenotype than single gene mutation patients. This suggests the importance of look into the phenotypic diversities among the family members and screening the entire panel of major genes causing HCM and DCM even after a single mutation has been identified.

## 2296/T/Poster Board #845

**Association of Ins/Del polymorphism of the angiotensin-I converting enzyme gene with nonfamilial atrial fibrillation in Koreans.** *D. Shin[1,3], A. Park[1], J. Im[1], N. Son[1], K. Lee[1], S. Kim[1], E. Shin[4], J. Lee[4], S. Lee[1,2], H. Park[1,2], Y. Jang[1,2].* 1) Cardiovascular Genome Ctr, Yonsei Col Medicine, Seoul, Korea; 2) Division of Cardiology, Yonsei University College of Medicine; 3) Yonsei University Research Institute of Science for Aging; 4) DNA Link Inc, Seoul, South Korea.

Atrial fibrillation (AF) is a common and serious clinic rhythmus disorder frequently accompanied by heart failure and thromboembolism. Recent studies have been reported renin-angiotensin system (RAS) may play a pivotal role in the pathogenesis of AF, and suggested that the development of AF has relation to activation of the RAS in the atria of humans and animal models of AF. These findings indicate that angiotensin II may be responsible for the mechanism of atrial structural and electrical remodeling, and angiotensin I-converting enzyme (ACE) inhibitors or angiotensin receptor anatagonists may reduce the risk of AF. We hypothesized that genetic variant in the ACE gene predicts risk condition of AF. The aim of this study was to elucidate whether the ACE I/D polymorphism is associated with nonfamilial AF. A total of 219 patients with documented nonfamilial AF, and age and sex-matched 250 controls without AF were recruited. All subjects were genotyped for ACE I/D polymorphism by polymerase chain reaction. The distribution of genotype and allele for I/D polymorphism was significantly different between the patients with AF and the controls in male group (p= 0.013 and 0.015, respectively). After adjustment for variables, the result of logistic regression analysis indicated that the ACE I/D polymorphism (DD+ID/II) was strongly associated with AF in the male subjects (OR, 0.455; 95% CI, 0.251-0.824, p = 0.009) and in all subjects (0.591, 0.371-0.940, p = 0.026), whereas no significant effect was observed in the female subjects. ACE II genotype predicts a decreased risk of nonfamilial AF. This study demonstrates the association of ACE I/D polymorphism with nonfamilial AF in males and this variant may influence effect of ACE inhibitors on AF.

## 2297/T/Poster Board #846

**Evidence that the 9p21 locus confers susceptibility to coronary artery plaque vulnerability - The IBIS-2 and APPROACH studies.** *D.M. Waterworth[1], K. Song[1], X. Yuan[1], N. Lim[1], M. Mosteller[2], M. Dickson[3], C. Huang[4], H.M. Garcia-Garcia[5], P.W. Serruys[5], N.S. Kolatkar[6], A. Zalewski[7], L. Cardon[1], V.E. Mooser[1].* 1) Genetics, GlaxoSmithKline, King of Prussia, PA; 2) Genetics, GlaxoSmithKline, Research Triangle Park, NC; 3) Biomedical Data Sciences, GlaxoSmithKline, Harlow, UK; 4) Biomedical Data Sciences, GlaxoSmithKline, King of Prussia, PA; 5) Thoraxcenter, Erasmus Medical Center, Rotterdam, The Netherlands; 6) Medicine Development Center, GlaxoSmithKline, King of Prussia, PA; 7) Cardiovascular Clinical Science Unit, Novartis, East Hanover, NJ.

The mechanism whereby the GWAS-derived 9p21 locus confers an increased risk for coronary artery disease (CAD) of approximately 35% per risk allele independently of known cardiovascular risk factors remains elusive. We hypothesized that carriers of the 9p21 locus risk alleles may have larger coronary atherosclerotic plaques and therefore may respond better to LpPLA2 inhibition, as compared to non-carriers. To test this hypothesis, we investigated this locus in the IBIS-2 study, a phase II placebo-controlled clinical trial of darapladib (a Lp-PLA2 inhibitor), where intravascular ultrasound (IVUS) greyscale and IVUS virtual histology (VH) parameters of single plaques were available at baseline and after approximately one year of follow-up (up to n=258). We found that 9p21 risk alleles were associated with smaller rather than larger VH total plaque volumes (rs1075274 p=0.006) and a corresponding lower necrotic core volume (p=0.006) at baseline. IVUS greyscale measures of total plaque volume were less significant (p=0.015). No clear treatment by genotype effect was observed when changes from baseline in the two treatments were evaluated. In addition, MMP9 levels, a surrogate measure of plaque vulnerability, were higher in 9p21 carriers over the entire course of the study and in both treatment groups. To confirm these results, we genotyped the same 9p21 SNPs in the APPROACH study, a 18-month randomized trial of rosiglitazone or glipizide on atherosclerosis progression in patients with type 2 diabetes and CAD where IVUS greyscale but not VH data and MMP9 levels were available at baseline (up to n=342). The IVUS greyscale total plaque volume trended in the same direction as in IBIS-2, though was not significant. As in IBIS-2, risk allele carriers had elevated baseline MMP9 levels, especially in subjects with poor glucose control (rs1075274-HbA1c interaction p=0.006). In summary, the potential association between 9p21 SNPs with smaller atherosclerotic plaques on IVUS virtual histology parameters, and the robust association with MMP9 provide evidence that the 9p21 locus predisposes to coronary artery plaques that are more vulnerable and prone to rupture.

## 2298/T/Poster Board #847

**Association of polymorphisms in NPR1 and NPPA genes and essential hypertension: single gene and gene-gene interaction.** *S. Wu, W. Niu, P. Gao, D. Zhu.* Shanghai Institute of Hypertension, Shanghai, China.

Objectives: Previous studies show that natriuretic peptides have blood pressure lowering properties. Recently, a study found that common variants in NPPA and NPPB loci were associated with circulating natriuretic peptides and blood pressure. We aimed to identify the genetic variants in NPPA and the NPR1 gene and gene-gene interaction which might play role in occurrence of essential hypertension. Methods: We enrolled 1023 unrelated essential hypertensives and 1004 age-, gender-matched normotensives. Six single nucleotide polymorphisms were selected. Results: We found that genotype and allele frequencies of rs11264236 in NPR1 gene distributed significantly different between hypertensive and normotensive groups (P= 0.004 and P=0.04 for genotype and allele respectively). More importantly, in the recessive model AA genotype showed significant association with the risk of essential hypertension (P=0.002, OR=3.329). In addition, genotype of rs7552330 in NPPA gene shows significantly association with occurrence of essential hypertension (P=0.04). We did not find any association of haplotype in the two genes or gene-gene interaction with essenthila hypertension. Conclusion: Our study suggests that NPR1 might be an important factor for the etiology of essential hypertension. More SNPs will be included in the future study.

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## 2299/T/Poster Board #848

**Genetic Variants in Angiotensinogen (*AGT*) are Associated with Plasma AGT Levels in Healthy Mexican.** *E. Balam[1,2], A. Esquivel-Villarreal[2], L. Alfaro[1], K. Carrillo[1], A. Elizalde[2], T. Gil[2], M. Urushihara[3], H. Kobori[3], G. Jimenez-Sanchez[1].* 1) National Inst Genomic Medicine, Mexico; 2) Central North Hospital, Petroleos Mexicanos, Mexico; 3) Tulane Univ, New Orleans, LA.

In Mexico, hypertension has a prevalence of 30.8%; in olders than 20 years. Different genetic variants including those in AGT are reported to be associated with hypertension. This study was performed to examine the association between AGT variants and plasma AGT levels in healthy Mexican. Sixty-one normotensive subjects (SBP/DBP <130/80 mmHg) were recruited and the following nine single nucleotide polymorphisms (SNPs) were genotyped: C-532T (rs5046), G-217A (rs5049), A-20C (rs5050), A-6C (rs5051), C3389T (rs4762), C4072T (rs699), G6309A (rs2493132), C11535A (rs7079), and A1240G (rs943580). Plasma AGT levels were determined by ELISA. we performed pairwise comparison between the AA genotype as reference group and others genotypes to further elucidate their association with plasma AGT. We observed differences in plasma AGT levels with genotypes of C3389T (CC: 25.3±5.3 vs. CT: 20.8±2.4 μg/ml, p= 0.003). Ten haplotypes covered 97%;; of the variability. The first haplotype was considered as the intercept for linear regression analysis. Significant associations detected by the single-SNP analyses were largely retained in the haplotype analysis. We observed that the seventh haplotype (CGCTCCCCG) is associated with high plasma AGT levels (6.9, 2.7-11.1, p=0.001). Furthermore, we observed that alleles of two SNPs are related to AGT plasma levels (A-20C: 6.9, 2.7-11.1, p=0.001 and C3389T: -11.1, -17.7--4.5, p=0.0009). These findings suggest putative interactions between haplotypes that contain these alleles (CGATCCCCG: 2.6 μg/ml and CGCTCCCCG: 6.9 μg/ml). The association of these haplotypes with plasma AGT levels remained after the full adjustment for covariates (age, abdominal circumference, and body mass index). Also, the total effect of significant haplotypes in plasma AGT level variance was 16.5%. We perform Likelihood Ratio Test (LRT) for haplotype-phenotype association adjusted for above covariates (X2=38.9 with 11 df, p=0.0005). These results support that the LTR are independent of allele frequency. Finally, we performed pairwise linkage disequilibrium (LD) analysis to determine the relationship between SNPs and found that A-20C and C3389T showed strong LD (D'=1.0, LOD= 0.98, 95% confidence bounds: 0.78-1, r2=0.76), suggesting unique locus with high degree of LD associated with plasma AGT level variance in healthy Mexican. While the present analysis only includes controls, other differences could be identified when patients with hypertension are included.

## 2300/T/Poster Board #849

**NIPBL is a Candidate Gene for Isolated Septal Heart Defects in an Inuit Population.** *D. Chai[1], L. Arbour[1], L. Brown[2], P. Eydoux[1,2].* 1) Med Gen, Univ British Columbia, Vancouver, Canada; 2) Pathology, Univ British Columbia, Vancouver, Canada.

The prevalence of congenital heart defects (CHDs) in the Inuit from Nunavut is higher than in the rest of the Canada . Although the rate of complex heart malformations decreased after folic acid fortification, the prevalence of ASDs and VSDs remained essentially unchanged. This high rate of cardiac septal defects could be related to other environmental or genetic factors. In search of a cause for this high rate of CHDs, we studied 15 trios (patients and their parents) using chromosome microarrays. Patients and parents consenting to the study were recruited because of an isolated auricular or ventricular septal defect (ASD, VSD) or association of both these malformations, confirmed with echocardiographic examination in each case. DNA was extracted, labeled and hybridized to the Affymetrix 6.0 array. Data generated from scanning the arrays were analyzed using the Affymetrix Genotyping Console software. In this sample, we found 2 probands with VSD carrying a de novo partial deletion of NIPBL: one deletion was 48,356 base pairs, the other one 114,132 base pairs in size. NIPBL encodes the delangin, a protein playing a role in chromatid cohesion, chromosome condensation and DNA repair. It is highly expressed in the developing heart. NIPBL frameshift, nonsense, splice-site and missense mutations, spanning most of the gene, are seen in ~50% of patients with Cornelia de Lange Syndrome (CdLS), a multisystem malformation syndrome with mental retardation. Up to 1/3 of individuals with CdLS have a CHD; VSDs and ASDs are the most frequent and are a significant cause of death in these patients. Missense mutations and absence of mutation seem to be associated with a milder disease. Deletions in this gene have not, to date, been reported in non-syndromic heart defects. In our Inuit patients, the two mutations, although different, deleted exon 2 to exon 9 included. Both leaved intact the distal, highly conserved part of the gene containing the 5 HEAT repeats. Most mutations resulting in CdLS described to date are distal to exon 9 and/ or are truncating mutations. Further studies in this population are underway to confirm the association of deletions in NIPBL to non-syndromic heart defects.

## 2301/T/Poster Board #850

**The M235T Polymorphism in the Angiotensinogen Gene is Associated with Susceptibility to Myocardial Infarction in the INTERHEART Study.** *N.M. Chami[1], S.W. Bailey[1], C. Xie[4], R. Do[1], G. Pare[2], A. Montpetit[2], T.J. Hudson[3], S. Yusuf[4], S. Anand[4], J.C. Engert[1] On behalf of the Interheart Individuals.* 1) Human Genetics, McGill University, Montreal, Quebec, Canada; 2) McGill Univ. and Genome Quebec Innovation Centre, Montreal, QC, Canada; 3) Ontario Inst. of Cancer Res., Toronto, ON, Canada; 4) Dept. of Medicine, McMaster Univ., Hamilton, O, Canada.

Hypertension is a well-known risk factor for cardiovascular disease and myocardial infarction (MI). Angiotensinogen (AGT) is a vasopressor agent and part of the renin-angiotenin system which plays an essential role in the regulation of blood pressure. Although many studies have shown a strong association between genetic variants of AGT and hypertension, there have been some conflicting results and only a few studies have investigated their role in cardiovascular disease. We assessed the association between the coding variant M235T and MI in INTERHEART, a global case/control study that has identified associations between a host of risk factors and MI among different countries, ethnicities, and socioeconomic groups. The study includes individuals from 52 countries. We genotyped a subset of 7232 individuals from the INTERHEART study and performed an association analysis with five different ethnicities (European, South Asian, Arab, Nepalese, and Iranian). Large differences in allele frequencies were observed for M235T between the different ethnicities. Specifically, the MAF ranged from 0.31in Nepalese to 0.48 in Europeans. In association analysis, we found that M235T is significantly associated with MI in all ethnicities combined (P<0.0002) as well as in Europeans (P< 0.04) and South Asians (P< 0.0004), the ethnicities with the largest number of samples. Interestingly, we observed greater significance in females (P< 0.0009) than in males (P< 0.01). In addition, we were able to replicate previous results and show that M235T is associated with hypertension (combined P value=0.02). These results confirm that M235T is involved in the pathogenesis of MI and at least part of that increased risk is probably mediated through hypertension.

## 2302/T/Poster Board #851

**High-density genome-wide linkage analysis of left-ventricular outflow tract obstruction.** *M.P. Dubé[1,2], L.P. Lemieux-Perreault[1,2], R. Gendron[3], G. Asselin[1], J.L. Bigras[3], M. Hitz[3], S. Yang[3], A. Richter[2,3], A. Montpetit[4], P. Chetaille[3], G.U. Andelfinger[2,3].* 1) Montreal Heart Institute, Research Centre, Montreal, QC, Canada; 2) Université de Montréal, Faculté de Médecine, Montreal, QC, Canada; 3) Centre Hospitalier Universitaire Sainte-Justine, Montreal, QC, Canada; 4) Genome Quebec Innovation Centre, Montreal, QC, Canada.

**INTRODUCTION**: Congenital heart disease is the most common birth defect and the second cause of infant mortality in North America. Left ventricular outflow tract obstruction (LVOTO) is a wide spectrum of congenital heart defects ranging from bicuspid aortic valve to aortic stenosis and hypoplastic left heart syndrome. The current lack of knowledge of the underlying aetiology limits our ability to modify disease course and develop primary or secondary prevention intervention strategies.

**METHODS**: We have recruited a large cohort of multiplex families with LVOTO. All individuals gave informed consent and underwent physical exam, ECG and echocardiography. We have chosen 42 highly informative families with 300 individuals for high-density genotyping on the Affymetrix 6.0 SNP array. Affection status was defined according to two phenotypes: LVOTO only (stringent) or LVOTO plus other cardiovascular malformation or arrhythmia (broad phenotype). Two-point and multipoint linkage analysis was carried out using MERLIN with parameters for an autosomal dominant disease. Unlinked SNPs were selected for multipoint linkage at a linkage disequilibrium threshold of $r^2 < 0.2$. We developed a new algorithm that converts Birdsuite's Fawkes integrated SNPs and copy number variants (CNV) into copy number polymorphisms (CNP) which we partitioned based on familial pedigree data and analyzed as multi-allelic markers for linkage. Run-of-homozygosity analysis was performed using GoldenHelix with a family-based case-control design. **RESULTS**: In the stringent phenotype scenario, we identified a novel locus on chromosome 3p26 (LOD score 2.95). 20/42 families were found to contribute to this locus. Linkage analysis of CNP shows that this locus is co-segregating with genomic structural variation. The broad phenotype scenario yields a locus on chromosome 16p12 (LOD score 3.15). 15/42 families contributed to this locus. Seven families contributed to both loci. No significant association with extended runs-of-homozygosity could be identified.

**CONCLUSIONS**: We provide the first high-density linkage scan of LVOTO, which identified two candidate loci in the French-Canadian population. Detailed haplotype analyses show conservation in a subset of families. We identified structural genomic variation that co-segregates with LVOTO. Ongoing work aims at evaluating positional candidates in LVOTO.

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## 2303/T/Poster Board #852

**MicroRNA microarray expression profiling of human myocardial infarction patients revealed potential microRNAs involved in downregulation of cardiac sarcoplasmic reticulum calcium ATPase-2 (SERCA2).**
*D. Glavac, E. Bostjani.* Department of Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia.

Using miRNA microarrays and bioinformatic tools, miRNA profiling was performed on human myocardial infarction (MI) , foetal hearts and healthy adult hearts. Of 719 miRNAs analyzed, ~50% were expressed in human hearts, 77 miRNAs were absent from all tested tissues and 68 were confidently dysregulated in at least one tested group. Some expression patterns appeared to be similar in MI and foetal hearts. Bioinformatic analysis revealed at least 15 novel dysregulated miRNAs not yet described in MI patients. On the other hand cardiac sarcoplasmic reticulum calcium ATPase-2 (SERCA2) plays a central role in myocardial contractility. SERCA2 mRNA and protein levels are reduced in various forms of heart disease, but a correlation between decreased SERCA2 mRNA and protein levels is not always observed. SERCA2 is regulated at post-translational level by phospholamban, but may be also regulated at post-transcription level. Since protein SERCA2 reduction may contribute to heart disease, we focused on miRNAs that could potentially involved in regulation of SERCA2 expression especially in human myocardial infarction (MI) patients. SERCA2 protein expression and microRNA expression were analyzed by Western blot and qPCR. MicroRNAs binding on SERCA2 mRNA was predicted using several in silico approaches. In infracted tissue when compared to remote myocardium, the protein SERCA2 was decreased, 64 miRNAs were dysregulated, and of these 21 miRNAs were upregulated. By combining miRNA microarray, target prediction, Western blot and Northern blot, we identified 8 miRNAs (miR-34a, miR-214, let-7i, miR-100, miR-122, miR-199-3p, miR-199-5p and miR-497) with a potential influence on SERCA2 regulation. In proportion of samples the up-regulation of miR-122 and miR-100 was confirmed by Northern blot and qPCR analysis. At the same time SERCA2 downregulation was observed by Western blot and qPCR. This results is in concordance with some other miRNA analysis obtained on cardiomyopathies and neonatal cardiomyocytes. Our results indicate that various miRNAs, in addition to phospholamban, may be involved in the regulation of SERCA2 in myocardial infarction patients.

## 2304/T/Poster Board #853

**Sequence variation in the APOA1 and APOA4 genes and their relationship with plasma HDL-cholesterol levels.**
*S.E. Hill[1], F.Y. Demirci[1], A.S. Dressen[1], C.M. Kammerer[1], C.H. Bunker[2], J.E. Hokanson[3], R.F. Hamman[3], M.I. Kamboh[1].* 1) Human Genetics, GSPH, Univ Pittsburgh, Pittsburgh, PA; 2) Epidemiology, GSPH, Univ Pittsburgh, Pittsburgh, PA; 3) Epidemiology, Colorado School of Public Health, Univ Colorado Denver, Aurora, CO.

Heart disease continues to be the leading cause of death in the United States, making it one of the foremost public health concerns. Many factors influence the risk to develop heart disease, including abnormal blood lipid levels. High levels of plasma high-density lipoprotein cholesterol (HDL-C) have been shown to have a protective effect. Genetic variation in *APOA1* and *APOA4* has been evaluated for association with HDL-C levels in multiple studies with somewhat inconsistent results. The aim of this study was to comprehensively investigate the role of both common and rare variation in these genes by first sequencing them (entire gene and flanking regions) in individuals having extremely low and high HDL-C levels in two epidemiological samples, non-diabetic U.S. Non-Hispanic Whites (NHWs) from the San Luis Valley Diabetes Study and African Blacks from Nigeria, and then screening the identified variants in the entire sample sets. The analysis of sequencing data from individuals with HDL-C levels in the upper $5^{th}$ percentile (47 NHWs and 48 African Blacks) and the lower $5^{th}$ percentile (48 NHWs and 47 African Blacks) revealed a total of 54 variants in *APOA1* and its flanking regions and 43 in *APOA4* and its flanking regions. The total number of identified sequence variants was higher in African Blacks as compared to NHWs. A dramatically higher number of coding region variants were detected in *APOA4* (n=17) versus *APOA1* (n=5). When individuals were compared between high and low HDL-C groups for carriership of rare variants unique to each group, the percentages were either similar or slightly higher in the high HDL-C group. The sequencing data also showed differences in minor allele frequencies of some common variants between low and high HDL-C groups that await confirmation by genotyping the entire sample sets. Genotyping of all identified rare variants and common tag SNPs in the entire NHW and Black samples is underway and will reveal the extent to which the *APOA1* and *APOA4* variants affect the HDL-C levels.

## 2305/T/Poster Board #854

**Genetic Risk Scores Weighted by SNP Effect on Intermediate Lipid Phenotypes for Application to Coronary Artery Disease Associations.**
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Most single nucleotide polymorphism (SNP) associations with common, complex diseases have small risk effects. Genetic risk scores (GRS) aggregate SNP information from many genes but only our prior work has used weighting and not simply summed the number of risk alleles. This study evaluated SNPs that are candidates for low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), or triglycerides (TG) and aggregated them into a GRS based on relative effects. GRS associations with coronary artery disease (CAD) were then tested. SNPs (total=54) were studied from a tagging SNP study of lipid genes (39 SNPs) and from genome-wide association studies (15 SNPs). Genotyping included untreated population normals (N=748) and patients undergoing coronary angiography (N= 2,950, 65% with CAD). Among normals, each SNP's risk allele was determined from LDL-C, HDL-C, and TG associations of $p \le 0.20$ (17 for LDL-C, 21 for HDL-C, and 12 for TG). GRS variables were computed separately for LDL-C, HDL-C, and TG. Deciles of GRS-LDL were associated with LDL-C (normals: p-trend=$3.8x10^{-6}$, but not in patients: p-trend=0.86), GRS-HDL with HDL-C (normals: p-trend=$1.3x10^{-12}$, patients: p-trend=$1.7x10^{-5}$), and GRS-TG with TG (normals: p-trend=$6.3x10^{-7}$, patients: p-trend=$1.4x10^{-6}$). GRS-LDL ranged from -24.9 to 22.6 mg/dL and for CAD had odds ratio (OR)=1.013 per mg/dL (95% CI=1.00, 1.03; p=0.055) for continuous values, while GRS-LDL deciles had OR=1.03 per decile (CI=1.00, 1.06; p-trend= 0.050). GRS-HDL ranged from -14.6 to 19.9 mg/dL and for CAD had OR= 0.991 per mg/dL (CI=0.97, 1.01; p=0.36) and OR=0.98 per decile (CI= 0.95, 1.02; p-trend=0.30). GRS-TG was measured in natural log-transformed values, with range=-0.66 to 0.05 and for CAD had OR=2.09 per natural log (mg/dL) unit (CI=0.99, 4.39; p=0.053) and OR=1.03 per decile (CI=1.01, 1.06; p-trend=0.042). GRS variables weighted by intermediate phenotype were associated with LDL-C, HDL-C, and TG in population normals and replicated in an independent sample of angiography patients for HDL-C and TG (failure of GRS-LDL to predict LDL-C in patients may be due to lipid-lowering treatment). Weak CAD associations existed for GRS-LDL and GRS-TG (but not GRS-HDL). Intermediate-phenotype weighted polygenic GRS metrics deserve further evaluation.

## 2306/T/Poster Board #855

**A novel stroke susceptibility locus in a pedigree from northern Sweden.**
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**Objectives** - The population of northern Sweden is characterized by reduced genetic diversity and a high incidence of stroke. We sought to reduce genetic variation further, using genealogical analysis in a set of nuclear families affected by stroke and we subsequently performed a genome wide scan to identify novel stroke susceptibility loci. **Methods** - Through genealogy, seven nuclear families with a common ancestor, connected over eight generations were identified. A genome wide scan, utilizing 449 microsatellite markers, was performed with subsequent haplotype analyses. **Results** -A maximum allele-sharing LOD score of 4.81 on chromosome 9q31-q33 was detected. Haplotype analysis identified a common 2.2 Mb interval in the chromosomal region in four of the nuclear families, where an overrepresentation of intracerebral hemorrhage was observed. **Conclusions** - We have identified a novel susceptibility locus for stroke. Haplotype analysis suggests that a shared genetic factor is of particular importance for intracerebral hemorrhage.

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## 2307/T/Poster Board #856

**Association between genetic variants on chromosome 9p21 and aneurysmal subarachnoid hemorrhage.** *C. Jern[1,2], S. Olsson[1,2], L.Z. Csajbok[3], K. Jood[1], K. Nylén[1], B. Nellgård[4].* 1) Dept of Clinical Neuroscience and Neurorehabilitation, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at Gothenburg University, Sweden; 2) Department of Clinical Genetics, Sahlgrenska University Hospital, Göteborg, Sweden; 3) Department of Anaesthesiology and Intensive Care, Sahlgrenska University Hospital, Göteborg, Sweden; 4) Department of Anaesthesia and Intensive Care, University Hospital Malmö, Sweden.

**Background:** Family studies have suggested a role for genetic factors in susceptibility to subarachnoid hemorrhage, but little is known about which genes are involved. Recently, genomewide association studies have identified a locus for risk for intracranial aneurysms (IA), coronary artery disease and ischemic stroke on chromosome 9p21. The aim of the present study was to examine the possible association between sequence variants at 9p21 and ruptured intracranial aneurysms, i.e. aneurysmal subarachnoid hemorrhage (aSAH), in a Swedish population. **Methods:** The study comprises 183 patients presenting with aSAH at the neurointensive care Unit at the Sahlgrenska University Hospital and 366 healthy age and sex matched population-based controls. Because the functional gene in the region on 9p21 remains to be identified, an approximately 44 kbp region on 9p21 (between position 22071397 and 22115503) was tagged using HapMap CEU data and haploview (r2=0.8 and MAF=0.1), resulting in 6 tag single nucleotide polymorphisms (SNPs). Genotyping was performed by TaqMan assays. **Results:** The mean age of patients and controls was 55 years and 74% were females. Two SNPs, rs10757278 and rs1333045, and one common haplotype showed significant association with aSAH in univariate analyses. As expected, hypertension and smoking were overrepresented among aSAH cases compared to controls. There is one study showing association between variants on 9p21 and arterial stiffness, and there are data indicating that arterial stiffness plays a role in the development of hypertension. Furthermore, there is evidence of an interaction between familial aggregation of intracranial aneurysms and smoking. Therefore associations between 9p21 and aSAH were also tested in multivariate analyses. After adjustment for hypertension and smoking, the association between aSAH and rs10757278 remained significant with an OR for aSAH of 1.42 (95% CI 1.08-1.87, p=0.01) for the uncommon G allele. We did not find a significant interaction between rs10757278 and smoking. **Conclusions:** We confirm earlier results showing that 9p21 is a susceptibility locus for intracranial aneurysms, and we show that this association is present in a Swedish sample restricted to ruptured intracranial aneurysms. For the first time we show that this association is independent of hypertension. Furthermore the present data do not suggest that there is an interaction with smoking.

## 2308/T/Poster Board #857

**Linkage and association analyses of platelet function following aspirin treatment measured by the PFA-100 closure time.** *Y. Kim[1], R.A. Mathias[2], L.R. Yanek[2], N. Faraday[2], L.C. Becker[3], D.M. Becker[2], A.F. Wilson[1].* 1) IDRB/Genometrics Sec, NHGRI/NIH, Baltimore, MD; 2) Division of General Internal Medicine , The Johns Hopkins University School of Medicine, Baltimore, MD; 3) Division of Cardiology, The Johns Hopkins University School of Medicine, Baltimore, MD.

Low-dose aspirin (ASA, 81mg/day) inhibits platelet function and is used as a preventive treatment for coronary artery diseases (CAD). The platelet function analyzer (PFA) - 100 test is used to assess the effects of ASA; it measures the time in seconds to shear stress-induced platelet plug formation with a collagen-epinephrine cartridge. In this study, three methods are used to screen for genetic effects contributing to PFA: 1) two-point genome-wide linkage analysis of 546 microsatellite markers, 2) multipoint linkage analysis in regions with evidence of linkage using single nucleotide polymorphisms (SNPs), and 3) tests of association in regions identified with linkage analysis. The study included 363 whites in 190 pedigrees and 255 blacks in 120 pedigrees with premature CAD but healthy. PFA values post ASA were adjusted for age, sex, and pre-aspirin values within each race. Two point linkage analysis was performed on the deCODE 550 STR markers (average spacing = 8 cM) with Haseman-Elston regression models in SAGE v5.1.0 (44/47 sibs in blacks and whites, respectively). A 10 Mb region on 5q35.2-3 suggested linkage ($P < 0.005$) in both races, and 4599 SNPs in this region were selected from the Illumina1M SNP chip for subsequent analyses. Multipoint linkage analysis modeling linkage disequilibrium (LD) was performed with these SNPs (median spacing = 1.5 kb) with Merlin v1.1.2. Association tests of each single SNP were performed using linear mixed effect models in SAS v.9.1.3 under an additive model. In blacks, 105 linkage signals with $P < 0.001$ clustered in the region between 176 - 178.5 Mbp on chromosome 5, 45 of which map to the COL23A1 gene. In association studies, there were four highly significant markers: rs4323245 (p =1.9e-4, MAF = 0.07), rs11953588 (p = 2.9e-4, MAF = 0.28), rs6898657 (p = 9.75e-5, MAF = 0.12), and rs7731035 (p = 7.05e-5, MAF = 0.14). However there was no evidence for association with the SNPs in whites. There are six genes of interest on 5q35.2-3. COL23A1 is a member of the transmembrane collagens. CPLX2, SNCB and CLTB are vesicle associated proteins, the first two of which play a role in platelet excocytosis/degranulation. PCDH24 is a calcium dependent cell adhesion molecule, and FGFR4 is fibroblast growth factor. In conclusion, we have identified a novel genetic locus on chromosome 5 using linkage and association analysis that may cause a modification of the responsiveness of platelets to ASA.

## 2309/T/Poster Board #858

**Kalirin: A novel genetic risk factor for ischemic stroke.** *T. Krug[1,2], H. Manso[1,3], L. Gouveia[4], J. Sobral[1,3], J. Xavier[1,2], I. Albergaria[3], G. Gaspar[3], M. Correia[5], M. Viana-Baptista[6], A.N. Pinto[7], R. Taipa[5], C. Ferreira[8], J.R. Fontes[8], M.R. Silva[9], J.P. Gabriel[9], I. Matos[10], G. Lopes[5], J.M. Ferro[2,4], A.M. Vicente[1,3], S.A. Oliveira[1,2].* 1) Instituto Gulbenkian de Ciencia, Oeiras, Portugal; 2) Instituto de Medicina Molecular, Lisboa, Portugal; 3) Instituto Nacional de Saude Dr. Ricardo Jorge, Lisboa, Portugal; 4) Servico de Neurologia, Hospital de Santa Maria, Lisboa, Portugal; 5) Servico de Neurologia, Hospital Geral de Santo António, Porto, Portugal; 6) Servico de Neurologia, Hospital Garcia de Orta, Almada, Portugal; 7) Servico de Neurologia, Hospital Fernando Fonseca, Amadora, Portugal; 8) Servico de Neurologia, Hospital Sao Marcos, Braga, Portugal; 9) Servico de Neurologia, Hospital de Sao Pedro, Vila Real, Portugal; 10) Servico de Neurologia, Hospital Distrital de Mirandela, Mirandela, Portugal.

Cerebrovascular and cardiovascular diseases are the leading cause of death and disability worldwide. They are complex disorders resulting from the interplay of genetic and environmental factors, and may share several susceptibility genes. Several recent studies have implicated variants of the Kalirin (KALRN) gene with susceptibility to cardiovascular and metabolic phenotypes but no studies have yet been performed in stroke patients. KALRN is involved, among others, in the inhibition of inducible nitric oxide synthase, in the regulation of ischemic signal transduction, and in neuronal morphogenesis, plasticity and stability. The goal of the present study was to determine whether SNPs in the KALRN gene region on 3q13, which includes the Ropporin gene (ROPN1), predispose to ischemic stroke (IS) in a cohort of Portuguese patients and controls. We genotyped 34 tagging SNPs in the KALRN and ROPN1 chromosomal region on 565 IS patients and 517 unrelated controls, and performed genotype imputation for 405 markers on chromosome 3. We tested the single-marker association of these SNPs with IS. One SNP (rs4499545) in the ROPN1-KALRN intergenic region and two SNPs in KALRN (rs17286604 and rs11712619) showed significant ($P<0.05$) allelic and genotypic (unadjusted and adjusted for hypertension, diabetes and ever smoking) association with IS risk. A cluster of four imputed SNPs (represented by rs7620580) in complete linkage disequilibrium were the most significantly ($P<0.002$) associated with IS (confirmed by genotyping). This study suggests that variants in the KALRN gene region constitute risk factors for stroke in the Portuguese population and that KALRN may represent a common risk factor for vascular diseases. Currently we are following up our results in the Spanish population.

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## 2310/T/Poster Board #859

**Association studies of candidate genes involved in oxidative stress regulation pathways in Lithuanian population.** *V. Kucinskas[1], K. Grigalioniene[1], A. Laucevicius[2], Z.A. Kucinskiene[3].* 1) Department of Human & Medical Genetics, Vilnius University, Vilnius, Lithuania; 2) Clinics of Heart & Vessel Diseases, Vilnius University, Vilnius, Lithuania; 3) Department of Physiology, Biochemistry & Laboratory Medicine, Vilnius University, Vilnius, Lithuania.

A number of biological systems involving proteins encoded by numerous genes are involved in the process of atherogenesis. Unraveling their genetic diversity is important for the understanding its mechanisms and finding new molecular markers for early prediction of the increased risk of future cardiovascular events. Hypothesis of atherosclerosis was formulated and in order to support it with the data the study was planned. The scheme of the study included the bioinformatics, genotyping stages and data analysis. Metabolic / signalling pathways most widely represented in atherosclerosis form a network in which association with inflammation is an important feature. This is consistent with our hypothesis of the role of oxidative stress in the initiation and development of atherosclerosis phenotype. Thus a number of genes participating in the above-stated network can be related to the susceptibility to atherosclerosis via alleles leading to increased vulnerability to oxidative stress. After the large scale analysis of literature and bioinformatic databases 150 single nucleotide polymorphisms (SNPs) of 89 candidate genes, mostly involved in oxidative stress regulation and oxidative homeostasis, were selected to develop a microarray for arrayed primer extension (APEX) resequencing technology. Most of the selected SNPs were in promoter regions or exons that might be expected to cause changes in the function or level of expression of the encoded protein. Out of 150 analyzed SNP's 116 were polymorphic and 34 SNPs were not polymorphic in the studied group. Association analysis using TDT and S-TDT statistics showed statistically significant association of 10 SNP's: rs1376251, rs1800795, rs3811699, rs5882, rs28573147, rs3918242, rs1151640, rs9551963, rs1143627 and rs1126643 in candidate genes: ITGA2, IL1B, ALOX5AP, OR13G1, MMP9, NFKB1, CETP, GPX1, UCP2 and TAS2R50 respectively. The main gene groups are related to: thrombocyte adhesion and vessel damage (ITGA2, MMP9), inflammation response (IL1B, NFKB1), oxidation mechanisms (UCP2, GPX1), cholesterol and lipoxygenase metabolic pathway (ALOX5AP, CETP), and nutrition (TAS2R50, OR13G1). The results of the study support the assumption that different genetic history leads to different spectrum and different frequencies of SNPs across populations.

## 2311/T/Poster Board #860

**Detection of Recurrent Copy Number Variants in Congenital Heart Disease using Multiplex Ligation-dependent Probe Amplification.** *L.A. Larsen[1], K.M. Sørensen[1,2], M. El-Segaier[3], E. Fernlund[3], P. Bouvagnet[4], N. Nehme[4], J. Steensberg[5], A. Errami[6], J. Schouten[6], V. Hjortdal[7], N. Tommerup[1], P.S. Andersen[8].* 1) Wilhelm Johannsen Centre for Functional Genome Research, University of Copenhagen, Copenhagen, Denmark; 2) Department of Clinical Biochemistry, Statens Serum Institut, Copenhagen, Denmark; 3) Department of Paediatric Cardiology, Lund University Hospital, Lund, Sweden; 4) Department of Pediatric Cardiology, Groupe Hospitalier Est, Lyon, France; 5) Department of Paediatric Cardiology, Copenhagen University Hospital, Copenhagen, Denmark; 6) MRC-Holland, Amsterdam, The Netherlands; 7) Department of Cardiothoracic Surgery, Aarhus University Hospital, Skejby, Denmark; 8) Molecular Biology Lab, National Center for Antimicrobials and Infection Control, Statens Serum Institut, Copenhagen, Denmark.

Recent studies have suggested that chromosomal deletions and duplications may be found in a proportion of patients with congenital heart disease (CHD). We have designed an assay for simultaneous analysis of deletions and duplications in 26 genomic regions which have previously been associated with CHD. The assay is based on the Multiplex Ligation-dependent Probe Amplification (MLPA) technique. The assay is performed in a single reaction and targets the following genomic regions: 1p36, 1q21, 2q37, 2p22, 3q22, 4q32, 4p16, 5q35.2, 5q35.3, 6p25.3, 7q22, 7q11.23, 8q12.2, 8p23.2, 8p23.1, 8q34.13, 8q34.11, 10q25.2, 10q25.3, 11q25, 13q14, 15q26, 17p13, 17p11, 20p12, 22q11. At the time of abstract submission we have screened 219 patients with CHD using the MLPA kit. The screening revealed copy number variants (CNVs) in nine patients (4.3%). None of these CNVs were found in 96 controls. Three CNVs were *de novo*: dup(2)(p22.3), dup(5)(q35.-3qter) and del(8)(p23.1). Interestingly, the 2p22.3 duplication was previously detected in another CHD patient as inherited from a healthy parent (Erdogan et al., 2008, J. Med. Genet, 45:704-9). We detected the recurrent dup(22)(q11.21) in four patients. All four 22q11 duplications were inherited from a healthy parent, but the 22q11 duplication was not present in 600 controls. In conclusion the MLPA analysis shows that recurrent CNVs are present in a significant proportion of CHD patients.

## 2312/T/Poster Board #861

**Genetic association analysis of heart rate on chromosome 9p21 in American Indians: The Strong Heart Family Study.** *P.E. Melton[1], S. Rutherford[1], V.S. Voruganti[1], H.H.H. Göring[1], S. Laston[1], K. Haack[1], A.G. Comuzzie[1], J.E. Curran[1], J. Blangero[1], E.T. Lee[2], L.G. Best[3], R.R. Fabsitz[4], R.B. Devereux[5], P.M. Okin[5], J.N. Bella[5], U. Broeckel[6], B.V. Howard[7], J.W. MacCluer[1], S.A. Cole[1], L. Almasy[1].* 1) Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Center for American Indian Health Research, College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma; 3) Missouri Breaks Industries Research, Inc., Timber Lake, South Dakota; 4) Epidemiology Branch, National Heart, Lung, and Blood Institute, Bethesda, Maryland; 5) Department of Medicine, Weill Cornell Medical College of New York, New York; 6) Medical College of Wisconsin, Milwaukee, Wisconsin; 7) MedStar Research Institute, Washington, DC.

Several studies have identified higher heart rate as an independent risk factor for cardiovascular disease (CVD) and hypertension. However, little is known regarding the genetic architecture of heart rate. The Strong Heart Family Study (SHFS) was designed to identify genetic risk factors for CVD in American Indians (AIs) ascertained without regards to disease status, from large multigenerational families located in Arizona, Oklahoma, and North/South Dakota. Previous genetic linkage research of SHFS participants had identified a significant QTL for heart rate (LOD=3.3; genome wide *p*-value= 0.016) on chromosome 9p21. The greatest support for this signal came from the Oklahoma center (LOD = 4.2). In this study, we examined 2,249 SNP genotypes in the 1 LOD support region of the 9p21 SHFS heart rate QTL from 1,122 AI participants. Heart rate (beats per minute) was measured from echocardiographic Doppler recordings and were inverse normalized and adjusted for effects of age and sex prior to statistical analysis. Fat-free body mass, alcohol use, smoking status, HOMAIR, insulin, HbAIC, antihypertensive medication, systolic, diastolic and mean arterial pressures were assessed but were not significant covariates and were not included in subsequent analysis. We applied a gene-centric joint statistical test that uses the effective number of SNPs within a single gene and is better able to determine significance under multiple testing. Two SNPs (rs7875153 and rs10757082) remained significant after correction among Oklahoma SHFS participants. The SNP rs7875153 ($p$ = 7.95 X 10-5) is located near the gene for KIAA1797 a hypothetical protein that may be involved in mitotic chromosomal condensation. To further investigate this relationship we used RNA expression data available from the San Antonio Family Heart Study (SAFHS), a longitudinal genetic epidemiologic study of CVD in Mexican Americans from San Antonio, TX. Expression levels of the KIAA1797, transcript were significantly associated ($p$ = 0.012) with electrocardiographic heart rate in SAFHS participants. These findings suggest that genetic variation within KIAA1797 is associated with heart rate and may influence risk for heart disease.

## 2313/T/Poster Board #862

**Mutation screening of sarcomere genes MYH7, MYBPC3, LDB3, and TNNT2 in a large cohort of dilated cardiomyopathy families.** *M. Merlo[1], E. Carniel[2], D. Slavov[2], X. Zhu[2], D. Ferguson[2], E. Salcedo[2], J. Cavanaugh[2], G. Sinagra[1], A. Di Lenarda[1], L. Mestroni[2], M. Taylor[2].* 1) Ospedale Maggiore and University of Trieste, Trieste, Tireste, Italy; 2) University of Colorado Denver, Aurora, CO.

**Background:** Idiopathic dilated cardiomyopathy is a severe cause of cardiac ventricular dilation and systolic dysfunction that shows extensive genetic heterogeneity. Mutations in sarcomere proteins have been well-studied in hypertrophic cardiomyopathy but the prevalence of and phenotypic characteristics of sarcomere protein mutations in the dilated phenotype are less understood. **Methods:** We undertook mutation screening of 242 well-phenotyped subjects with dilated cardiomyopathy from 179 families (129 familial and 50 sporadic cases) using denaturing high-performance liquid chromatography and sequence analysis. Four sarcomeric genes (MHY7, MYBPC3, LDB3, and TNNT2) were tested. Putative mutations were evaluated for pathogenicity by testing samples from available relatives and excluding the mutations from healthy controls and available polymorphism databases. **Results:** 2 mutations were detected in 86 subjects tested for MHY7 (2.3%) (M982T, R1434C) and 3 mutations in 209 (1.4%) tested for MYBPC3 (3 missense mutations: S217G, R458H, Ser956Thr) for an overall prevalence of 5/242 (4.1%). No clear mutations were identified in LDB3 or TNNT2. The phenotypes were severe with two mutation carriers having arrhythmias and four mutation carriers have undergone cardiac transplantation. **Conclusions:** Our study, which includes the largest screen of MYBPC3 in dilated cardiomyopathy, found that mutations in MHY7 and MYBPC3 account for approximately 2% of this phenotype. While rare, the mutations found in our study suggest that sarcomeric mutations leading to dilated cardiomyopathy have a severe phenotype.

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## 2314/T/Poster Board #863

**Interaction of NPPB genotype on the relationship between left ventricular hypertrophy and plasma BNP levels.** *T. Miki[1,2], Y. Tabara[1,3], M. Igase[2], A. Oghimoto[4], T. Kido[2], E. Uetani[2], N. Ochi[2], K. Kohara[1,2].* 1) Ehime ProteoMedicine Research Center; 2) Department of Geriatric Medicine, Ehime University Graduate School of Medicine; 3) Department of Basic Medical Research and Education, Ehime University Graduate School of Medicine; 4) Department of Integrated Medicine and informatics, Ehime University Graduate School of Medicine.

**Backgrounds:** Recent genome wide association study identified NPPB (natriuretic peptide precursor B) rs632793 as a susceptible polymorphism for plasma brain natriuretic peptide (BNP) levels in Caucasians. Findings from previous GWAS, however, cannot be extrapolated to other populations with different lifestyles and environmental backgrounds. Here we investigated a cross-validation of the relationship between NPPB polymorphism and plasma BNP levels in Japanese. Further we investigated interactions of NPPB genotype in correlation between plasma BNP levels and electrocardiographic left ventricular hypertrophy (LVH).

**Methods:** The study subjects comprised 591 apparently healthy middle-aged to elderly persons (67±8 years old; 37.9% male). All subjects attended the medical check-up program at Ehime University Hospital, and clinical data used in this study, including plasma BNP levels, were obtained through the check-up process. Genomic DNA was extracted from peripheral blood and all SNPs were analyzed by TaqMan probe assay.

**Results:** Plasma BNP levels were significantly different among the genotype (AA (n=457) 28±25, AG (n=119) 32±26, GG (n=15) 56±30 pg/ml, p= $3.0*10^{-5}$). Age (r=0.343, p<0.001), body mass index (r=-0.131, p=0.001), plasma hematocrit levels (r=-0.149, p<0.001) were also correlated with BNP levels. Multiple regression analysis identified the NPPB genotype as an independent determinant for plasma BNP levels (GG genotype: $\beta$=0.156, p<0.001) independent of following confounding factors; age ($\beta$=0.326, p<0.001), BMI($\beta$=-0.123, p=0.002), plasma hematocrit levels ($\beta$=-0.097,p= 0.011), treatment with diuretics ($\beta$=0.094, p=0.013), and electrocardiographic LVH ($\beta$=0.103, p=0.006). Subjects with electrocardiographic LVH showed significantly higher BNP levels (LVH (n=76) 38±32, non-LVH (n= 515) 28±24 pg/ml, p=0.001). However, in subjects without LVH, GG genotype carriers showed predominantly higher BNP levels (51±30 pg/ml) as compared with that of LVH subjects with GA or AA genotype (36±4 pg/ml).

**Conclusion:** Association between NPPB rs932793 polymorphism and plasma BNP levels was replicated in Japanese. The genotype data is essential in order to ensure accurate interpretation of plasma BNP value.

## 2315/T/Poster Board #864

**A functional variation in *BRAP* confers risk of myocardial infarction in Asian populations.** *K. Ozaki[1], H. Sato[2], K. Inoue[3], T. Tsunoda[4], Y. Sakata[2], Y. Onouchi[1], S. Ikegawa[5], S.H. Juo[6], M. Hori[2], Y. Nakamura[7], T. Tanaka[1].* 1) Laboratory for Cardiovascular Diseases, Center for Genomic Medicine, RIKEN, Yokohama, Japan; 2) Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Japan; 3) Department of Cardiology, Kokura Memorial Hospital, Kitakyushu 802-8555, Japan; 4) Laboratory for Medical Informatics, Center for Genomic Medicine, RIKEN, Yokohama, Japan; 5) Laboratory for Bone and Joint Disease, Center for Genomic Medicine, RIKEN, Tokyo, Japan; 6) Graduate Institute of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan; 7) Center for Genomic Medicine, RIKEN, Yokohama, Japan.

Development and progression of coronary artery diseases including myocardial infarction (MI) depend on complex interactions multiple genetic and environmental factors. We previously showed that a functional variation in *LGALS2* encoding galectin-2 was associated with susceptibility to MI by a case-control association study using a Japanese population. Here we show that galectin-2 binds to BRAP, BRCA1 associated protein, and SNPs in *BRAP* on chromosome 12q24 also confer risk of MI with marked significance in a large Japanese cohort (P = $3.0 \times 10^{-18}$, OR=1.48, 2,475 cases and 2,778 controls). Replication study confirmed this association (P = $4.4 \times 10^{-6}$, 862 cases and 1,113 controls). Furthermore, another Asian population, Taiwanese population, also showed significant association (P = $4.7 \times 10^{-3}$, 349 cases and 994 controls). One SNP, located within intron3 in *BRAP*, enhanced the transcriptional level of this gene. Moreover, suppression of *BRAP* expression level using siRNA in cultured coronary artery endothelial cells suppressed activation of NFkB, a central mediator of inflammation. Smooth muscle cells (SMCs) and macrophages in the human atherosclerotic lesions also expressed BRAP. These findings indicate that *BRAP* is a novel genetic risk factor for MI. Moreover, by combination of this genetic risk factor and five ones (*LTA*, *LGALS2*, *ITIH3*, *MIAT* and *PSMA6*) previously identified by us, the odds ratio increased to about 9. The result indicates that combination of genetic risk factors contributes to the pathogenesis of MI.

## 2316/T/Poster Board #865

**Familial isolated cardiomyopathy caused by a mutation in the flavoprotein subunit of succinate dehydrogenase.** *R. Parvari[1,2], E. Muhammd[1], G. Harel Levy[1], A. Levitas[3], V. Chalifa Caspi[2], E. Manor[4], J.C. Beck[6], A. Saada[5], V.C. Sheffield[6].* 1) Dept Gen & Virology, Ben Gurion Univ, Beer Sheva, Israel; 2) National Institute of Biotechnology Negev, Ben Gurion University of the Negev, Beer Sheva 84105, Israel; 3) Division of Pediatrics, Soroka Medical Center, Beer Sheva 84105 and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva 84105, Israel; 4) Institute of Genetics, Soroka Medical Center, Beer Sheva 84105 and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva 84101, Israel; 5) Deparment of Human Genetics and Metabolic Diseases. Hadassah-Hebrew University Medical Center Jerusalem Israel; 6) Dept. of Pediatrics - Division of Medical Genetics; Howard Hughes Medical Institute, University of Iowa, Iowa City, IA 52242, USA.

Cardiomyopaties are the most common disorders resulting in heart failure. Dilated cardiomyopathy (DCM), a disorder characterized by cardiac dilatation and reduced systolic function, is the most frequent cause. However, recessive neonatal isolated dilated cardiomyopathy has scarcely been associated with a mutation. A defect of the human succinate dehydrogenase (SDH) is a rare condition in human, representing 2% of respiratory chain (RC) deficiencies. Its clinical presentation is highly variable, ranging from early onset encephalomyopathies to tumor susceptibility in adults. We present the association of a mutation in the SDHA gene with the clinical manifestation and interfamilial variability of 15 patients diagnosed with dilated and hypertrophic cardiomyopathy. The cardiomyopathy is presumably caused by the significant specific reduction of the SDH enzymatic activity in heart muscle whereas substantial activity is retained in skeletal muscle and lymphoblastoid cells. Identification of the SDHA as the mutated gene was hindered since patients with a similar clinical presentation belonging to the same enlarged family proved to have mutations in another gene and a father homozygous for the mutation is not affected. The same mutation was previously reported to cause a multi-systemic failure leading to neonatal death and to Leigh syndrome. Thus this study highlights the extreme variability that results from homozygosity of the same mutation in a nuclear encoded respiratory complex.

## 2317/T/Poster Board #866

**Large scale candidate gene analysis of lipid traits in the Pakistan Risk of Myocardial Infarction Study and comparison with participants of European descent.** *D. Saleheen[1,3], N. Soranzo[2], M. Zaidi[3], S. Kaptoge[1], E. Angelantonio[1], A. Rasheed[3], M. Alexander[1], P. Haycock[1], N. Sarwar[1], A. Butterworth[1], N. Shah[3], M. Samuel[3], A. Samad[4], M. Ishaq[4], P. Frossard[3], J. Danesh[1], P. Deloukas[2].* 1) Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom; 2) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 3) Center for Non-Communicable Diseases, Karachi, Pakistan; 4) Karachi Institute of Heart Diseases.

Background: Numerous candidate gene studies and high-throughput genotyping approaches have yielded several loci regulating lipid levels in participants of European descent, with little validation and quantification of these associations in other ethnicities. This is specifically important for 1.5 billion people living in South Asia where cardiovascular disorders are expected to double by 2020. Methods: We tested for associations with lipid traits over 45,000 variants across 2000 candidate genes pertinent to cardiovascular and metabolic syndromes, by using the IBC Illumina 50K array, in 3200 participants of the Pakistan Risk of Myocardial Infarction study (PROMIS). For comparison, we genotyped 2450 participants of European ancestry in the LURIC study by using the same array and performed meta-analyses of previously published studies in Europeans. Associations were tested through an additive genetic model adjusting for age, gender, the first two PCs and disease status. Results: Principal components analyses involving data from PROMIS and HAPMAP3 revealed Pakistani ethnicities to be unique from other worldwide groups. We identified 27 variants, representing five genomic regions, associated with HDL-C, LDL-C or triglycerides with a bonferroni adjusted P-value of < 10-6 and overall validated the associations of 15 genomic regions in South Asians. In particular, we identified CETP to be associated with HDL-C (rs711752; P < 10-14), APOA5, ZNF259 (rs651821; P < 10-14) and GCKR (rs1260326; P < 10-10) to be associated with triglycerides and CELSR2 to be associated with LDL-C (rs646776; P < 10-10). The minor allele frequency and associations observed in South Asians were observed to be consistent with the results obtained in Europeans. The minor allele frequency for APOA5 variants associated with triglycerides, however, was twice as common in PROMIS participants compared to Europeans with stronger associations in the Pakistani population. Conclusions: These findings suggest that several loci associated with lipid traits in Europeans also extend to South Asians, with minor quantitative differences for some loci. South Asians can therefore provide a useful resource for the discovery and validation of QTLs discovered in participants of European descent. More detailed hypothesis-free investigations such as a GWAS in South Asians will enable identification of additional novel loci due to their unique ancestry and differences in allelic architecture.

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## 2318/T/Poster Board #867

**Functional Mutation in *ABCG1* Predicts Risk of Ischemic Heart Disease in the General Population.** *J. Schou[1], R. Frikke-Schmidt[1], B.G. Nordestgaard[2, 4], D. Kardassis[3], E. Thymiakou[3], G. Jensen[4], P. Grande[5], A. Tybjærg-Hansen[1, 4].* 1) Dept. of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; 2) Dept. of Clinical Biochemistry,Herlev Hospital, Copenhagen University Hospital, Herlev, Denmark; 3) Dept. Biochemistry, University of Crete Medical School, Heraklion, Greece; 4) The Copenhagen City Heart Study, Bispebjerg Hospital, Copenhagen University Hospital, Copenhagen, Denmark; 5) Dept. of Cardiology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark.

The ATP-Binding Cassette transporter G1 (ABCG1) has recently been identified as a facilitator of cholesterol and phospholipid efflux from macrophages to HDL. In studies of *ABCG1*-/- versus *ABCG1*+/+ mice, total body expression of *ABCG1* protected against atherosclerosis, without affecting HDL cholesterol (HDL-C) or other lipid or lipoprotein levels. To determine the role of ABCG1 in atherogenesis in humans, we investigated the ability of a functional variant in the *ABCG1* promoter to predict risk of ischemic heart disease (IHD) in the general population. We resequenced the *ABCG1* promoter in 380 individuals with extreme levels of HDL cholesterol, and determined the ability of a new variant, g.-376C>T located in a putative SP1 binding site, to predict risk of IHD in a large prospective study of the general population, The Copenhagen City Heart Study (N=10,313, 31 years follow-up). In the prospective study, the cumulative incidence of IHD was increased in g.-376C>T heterozygotes (frequency: 0.5%) versus non-carriers (P= 0.002). The age and gender adjusted hazard ratio for IHD was 2.4 (95% confidence interval 1.5-3.9). These results for IHD were verified in an independent case-control study (N=10,585; odds ratio: 2.7 (1.4-5.0)). Genotype did not affect HDL-C or any other lipid or lipoprotein levels. Furthermore, in functional studies *in vitro* and *in vivo*, the presence of the -376T mutation completely abolished the binding of Sp1 to the *ABCG1* promoter. **Conclusion:** We have identified a loss of function mutation in the *ABCG1* promoter which reproducibly predicts a 2-3 fold risk of IHD in the general population. This is the first study to demonstrate a role for *ABCG1* in atherogenesis in humans.

## 2319/T/Poster Board #868

**Chromosome 9p21 CAD and Diabetes SNPs Identified through Genomewide Association are Associated with Different Clinical and Molecular Profiles.** *S.H. Shah[1,2], D.R. Crosslin[2], T. Wang[2], J. Johnson[2], C. Haynes[2], S. Nelson[2], D. Seo[3], W.E. Kraus[1], C.B. Granger[1], E.R. Hauser[2].* 1) Dept Medicine, Division Cardiology, Duke Univ Med Ctr, Durham, NC; 2) Center for Human Genetics, Duke University Medical Center, Durham, NC; 3) University of Miami School of Medicine, Miami, FL.

**Background.** Genomewide association studies (GWAS) in CAD and type II diabetes mellitus (DM) have identified two SNPs on chromosome 9p21 near *CDKN2a/b.* We hypothesized that they report on independent genetic effects. **Methods.** We genotyped the CAD SNP rs10757278 and the DM SNP rs10811661 in 1864 subjects ascertained through the cardiac catheterization lab and 78 human aortas. Microarray transcriptome profiling (Affy U95 chip) was performed on aortas. Chi square, linear and logistic regression models were used to compare clinical characteristics by genotype. Mixed models were used to assess correlation between expression tag and genotype. **Results.** SNPs were not in linkage disequilibrium (r2 0.003). Rs10757278 (but not rs10811661) was associated with CAD phenotypes: overall CAD (p=0.0003), 3 vessel CAD (p=0.0004), higher CAD severity as measured by CADindex (<0.0001), left main CAD (0.008), CAD family history (p=0.005), MI (p=0.05), and risk of future MI (p=0.06). Rs10811661 (but not rs10757278) was associated with DM (p=0.03). Both SNPs were associated with lower HDL (p=0.02 for both). In multivariate logistic regression variables independently associated with rs10757278 genotype were race (p<0.0001), cadindex (p=0.001), history of MI (p=0.002) and overall CAD (p=0.04). For rs10811661 race (p=0.003) and DM (p=0.0009) were independently associated with genotype. Each SNP was associated with different expression profiles. Genes most differentially expressed by rs10757278 genotype included *lamin B2* (lipodystrophy gene p=0.001); *PLA2G4C* (phospholipase catalyzing release fatty acid release p=0.006); *KLKB1* (causes prekallikrein deficiency coagulation defect p=0.007); and *BMPR2* (bone morphogenetic protein p=0.007). Transcripts most differentially expressed by rs10811661 genotype included BAIAP2 (insulin receptor tyrosine kinase substrate p= 0.0007); SREBF1 (transcription factor p=0.0007); PTGDS (glutathione independent prostaglandin D synthase p=0.001); and FLNB (filamin member may have role in GPIb$\alpha$function in endothelial cells and platelets p=0.002). **Conclusions.** In conclusion, it appears that the two SNPs identified from GWAS for CAD and DM appear to report on different clinical and molecular phenotypes. Further functional characterization of these SNPs is necessary.

## 2320/T/Poster Board #869

**Massively parallel sequencing in pediatric cardiomyopathy patients.** *S.M Ware, B. Schrand, T. Le, D.C. Jamison, P. Putnam, M. Keddache, J.A. Towbin, S. Kindel.* Cincinnati Children's Hospital and University of Cincinnati College of Medicine, Cincinnati, OH.

Cardiomyopathy is a clinically and genetically heterogeneous disease that affects the cardiomyocyte, leading to decreased ventricular systolic function, diastolic function, or both. Pediatric cardiomyopathy is a particularly malignant subset of this disease with up to 40 percent of symptomatic children progressing to transplant or death within 5 years of diagnosis. The majority of pediatric patients are affected prior to one year of age, complicating diagnosis and treatment. While descriptive measures including physical exam, echocardiogram, cardiac catheterization, and MRI offer data regarding a patient's phenotype, genetic analyses are rarely part of diagnostic protocols. Currently, very limited prevalence data exist to guide design of molecular diagnostics resulting in the majority of pediatric cases being labeled idiopathic. Nevertheless, preliminary evidence suggests that potential treatments for pediatric patients will be etiology specific, reinforcing the need for an understanding of the genetic basis of disease. Here we report the use of massively parallel sequencing to interrogate 30 genes and the mitochondrial genome in 80 patients under age ten with cardiomyopathy. Paired end high depth sequencing was performed using the Illumina Genome Analyzer. Alignment, base pair consensus calling including single nucleotide polymorphism calls, and quality scoring were performed using a novel algorithm. Results of the sequencing screen will be presented including categorization of single nucleotide polymorphism subtype, frequency, and predicted pathogenicity. We assess the relative contribution of amino acid variation in the pediatric cardiomyopathy population and compare rare variant discovery rates to those in the adult cardiomyopathy population. These results provide important information on rare and common variants in candidate genes in the pediatric cardiomyopathy population, establish baseline prevalence data, and serve as an important benchmark for the development of clinically relevant diagnostic testing.

## 2321/T/Poster Board #870

**High-resolution Microarray Analysis in Children with Conotruncal Heart Defects and Hypoplastic Left Heart Syndrome.** *D. Warburton[1], J. Kline[1,3], N. Wong[1], A. Kinney[1], V. Jobanputra[1], C-Y. Yu[1], K. Anyane-Yeboa[1], W. Chung[1], S-H. Lee[2], M. Wigler[2], M. Ronemus[2].* 1) Columbia University, New York, NY; 2) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 3) New York State Psychiatric Institute.

We systematically ascertain all babies ≤1 month old with a conotruncal heart lesion (CNT) and all children ≤5 years old with hypoplastic left heart syndrome (HLHS) from the pediatric cardiology service of Morgan Stanley Children's Hospital of New York. Thus far, we have identified 169 patients with CNT, 90 with HLHS and 7 with both. Of completed cases, 51% were identified at birth, 38% during the prenatal period and 11% at outpatient visits. We excluded cases with complete chromosome aneuploidy or where DNA from both biological parents was not available. We included cases with known DiGeorge syndrome both as internal controls and to search for genetic variability that might be correlated with phenotype. We have enrolled 177 (73%) of contacted families. Completion of the study involves microarray analysis of the family trio, genetic and cardiac work-up of the proband, and echocardiograms of the parents. The goal of our project is to analyze a total of 350 families. To date we have performed microarray analysis of 124 trios using the Nimblegen 2.1 million platform and an analytical method developed by the team at Cold Spring Harbor Laboratory. In preliminary analyses only de novo copy number changes (CNCs) were evaluated. All 6 cases of CNT with DiGeorge syndrome were identified on the array as >2Mb deletions in 22q11.2 (8% of CNT cases). Six cases out of 118 trios without DiGeorge syndrome (5.1%) showed de novo CNCs: 1 del(1q) (8.2 Mb); 1 dup(1q) (15Mb); 1 dup(16p) (759kb); 1 del(12q) (356kb) and 2 distinct cases of del(19p) (43kb and 33 kb). All de novo CNCs contain genes with known function, including two small deletions that include only one gene each (ARID3A, GNG7). Both genes encode DNA-binding proteins believed to be important in embryogenesis. There was overlap between the large deleted and duplicated regions of 1q, but not between the two small deletions in 19p. All three patients with >1Mb CNCs had other anomalies such as absence of the corpus callosum, developmental delay and microcephaly. FISH was used to verify the larger lesions, and revealed a tandem duplication (16p) and an unbalanced translocation (1q). The rate of de novo non-DiGeorge CNCs in patients with congenital heart disease is much higher than the rate (≈1%) found in control series using the same array. Thus small CNCs contribute significantly to the etiology of CNT and HLHS. Numbers are as yet too small to determine whether the rates differ for the two anomalies.

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## 2322/T/Poster Board #871

**Submicroscopic genomic aberration in fetus with congenital heart defects detected by array comparative genome hybridization.** *Y. Chen[1], W. Wang[1], Y.P. Lei[2], H.B. Cheng[1], J. Ou[1], H.Y. Wang[2], K.W. Choy[3], H. Li[1].* 1) Center for Reproduction and Ge, Nanjing Medical University Affiliated Suzhou Hospi, Suzhou, Jiangsu, China; 2) State Key Laboratory of Genetic Engineering and MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai, 200433, P.R. China; 3) Department of Obstetrics and Gynaecology, and Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, P.R. China.

Background: Congenital heart defects (CHD) is the most common birth defect and affects nearly 1% of newborns. Environmental factors and genetic factors are involved in the etiology of CHD. Chromosomal imbalances and DNA copy number aberration have been identified in many forms of syndromic CHD and non-syndromic CHD. Method: One million resolution of array comparative genome hybridization (CGH) was applied in the detection of 16 karyotypic normal fetus with non-syndromic CHD. Putative chromosome copy number changes were defined by intervals of three or more adjacent probes with log2 ratios. The Quality-Weighted Interval Score algorithm (ADM2) with a value of 6.0 was used to compute and assist the identification of aberrations for a given sample. We only accept recurrent changes above the set log2 ratio in dye-flip replicates as significant changes in gain or loss of gene copy number. Results: Submicroscopic deletions and duplications, ranging in size from 0.4 to 511Mb, were detected in all the cases with CHD which are normal in G-banding karyotping. The vast majority of these are phenotypically indifferent polymorphisms without pathogenic significant according to the Database of Genomic Variants (http://projects.tcag.ca/variation/) and were not further investigated. 114 imbalances which do not coincide with common DNA copy number variants were listed as undescribed changes. Significant deletion, duplication found across the genome were analyzed further and confirmed by real-time PCR. Conclusions: Our data show that submicroscopic genomic aberration may play an important role in the etiology of these defects, either as direct causes or as genetic risk factors for CHD.

## 2323/T/Poster Board #872

**Investigating the association of Obesity with Ischemic Stroke using the FTO genotype as an Instrumental Variable.** *M.N. Cooper[1], E.J. Jamrozik[1], P.E. Norman[2], G.J. Hankey[3,4], L.J. Palmer[1].* 1) Centre for Genetic Epidemiology and Biostatistics, The University of Western Australia, WA, Australia; 2) School of Surgery, University of Western Australia, Perth, WA, Australia; 3) School of Medicine & Pharmacology, University of Western Australia, Perth, WA, Australia; 4) Stroke Unit, Department of Neurology, Royal Perth Hospital, Perth, WA, Australia.

Obesity is well recognised as a major risk factor for many common diseases including cardiovascular disease, many cancers, type two diabetes and respiratory disorders. The association between obesity and ischemic stroke (IS) remains unclear. Research to date has found both strong, moderate, and no association between obesity and IS. The Western Australian Health in Mens Study (HIMS) is a population based cohort of men aged 65-years and older (n=12,203). Stroke cases were self-reported by questionnaire and confirmed by inspection of hospital records by a stroke neurologist. Using these data, we investigated the association between obesity and IS through the use of a strongly associated instrumental variable, FTO (fat mass and obesity associated) genotype. Multivariate linear regression was used to investigate the association of FTO genotype with BMI. Logistic regression was then used in sensitivity analysis to investigate the association of BMI with self-reported stroke and confirmed IS, using FTO as an instrumental variable for BMI. Multivariate linear regression analysis was completed on data from 3,323 older men (mean age=71.4, SD 4.2). BMI was log transformed to normalise residuals. FTO (MAF=0.38) was associated with BMI (P<0.001) independent of other risk factors (age, HDL, DBP, WHR and hypertension). The F-statistic for FTO on BMI (F=11.1) was above the recommended minimum to ensure validity of use in analysis as an instrumental variable. When adjusting for known risk factors (age, history of diabetes, cholesterol and hypertension) BMI showed no association with stroke (confirmed IS P=0.65, self-reported stroke P=0.34). The association of FTO (modelled additively) as both an interaction term with BMI (P=0.31) and as a main effect term (P=0.77) yielded no significant findings with confirmed IS. Sensitivity analysis to fully investigate the existence of an association of BMI with IS included defining obesity through dichotomising BMI (≤30/>30, P=0.94), as well as categorising BMI according to WHO categories (normal <25; overweight 25-29.9; obese ≥30) as per previous publications, again yielded no significant results. Our findings show no significant association of FTO genotype or obesity with IS within this cohort of older Caucasian men and suggests that obesity is not a causal risk factor for IS. However, a causal association will only be excluded by showing, in randomised trials, that modification of obesity does not lower incidence of IS.

## 2324/T/Poster Board #873

**Association of TCF7L2 with Prospective Risk of Cardiovascular Disease.** *J. Hartiala[1,2], RM. Watanabe[1,3], ML. Brennan[4,5,6], DM. Brennan[5], SL. Hazen[4,5,6], H. Allayee[1,2].* 1) Department of Preventive Medicine, USC Keck School of Medicine, Los Angeles, CA; 2) Institute for Genetic Medicine, USC Keck School of Medicine, Los Angeles, CA; 3) Department of Physiology and Biophysics, USC Keck School of Medicine, Los Angeles, CA; 4) Department of Cell Biology, Cleveland Clinic, Cleveland, OH; 5) Department of Cardiovascular Medicine, Cleveland Clinic, Cleveland, OH; 6) Center for Cardiovascular Diagnostics and Prevention, Cleveland Clinic, Cleveland, OH.

Objective: We sought to evaluate the genetic contribution of the type 2 diabetes (T2D) susceptibility gene, transcription factor 7-like 2 gene (TCF7L2), to cardiovascular disease (CVD) and its complications. Background: CVD is a major complication for T2D patients but the role of TCF7L2, a validated T2D susceptibility gene, in the development of CVD has not been fully explored. Methods: Two TCF7L2 variants, rs12255372 and rs7903146, were genotyped in 1415 individuals ascertained through elective diagnostic coronary angiography. Multiple logistic regression analyses were used to test for association with CVD and T2D traits. Cox proportional models were used for assessing risk of experiencing incident major adverse cardiac events (MACE), including revascularization, myocardial infarction (MI), stroke, or death. Results: In a prospective analysis with the entire sample, the number of MACE in subjects carrying the GT/TT genotypes of TCF7L2 rs12255372 was significantly higher than in GG homozygotes (26% vs.17%; log-rank p=0.0001). This effect was particularly strong in impaired glucose tolerant (IGT) subjects (adjusted HR = 1.4; 95% CI, 1.1-1.7; p=0.01). Both TCF7L2 variants were also associated with T2D with an OR of ~1.5 and ~2.2 in heterozygote and homozygote carriers of the rare alleles, respectively (p < 0.00003). Conclusions: These studies extend the role of TCF7L2 to CVD complications and MACE, even within IGT subjects before the diagnosis of T2D, and add to the growing body of evidence that TCF7L2 is a validated T2D susceptibility gene. These findings may have important implications for preventive clinical management of CVD risk in individuals with IGT.

## 2325/T/Poster Board #874

**Genetic Variation at the *Proprotein Convertase Subtilisin/Kexin Type 5* Gene Modulates HDL Cholesterol Levels.** *I. Iatan[1], Z. Dastani[2], R. Do[2], D. Weissglass-Volkov[3], I. Ruel[1,2], J.C. Lee[3], A. Huertas-Vazquez[3], M.-R. Taskinen[4], A. Prat[5], N.G. Seidah[5], P. Pajukanta[3], J.C. Engert[1,2], J. Genest[1,2].* 1) Department of Biochemistry, Cardiology Division, Cardiovascular Research Laboratories, McGill University, Montreal, Quebec, H3A 1A1, Canada; 2) Department of Human Genetics, Cardiovascular Research Laboratories, Cardiology Division, McGill University, Montreal, Quebec, H3A 1A1, Canada; 3) Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA; 4) Department of Medicine, Helsinki University Central Hospital, HUS-00029, Helsinki, Finland; 5) Laboratory of Biochemical Neuroendocrinology, Clinical Research Institute of Montreal, Montreal, Quebec, H2W 1R7, Canada.

A low level of plasma high-density lipoprotein cholesterol (HDL-C) is a risk factor for cardiovascular disease. HDL particles are modulated by a variety of lipases, including endothelial lipase (EL), a phospholipase present on vascular endothelial cells. The proprotein convertase subtilisin/kexin type 5 (PCSK5) is known to directly inactivate EL, and, indirectly, cleave and activate angiopoetin-like protein 3, a natural inhibitor of EL. We therefore investigated the effect of human *PCSK5* genetic variants on plasma HDL-C levels. Haplotypes at the *PCSK5* locus were examined in nine multi-generational families that included 60 individuals with HDL-C<$10^{th}$ percentile. Segregation with low HDL-C in one family was found. Sequencing of the *PCSK5* gene in 12 probands with HDL-C<$5^{th}$ percentile identified seven novel variants. Using a two-stage design, we first genotyped these single-nucleotide polymorphisms (SNPs) along with 163 tagSNPs and 12 additional SNPs (n=182 total) in 457 individuals with documented coronary artery disease. We identified nine SNPs associated with HDL-C (P<0.05), with the strongest results for rs11144782 and rs11144766 (P=0.002 and P=0.005 respectively). The SNP rs11144782 was also associated with very low-density lipoprotein (P=0.039), triglycerides (P=0.049) and total apolipoprotein B levels (P=0.022). In stage 2, we replicated the association of rs11144766 with HDL-C (P=0.014) in an independent sample of Finnish low HDL-C families. In a combined analysis of both stages (n=883), region-wide significance of rs11144766 and low HDL-C was observed (unadjusted P= $1.86 \times 10^{-4}$ and Bonferroni adjusted P=0.031). We conclude that variability at the *PCSK5* locus influences HDL-C levels, possibly through the inactivation of EL activity and consequently, atherosclerotic cardiovascular disease risk.

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## 2326/T/Poster Board #875

**Chromosome 9p21.3 Coronary Artery Disease Risk Allele alters activity of a functional regulatory element.** *O. Jarinova, P. Lau, T. Naing, R. McPherson.* University of Ottawa Heart Institute, Ottawa, Canada.

The 9p21.3 risk locus, identified in several genome-wide association studies for coronary artery disease (CAD) encompasses multiple single nucleotide polymorphisms in tight linkage disequilibrium, lies centromeric to *CDKN2A* and *CDKN2B* genes and overlaps *ANRIL*. We previously reported an upregulation of several cellular proliferation pathways as well as increased expression of short variants of *ANRIL* and reciprocally lower expression of *CDKN2A* and *CDKN2B* in subjects homozygous for the risk allele. These data suggested that the 9p21.3 risk region may contain *cis*-acting regulatory elements responsible for allele-specific gene expression differences. Using phylogenetic footprinting, we have identified four conserved non-coding sequences (CNSs) within the 9p21.3 risk locus for coronary artery disease that may contain such regulatory elements. We demonstrate that one of the CNSs, termed CNS3, has enhancer activity and that the risk variant significantly increases reporter gene expression in human primary aortic smooth muscle cells (AoSMCs). By sequencing the CNS3 regions from the reference and the risk haplotypes, we demonstrated that the observed difference in the CNS3 regulatory activity is attributable to the presence of a single nucleotide polymorphism, rs1333045, which shows strong LD with representative SNPs of the previously defined 9p21.3 risk region (r2=0.85). Moreover, rs1333045 has a similar allele frequency to that of rs1333049 in premature angiographic CAD cases and healthy elderly controls (MAFcases = 0.605 and 0.598; MAFcontrols = 0.445 and 0.441, rs1333045 and rs1333049, respectively) and a virtually identical allele specific odds ratio for CAD (OR = 1.28). Electrophoretic mobility shift assays of AoSMCs nuclear extracts indicated that CNS3 encompasses a specific transcription factor binding site and that the risk allele of rs1333045 has a lower binding affinity compared to its reference counterpart. These findings demonstrate that the 9p21.3 risk allele alters activity of at least one regulatory sequence which in turn may lead to changes in expression levels of *ANRIL* and/or other genes relevant to atherosclerosis.

## 2327/T/Poster Board #876

**Genetic variation affects the correlation patterns between t-PA and PAI-1 concentrations and traditional cardiovascular risk factors in a Ghanaian population.** *N. KODAMAN[1], F.W. ASSELBERGS[2], K.A. POKU[3], D.E. VAUGHAN[4], N.J. BROWN[4], S.M. WILLIAMS[1].* 1) HUMAN GENETICS, VANDERBILT UNIVERSITY, NASHVILLE, TN; 2) DEPARTMENT OF CARDIOLOGY, UNIVERSITY MEDICAL CENTER GRONINGEN, UNIVERSITY OF GRONINGEN, GRONINGEN, THE NETHERLANDS; 3) HUMAN SERVICES MANAGEMENT AND PUBLIC ADMINISTRATION, BUSINESS SCHOOL, UNIVERSITY OF GHANA, LEGON, GHANA; 4) DEPARTMENT OF MEDICINE, VANDERBILT UNIVERSITY, NASHVILLE, TN.

The fibrinolytic system is activated by tissue-type plasminogen activator (t-PA), which is neutralized by plasminogen activator inhibitor type 1 (PAI-1), promoting thrombus formation. The balance between plasma levels of PAI-1 and t-PA has emerged as a risk factor for cardiovascular disease. We analyzed plasma and DNA samples from a Ghanaian population to assess the relationship between t-PA and PAI-1 concentrations and several well-known cardiovascular disease-related traits (blood pressure, BMI, glucose, LDL, HDL, total cholesterol and triglyceride levels). We found significant correlations between PAI-1 and t-PA levels and many of these variables, with significant heterogeneity between sexes. We also looked at 8 loci in 5 candidate genes involved in the fibrinolytic and renin-angiotensin systems, and found that the correlations between PAI-1 and t-PA levels and the cardiovascular disease-related traits varied significantly by genotype, again with significant heterogeneity between sexes. Overall, 59 of 576 within-sex comparisons were significant at the 0.05 level; 25 of 576 tests were significant at the 0.01 level. Importantly, a similar large-scale study of a Dutch cohort found a different pattern of correlations between PAI-1 and t-PA levels and the cardiovascular disease-related traits, and no comparably significant correlations with genotype. Given the predominantly West African ancestry of African-Americans, these findings provide insight into the environmental and genetic factors that contribute to the incidence of cardiovascular disease in African-American populations, and may have clinical implications for predicting cardiovascular disease risk.

## 2328/T/Poster Board #877

**Genome-wide linkage analysis of 23 multiplex Caucasian families identifies a novel susceptibility locus on chromosome 19q13.4 for coronary artery disease.** *G.-Q. Shen[1, 2], S. Rao[1, 2], L. Li[1, 2], Q. Xi[1, 2], E.J. Topol[3], Q.K. Wang[1, 2].* 1) Center for Cardiovascular Genetics, The Cleveland Clinic, Cleveland, OH; 2) Department of Molecular Cardiology, The Cleveland Clinic, Cleveland, OH; 3) Scripps Translational Science Institute, The Scripps Clinic, La Jolla, CA.

**Background:** Coronary artery disease (CAD) is a common complex disease caused by both various genetic and environmental factors as well as their interactions with each other. It is a leading cause of death in developed countries, but the responsible genetic determinants remain unidentified. The aim of this study was to identify novel genetic locus for CAD linked to the disorder using the approach of genome-wide linkage analysis. **Methods:** A total of 281 individuals from 23 multiplex families with familial CAD enriched with well-characterized CAD cases were genotyped. The average family size was 18.5 ± 10.2 members, and the sib-ship was 113 that produced 388 sib-pairs. Genotyping was performed at the National Heart, Lung, and Blood Institute Mammalian Genotyping Facility through the use of 408 polymorphic microsatellite markers that cover the entire human geneome by an average of 10 cM. Model-free linkage analysis was performed with the modified Haseman-Elston regression model through the use of the SIBPAL program of S.A.G.E. Two genomewide scans were conducted: single-point and multipoint analyses. Empirical *P*-value was also calculated using 100,000 Monte Carlo simulations. **Results:** One novel and significant susceptibility locus was identified for CAD on chromosome 19q13.4 region, with a multi-point *pP* value ($-\log_{10}$ *P*-value) of 4.70, *P*-value of $1.53 \times 10^{-5}$ and LOD score of 4.07. These data were further verified by an empirical *P*-value of $0.99 \times 10^{-5}$ (LOD = 4.25) at the same locus, which corresponds to a genome-wide significance of $P < 0.01$. **Conclusions:** These results indicate that our present study identifies a novel genetic-susceptibility locus for CAD and provides a framework to discover novel susceptibility gene(s) for the familial CAD.

## 2329/T/Poster Board #878

**Identification of SNPs in the LDLR and APOB genes linked with premature CAD in heterozygous familial hyperlipidemia.** *N. Dzimiri[1], S. Wakil[1], M. Al-NAjai[1], B. BAz[1], P. Muiya[1], B.F. Meyer[1], M. Alshaid[2], F. Almohanna[3].* 1) Genetics, King Faisal Spec. Hospital & R, Riyadh, Saudi Arabia; 2) King Faisal Heart Institute, King Faisal Spec. Hospital & R, Riyadh, Saudi Arabia; 3) Biological and Medical Research, King Faisal Spec. Hospital & R, Riyadh, Saudi Arabia.

The underlying genetic causes of early onset of coronary artery disease (early CAD) and heterogeneous familial hypercholesterolemia (HFH) are not fully understood. We employed the Affymetrix whole genome scan 250 sty1 array to characterize possible genomic linkage to these disorders and sequencing techniques to identify related mutations in the low density lipoprotein receptor (LDLR), apolipoprotein B (APOB) and protein convertase subtilisin/kexin type 9 (PCSK9), in a Saudi family of 11 individuals with 5 siblings affected with HFH. The propositus had early CAD, very significantly elevated cholesterol (Chol) level of 10.1 mmol/L and LDL-cholesterol (LDL-C) of 7.9 mmol/L as well as low HDL-C level of 0.51 mmol/L. Whole genome scan using the autosomal dominant pattern of inheritance showed high homology for the affected individuals in several regions including chromosomes 1 and 2 which harbour PCSK9 and APOB, respectively. Subsequent sequencing of the coding areas of the APOB and LDLR identified 11 single nucleotide polymorphisms (SNPs) in the LDLR, in which the propositus uniquely carried the homozygous mutant genotypes (haplotype) for all 11 SNPs, in direct contrast to a normolipidemic sibling and a control who carried the homozygous wild type genotypes at these loci. Interestingly, all family members were heterozygous for all except the SNP rs2228671 C>T of this gene, for which the mother shared the C/C genotype with the propositus, two other affected off-springs and a control, all of whom exhibited low HDL-C levels. A confirmatory experiment involving 70 individuals harbouring low HDL-C revealed 74.3% of them as C/C carriers. Also, another set of 7 SNPs in the APOB isolated with HFH. Our study identified a haplotype in the LDLR as a marker for early onset of CAD, and rs2228671 C>T in the LDLR in association with a reduction in HDL-C concentrations in HFH. The results also substantiate the notion of genetic heterogeneity in HFH, underlining the essence of recognizing ethnic-specific gene variability as a potential basis for appropriate management of FH.

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## 2330/T/Poster Board #879

**A cluster of SNPs in the apolipoprotein B region is associated with high serum apolipoprotein B levels.** *B.E. Haas[1], T. Tusié-Luna[2,3], C. Aguilar-Salinas[4], L. Riba[2,3], C.L. Plaisier[1], D. Weissglas-Volkov[1], M. Laakso[5], P. Pajukanta[1].* 1) Department of Human Genetics, David Geffen School of Medicine at UCLA, University of California, Los Angeles; 2) Molecular Biology and Genomic Medicine Unit, Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Mexico City, Mexico; 3) Instituto de Investigaciones Biomédics de la UNAM, Mexico City, Mexico; 4) Department of Endocrinology and Metabolism, Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Mexico City, Mexico; 5) The Department of Medicine, University of Kuopio and Kuopio University Hospital, Finland.

Coronary artery disease (CAD) is the leading cause of death worldwide. Lowering low-density lipoprotein cholesterol (LDL-C) levels has been shown to be the most effective way to prevent death due to CAD. In search of novel functional LDL-C variants, we selected SNPs found both in a meta-analysis of genome-wide association studies (GWAS) for LDL-C (Kathiresan et al. 2009) and a human liver cis-eQTL dataset (Schadt et al. 2008). We discovered that rs7575840 was one of the top SNPs identified in this comparison (GWAS rank=52; cis-eQTL rank =24). rs7575840 affects the expression of EST BU630349 in human liver. BU630349 is a predicted, primate-specific noncoding RNA, 872 bp upstream of apolipoprotein B (APOB). rs7575840 is in LD (r2>0.8) with 4 SNPs in the CEU HapMap sample: rs1367117, rs1429974, rs754523, and rs11687710. All SNPs reside in an intergenic region within 105 kb upstream of APOB except rs1367117. rs1367117 is a nonsynonymous SNP (T71I) in exon 4 of APOB with prior evidence of association with serum ApoB levels. To replicate the LDL-C association, we genotyped rs7575840 in METSIM (METabolic Syndrome In Men), a cross-sectional population cohort of 7,710 men from Finland. rs7575840 was significantly associated with LDL-C (p=1.16E-08), ApoB (p=1.80E-10), and ApoB/ApoA (p=7.64E-09) in METSIM. Furthermore, rs7575840 explains a larger ApoB variance in individuals with family history of CAD (1.46%) when compared with individuals without family history of CAD (0.42%). The variance explained in the entire METSIM sample was 0.73%. To determine whether this ApoB association replicates in non-Caucasian populations, we genotyped rs7575840 in a hyperlipidemia case-control study sample of 1376 males and females from Mexico. The same allele of rs7575840 as in Finns was significantly associated with ApoB (p=0.008) in Mexicans. To conclude, a cluster of SNPs in tight LD influences the expression of a non-coding RNA residing in the APOB region and is associated with high serum ApoB levels.

## 2331/T/Poster Board #880

**Haplotype analysis of angiotensinogen (AGT) genetic variation, plasma AGT levels, and essential hypertension.** *W.S. Watkins[1], A. Rohrwasser[1], Y. Zhang[1], W. Tolpinrud[1], A. Peiffer[1], E. Hillas[1], S.C. Hunt[2], G.H. Williams[3], M. Leppert[1], J-M. Lalouel[1], L.B Jorde[1].* 1) Department of Human Genetics, University of Utah, Salt Lake City, UT 84112, USA; 2) Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, UT 84112, USA; 3) Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA.

Linkage and association between the angiotensinogen (*AGT*) locus, plasma AGT levels, and hypertension have been reported in many studies but have not been replicated in some others. To better characterize the contribution of *AGT* genetic variation to plasma AGT levels and essential hypertension, we have genotyped 25 *AGT* SNPs in 426 individuals from 41 large 2-generation Utah Genomic Reference (UGRP) families. Plasma AGT is highly heritable ($h^2 = 0.68$), and males show higher heritability for plasma AGT than do females ($h^2$: 0.74 vs. 0.48). This may be due to the known interaction between *AGT* and estrogen levels. Blood pressure also shows higher heritability in male offspring than in female offspring (SBP: 0.28 vs. 0.17, DBP: 0.38 vs. 0.14). In these families, *AGT* genetic variation and the quantitative phenotype of plasma AGT are significantly linked, with a maximum LOD score of 4.4 for AGT SNP rs943580. The LOD score increased when haplotypes were used instead of single SNPs (maximum LOD of 5.5 for a haplotype denoted H4). Linkage to the quantitative traits of either systolic or diastolic blood pressure, however, was not significant in these families, which were not selected for disease status. To analyze the relationship between *AGT* and blood pressure in families selected for a hypertensive phenotype, we genotyped these SNPs in 382 individuals from the phenotypically well-characterized HyperPATH/SCOR hypertensive cohort. In hypertensives, significant associations between *AGT* SNPs and plasma AGT are found, but only under condition of sodium depletion ($p < 0.01$). Six common haplotypes (frequencies > 0.05), identical to those identified in the UGRP cohort, were identified. Hypertensives with haplotype H4 -- containing the known functional promoter allele -6A -- have higher plasma AGT levels ($p < 0.003$) and reduced renal plasma flow ($p < 0.0002$). Haplotype H4 is more common in hypertensive cases than in normotensive controls ($p < 0.054$), and haplotype group H1/H2 is more common in controls than hypertensive cases ($p < 0.030$). Thus, in two independent study populations, the same *AGT* haplotypes are linked or associated with plasma AGT levels (and hypertension in one cohort), and they provide more information than do single SNPs alone. Additionally, the association between *AGT* genetic variation and plasma AGT is stronger under conditions of sodium depletion, helping to explain previous conflicting linkage and association results.

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## 2332/T/Poster Board #881

**Mutations in Smooth Muscle Myosin Heavy Chain (MYH11) in Coronary Artery Disease, Stroke, and Moyamoya Disease: Evidence for a Common Pathogenesis for Arterial Diseases.** *H. Pannu[1], S.E. Scherer[2], L. Gong[1], E. Regalado[1], N. Avidan[1], D. Guo[1], J. Grotta[1], A.J. Marian[3], E. Boerwinkle[4], J. Grotta[5], S. Shete[6], G. Steinberg[7], D.M. Milewicz[1].* 1) Department of Internal Medicine, University of Texas Health Science Center at Houston, Houston, TX 77030; 2) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX 77030; 3) Center for Cardiovascular Genetic Research, Brown Foundation Institute of Molecular Medicine, Texas Heart Institute at St. Luke's Episcopal Hospital, Houston, TX 77030; 4) Human Genetics Center and Institute for Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX 77030; 5) Department of Neurology, University of Texas Health Science Center at Houston, Houston, TX 77030; 6) Department of Epidemiology, University of Texas M.D.Anderson Cancer Center, Houston, TX 77030; 7) Department of Neurosurgery and Stanford Stroke Center, Stanford University School of Medicine, Stanford, CA 94305.

Genome-wide association studies (GWAS) have indicated that common variants on chromosome 9p can predispose individuals to a variety of vascular diseases (coronary artery disease, strokes, and abdominal aortic aneurysms), suggesting a common vascular disease pathogenesis. We have previously demonstrated that mutations in the smooth muscle specific isoform of β-myosin heavy chain (MYH11) cause thoracic aortic aneurysms, but we also noted other premature occlusive vascular diseases in MYH11 mutation carriers. MYH11 resequencing was performed in DNA from 669 patients with early onset coronary artery disease (216), early onset stroke (271), and Moyamoya disease (182), along with 188 controls, to determine if novel, non-synonymous variants were more common in DNA from patients with premature occlusive disease than in controls. We identified rare, novel, and non-synonymous MYH11 variants in all three vascular disease cohorts sequenced, but failed to identify these variants in the control group. Analyzing the effects of the rare MYH11 variants on the structure and biochemical function of MYH11 supports causality of these MYH11 mutations for CAD, stroke and MMD. These analyses, along with GWAS studies of vascular diseases, imply a need for a change in the approaches to identifying genetic predisposition and molecular pathways for vascular diseases, viewing the vascular system as a unified disease entity, and not categorizing diseases exclusively on the basis of the vascular bed affected.

## 2333/T/Poster Board #882

**Relationship of rs4420638 in the *APOE/C1/C4/C2* locus to coronary artery disease.** *SS. Bhatia, RW. Davies, R. McPherson.* Ottawa Heart Institute, Ottawa, Ontario, Canada.

**Background:** Genome-wide association studies have reported a significant effect of rs4420638 in the 19q13.2 *APOE/C1/C4* gene cluster to plasma lipid traits and CAD risk. This has been attributed to linkage disequilibrium (LD) with the *APOE* single nucleotide polymorphisms (SNPs) rs7412 and rs429358 which give rise to 3 apoE variants E2, E3 and E4. However in a recent meta-analysis E4 was associated with CAD risk with an odds ratio of 1.06 whereas E2 showed a protective effect (OR 0.8) (JAMA2007;298:1300-11). In contrast rs4420638 lies 3' of *APOC1* and has a reported OR for CAD of 1.17 (NatGen2008;40:161-9) and is in partial LD with rs429358 (r2=0.70). The minor allele frequency of rs4420638 is 0.18 versus 0.14 for rs429358 and 0.07 for rs7412. We tested the hypothesis that the effect of rs4420638 on CAD risk is in part independent of rs429358 and rs7412. **Methods:** The above SNPs were genotyped using standard sequencing methods in 600 CAD patients and 1600 healthy control subjects enrolled in the Ottawa Heart Study (OHS). Additional SNPs in the region lie within enhancer sites (rs483082, rs3513657) the *APOE* gene (rs769449, rs769450) and non-coding regions (rs445925) and were similarly genotyped. Genotypes for rs4420638 were obtained using the Affymetrix 6.0 array. Plasma concentrations of apoE and apoC1 were determined by ELISA. **Results:** In the OHS population rs429358 and rs4420638 (r2=0.71) and rs429358 and an intronic *APOE* SNP rs769449 (r2=0.81) were in moderate LD. The strongest D' values were amongst the *APOE* SNPs and rs4420638 at 1.0. Other SNPs showed weak LD to the *APOE* SNPs and rs4420638. Haplotype analysis demonstrated a stronger association of rs429358 with CAD as compared to rs4420638; the frequency of individuals bearing risk alleles for both SNPs was 0.17 for cases and 0.11 for controls. Carrier status for both rs4420638 risk and rs429358 reference alleles was 0.036 in cases and 0.042 in controls. Thus the minor allele of rs429358 tends to confer a greater CAD risk than does the minor allele of rs4420638. Plasma apoE but not apoC1 concentrations were associated with rs429358; CC: 13ug/ml; CT: 90ug/ml; TT: 149ug/ml (n=20; p=0.0009). Similar results were obtained for rs4420638. **Conclusions:** The relationship of rs4420638 to CAD risk is explained by LD with rs429358 and is not due to linkage with other common SNPs in the same locus. Both rs429358 and rs4420638 are associated with altered plasma concentrations of apoE.

## 2334/T/Poster Board #883

**Searching for a gene on chromosome 18q influencing systolic blood pressure in Mexican Americans.** *S. Rutherford[1], V.S. Voruganti[2], K. Haack[2], J.W. MacCluer[2], L. Griffiths[3], J. Blangero[2], A.G. Comuzzie[2], S.A. Cole[2].* 1) Dept of Anthropology, Penn State University, University Park, PA; 2) Dept of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 3) Griffith Institute for Health and Medical Research, Griffith University, Gold Coast Australia.

Since the etiology of hypertension begins several years before its clinical manifestation, it is important to identify the factors that are likely to have an early influence on blood pressure determination. We conducted genome wide scans on blood pressure phenotypes in Mexican American families from the San Antonio Family Heart Study (SAFHS) and in Australian Caucasian hypertensive sib pairs. Results from both studies identified a single region on chromosome 18q showing involvement with hypertension (LOD = 3.69; p = 0.00002, in Australian sibpairs) and systolic blood pressure (LOD = 2.09; p = 0.00096, in the SAFHS). Eight other studies also report modest evidence for linkage of blood pressure and/or hypertension within 20Mb of this chromosome 18q region. We hypothesize that this chromosome 18q region contains one or more polymorphic variants that account for hypertension in the Australian Caucasians and for the systolic blood pressure variation that we observe in our Mexican American participants. Advances in high-throughput genotyping, single nucleotide polymorphism (SNP) discovery, and haplotype map (HapMap) construction provide an unprecedented opportunity for comprehensive genetic analysis. We searched the 1-LOD score interval (a 19Mb region) and identified SNPs located in a microRNA (miRNA) locus (hsa-mir-122a) and a number of blood pressure and hypertension candidate genes such as *NEDD4L* and *MC4R*. We genotyped 480 SNPs using an Illumina BeadXpress instrument and conducted association analysis to explore the causative role these variants may play in affecting blood pressure related phenotypes. In an analysis of 24 SNPs, suggestive association (p = 0.02-0.046) between 2 SNPs in *NEDD4L* and systolic blood pressure was observed in our Mexican American participants. NEDD4L regulates the amiloride-sensitive epithelial sodium channels (ENaC) and is a key regulator of sodium reabsorption in the distal nephron. A functional SNP located in exon 1 of *NEDD4L* is known to result in premature truncation of a NEDD4L protein leading to a transcript that encodes a nonfunctional protein. Further analysis is needed to determine the role of the *NEDD4L* variants and the chromosome 18q region in systolic blood pressure regulation.

## 2335/T/Poster Board #884

**Transcriptional regulation of *MMAB* at a locus associated with HDL-C level.** *M.P. Fogarty[1], S. Bellendir[2], K.J. Gaulton[1,2], G.E. Crawford[3], T.S. Furey[3], J.D. Lieb[4], K.L. Mohlke[1].* 1) Department of Genetics, University of North Carolina, Chapel Hill, NC; 2) Curriculum in Genetics and Molecular Biology, University of North Carolina, Chapel Hill, NC; 3) Institute for Genome Sciences and Policy, Duke University, Durham, NC; 4) Department of Biology, University of North Carolina, Chapel Hill, NC.

Genome wide association studies have identified a locus associated with HDL-C levels that spans a 175 kb region and contains ≥ 70 strongly associated common SNPs, many of which are located in non-coding regions suggesting a possible regulatory effect on transcription. Using human hepatocytes, we recently observed allelic expression imbalance in only one of four genes at the locus, *MMAB* ($P$=1.4 × $10^{-13}$). We also demonstrated that SNPs associated with decreased HDL-C level were associated with increased *MMAB* mRNA and protein level ($P_{mRNA}$=.0081; $P_{protein}$=.002), suggesting *MMAB* as the most likely target of an HDL-C-associated SNP at this locus. We aim to identify the underlying functional SNP(s) affecting *MMAB* and ultimately to determine how variation in *MMAB* gene expression may affect HDL-C level. Given the evidence of *cis*-acting variation in *MMAB* expression, we hypothesize that the functional variant(s) is likely to have a modest effect on synthesis or stability of the mRNA transcript. For evaluation of transcriptional activity affecting mRNA synthesis, we first prioritized the HDL-C-associated SNPs using datasets that characterize potential regulatory elements including evidence of open chromatin in human hepatocyte-derived cell line HepG2, histone modifications, predicted transcription factor binding and sequence conservation. We identified four highly prioritized SNPs that were also predicted to alter transcription factor binding in an allele-specific manner. Regions of 200 bp surrounding each SNP were cloned into luciferase-reporter vectors and allele-specific transcriptional activity was assessed. Initial results in the HepG2 cell line demonstrated increased transcriptional activity for both alleles of rs1558804 ($P$= 1.0 x $10^{-4}$, ANOVA) compared to a control vector. Additionally, a significant differential allele-specific change in transcriptional activity was observed ($P$ = 8.2 x $10^{-5}$, t-test). These results demonstrate that rs1558804 has enhancer activity and that alleles of rs1558804 may differentially modulate transcription; protein-DNA interactions are currently being assessed. Additional studies will also evaluate HDL-C-associated SNPs in the 3'UTR of *MMAB* that may act to affect mRNA stability. Identification of a functional regulatory SNP would provide insight into the basis of *cis*-acting variation at this locus and may facilitate understanding of how *MMAB* may regulate HDL-C levels.

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## 2336/T/Poster Board #885

**Restrictive cardiomyopathy due to multiple mutations in sarcomeric genes usually associated with a hypertrophic phenotype.** *C. Brown[1,2], R. Sakhuja[3], N. Schiller[3], R. Nussbaum[1,2], J.E. Rame[1,3,4].* 1) Program in Cardiovascular Genetics, UCSF, San Francisco, CA; 2) Division of Medical Genetics, UCSF, San Francisco, CA; 3) Division of Cardiology, UCSF, San Francisco, CA; 4) Heart Failure Evaluation and Treatment Center, UCSF, San Francisco, CA.

Mutations in genes that encode components of the sarcomere are well established as the primary cause of hypertrophic cardiomyopathy. It has increasingly been recognized that mutations in sarcomeric genes may present with extensive variable expressivity, causing a range of cardiomyopathy phenotypes. One phenotype recently recognized as a primary sarcomeric disease is restrictive cardiomyopathy, with as many as half of such cases being caused by mutations in sarcomeric genes. We report two cases of restrictive cardiomyopathy caused by multiple mutations in sarcomeric genes.

Case 1 presented with NYHA Class III/IV heart failure at 22 years of age. She was diagnosed with restrictive cardiomyopathy and advanced heart failure requiring heart transplantation. Sequencing of 8 sarcomeric genes revealed homozygous Glu143Lys mutations in *MYL3*, and a novel Gly57Glu mutation in *MYL2*. The patient's mother is a double heterozygote for these two mutations and had a normal echocardiogram and electrocardiogram at 45 years of age. This is the first reported case of three sarcomeric mutations in a primary cardiomyopathy and the first *MYL2* mutation associated with restrictive cardiomyopathy. Case 2 presented at 35 years of age with volume overload while hospitalized for oophorectomy. She was later diagnosed with restrictive cardiomyopathy. She is currently being evaluated for heart transplant. Sarcomeric gene sequencing identified homozygous Asn279His mutations in *TPM1*. The patient's parents are consanguineous and both have echocardiographic evidence of mild cardiomyopathy, suggesting an incomplete dominant pattern of inheritance. While *TPM1* has been reported in cases of hypertrophic and dilated cardiomyopathy, mutations in this gene have not previously been associated with a restrictive phenotype.

The identification of mutations in sarcomeric genes not previously implicated in restrictive cardiomyopathy further supports the notion that sarcomeric mutations can cause different cardiomyopathy phenotypes and challenges the distinction of hypertrophic and restrictive phenotypes as distinct cardiomyopathies. These cases also suggest that restrictive cardiomyopathy may be more severe in patients with multiple mutations as compared to those with only one mutation, as has already been noted in families with hypertrophic cardiomyopathy.

## 2337/T/Poster Board #886

**Concealed Brugada Syndrome in a Long QT Syndrome family with the L1785Q mutation in SCN5A.** *P.L. Hedley[1], M. Christiansen[1], P.E.B Thomsen[2], M. Grunnet[3], T. Jespersen[3], J.K Kanters[2,3].* 1) Statens Serum Institut, Copenhagen, Copenhagen, Denmark; 2) Gentofte Hospital, Gentofte, Denmark; 3) University of Copenhagen, Copenhagen, Denmark.

Mutations in SCN5A, encoding the cardiac sodium channel Nav1.5, can result in several cardiac disorders including long QT syndrome (LQTS) type 3 (LQT3) and Brugada syndrome (BrS). The pivotal criterion for LQTS diagnosis is a prolonged QT interval, as measured by electrocardiogram (ECG). A prerequisite for a BrS diagnosis is ST segment elevation in the right precordial leads of the ECG. Both LQTS and BrS can cause sudden death. However, BrS patients are more often treated with an intracardiac defibrillator (ICD). We describe the genetic cause of disease in a patient with apparent LQTS in whom flecainide administration uncovered a type 1 ST segment elevation pattern. We subsequently characterize the mutant functionally. In a Danish LQTS family, where the proband had presented with an aborted cardiac arrest and a family history of sudden death, a mutation in SCN5A (c.5354T>A; p.L1785Q) was found. The mutation affects the c-terminal region of the protein; one other BrS-involving-complex-phenotype mutation has been identified in this region (p.E1784K). Additionally, two BrS-involving-complex-phenotype mutations (p.1500delK and p.1505_1507delKPQ) have been identified. Electrophysiological analysis was performed using HEK293 cells transiently transfected with Nav1.5-WT or Nav1.5-L1785Q. The mutant revealed a reduced peak current, a negative shift in inactivation properties and a positive shift in activation properties, compatible with BrS. Furthermore, blocking the sodium channel with TTX revealed a drastic increase in the sustained (INa,late), explaining the LQTS phenotype. The ECGs of the three gene carriers in this family indicate a clear QT prolongation with no ST segment elevation; however, flecainide administration revealed a type 1 ST segment elevation. Electrophysiological experiments of the mutant revealed functional characteristics explaining the two different phenotypes. Based on this study we hypothesize that a number of LQT3 patients may be affected by concealed BrS, which may indicate the use of an ICD. As there is no direct link between the location of SCN5A mutations and the clinical phenotype, the presence of a concealed BrS phenotype must always be suspected in SCN5A mutation carriers. However, the clinical prognosis of SCN5A mutation-carriers with combined LQTS and BrS phenotypes needs further investigation.

## 2338/T/Poster Board #887

**Control of VEGF Activity by CRELD1 as a Mechanism in the Cause of Cardiac Atrioventricular Septal Defects.** *C.L. Maslen, J. Redig, D. Babcock, B. Reshey, G.T. Fouad.* Cardiovascular Med, Oregon Hlth & Sci Univ, Portland, OR.

Vascular endothelial growth factor (VEGF) regulates endothelial cell growth and migration and plays a key role in heart development in the formation of endocardial cushions, the precursors to the atrioventricular (AV) valves and septa. Failure of AV cushion development results in the congenital heart defect known as an atrioventricular septal defect (AVSD). Increased expression of VEGF has been shown to interfere with AV endocardial cushion morphogenesis. A functional *VEGF* polymorphism -634G/C is associated with altered VEGF expression suggesting that this variant may modify susceptibility for AVSD. Using a case-control study we demonstrated that individuals with AVSD are more likely to carry the high expressing -634C allele than controls ($p$=0.0012). Missense mutations of *CRELD1* are also associated with AVSD, but are incompletely penetrant. Stratification of our AVSD cohort by *CRELD1* mutation revealed a specific association between *CRELD1* mutations and the *VEGF* -634C allele suggesting a causative interaction. From this we hypothesized that increased VEGF expression in combination with CRELD1-deficiency impairs atrioventricular endocardial cushion formation leading to AVSD. To test this hypothesis AV canals from Creld1$^{+/+}$, Creld1$^{+/-}$ and Creld1$^{-/-}$ mice were explanted, cultured and subjected to increased VEGF (200pg/ml) to mimic the over-expression associated with the -634C allele. Migration of stellate-shaped cells from the canals were tallied 48, 72 and 96 hours after explantation as a measure of epithelial to mesenchymal transformation, a critical step in endocardial cushion formation. We found that under endogenous levels of VEGF the cell counts from Creld1$^{+/-}$, Creld1$^{-/-}$ and Creld1$^{+/+}$ explants were indistinguishable at all time points (n>6); at 96 hours 256±119, 245±193 and 217±122 cells were observed respectively. However, when VEGF was added the Creld1$^{+/-}$ and Creld1$^{-/-}$ explants exhibited significantly higher numbers of cells than the Creld1$^{+/+}$ explants at both 72 hours and 96 hours ($p$<0.05, n>5) with 377± 76, 356± 62 and 221± 113 cells, respectively. This data demonstrates that Creld1-deficient epithelial cells from developing endocardial cushions have an enhanced response to small increases in VEGF. The hyper-reactivity of Creld1-deficient cells to VEGF indicates that CRELD1 is responsible for controlling cell response to VEGF. This interaction between CRELD1 and VEGF may explain the developmental origins of a subset of sporadic AVSD cases.

## 2339/T/Poster Board #888

**Endothelial nitric oxide synthase (eNOS) VNTR polymorphism and premature infants with maternal pregnancy induced hypertension (PIH).** *S. Ursin[1], J.G. Aryama[1], K. Yanamandra[1], H. Chen[1], A. Pramanik[1], J.A. Bocchini Jr.[1], R. Dhanireddy[2].* 1) Pediatrics, Gen Sec, LSUHSC- Shreveport, Shreveport, LA; 2) Dept Pediatrics, UT Health Science Center, Memphis, TN.

Endothelial nitric oxide (eNO) serves as a vasodilator, relaxes smooth muscle, prevents platelet aggregation, and facilitates improved blood flow, leading to vascular homeostasis. Reduced nitric oxide levels result in vasoconstriction and weak tone leading to decreased blood flow and hypoxia. Mutant eNO synthetase (eNOS) genotypes result in reduced nitric oxide levels by decreasing the enzyme activity. Pregnancy induced hypertension (PIH) has a multifactorial etiology. Mutant ENOS genotypes in the mother have been evaluated as risk factors for preeclampsia. However, eNOS genotypes were not studied in the infants with maternal PIH except from our laboratory. The aim of the present investigation was to seek how the fetal genotypes influence maternal circulation during pregnancy. In the present investigation we have studied the association of eNOS variable number of tandem repeats (VNTR) of 27bp in intron 4 region of the gene. We have collected peripheral blood specimens from a total of 173 premature infants consecutively from our NICU facility and genotyped by PCR. Our earlier findings in the study of eNOS genotypes in the promoter region have shown an elevation of -786C mutant alleles resulting in lower nitric oxide levels in infants with maternal PIH as compared to controls. However, in the present study the frequency of rear allele *a* consisting of 4 repeats was not elevated in patients as compared to controls (0.09 vs. 0.14, odds ratio 0.6) contrary to the expectations. Thus we speculate that the mutant genotype in promoter region of eNOS gene has a stronger role in premature infants with maternal PIH compared to the intron 4 VNTR genotype. Clinical data of the infants from NICU with ethnic stratification and distribution of alleles and genotypes and their significance will be presented.

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## 2340/T/Poster Board #889

**Distinct hypertrophic and survival pathways for IGF-1 and ANG II.** *A. Zhao, R. Duan, Z. Alvin, G.E. Haddad.* Physiology & Biophysics, Howard University, Washington, DC.

Cardiac hypertrophy is a compensatory mechanism for increased workload on the heart, which progresses into failure if not alleviated. Signaling pathways that regulate apoptosis may play a pivotal role in heart failure. We hypothesize that the signaling pathways related to the hypertrophic effects of IGF-I are distinct from those for its survival benefit. We have examined the role of PI 3-kinase and Akt on primary cardiocytes in in vitro models of cardiocyte apoptosis. Adenoviral vectors were used to express wild-type and mutant-forms of specific signaling molecules in cardiocytes. Performing qRT-PCR, we have found that treatment of cultured cardiomyocytes with IGF-1 had a biphasic response on Akt gene expression, with an increase at 30 min, 24h, 48h but a decrease at 2h. In addition, the gene expression profile with wild-type Akt and Ad-BD110 (constitutively active Akt) transfections translated into higher total Akt content as well as higher Akt activation (phosphorylated Akt to total Akt ratio), as measured by western blot. Incubation and transfection of cultured neonatal cardiomyocytes with IGF-1, wild-type Akt (Ad-Akt) or dominant positive PI 3-kinase (Ad-BD110) induced cellular hypertrophy within 24 hours using fluoroimmunoassay based BrdU incorporation (DELFIA, Perkin Elmer). Ad-BD110 transfection had lesser cellular hypertrophic effect than IGF-1. Wild-type Akt did not produce significant cellular hypertrophy on its own; however, when stimulated with IGF-1 it did. As a positive control, incubation with angiotensin II (ANG II) induced cellular hypertrophy, which seems to be independent from the PI-3K/Akt signaling as well. Addition of IGF-1 or Ad-BD110 in presence of ANG II produced a greater hypertrophic effect than ANG II alone. In addition, ANG II treatment showed a significant increase in ERK1/2 gene expression but a much smaller effect on that of Akt. On the other hand, ANG II and IGF-1 induced ERK1/2 activation, in an additive manner. Our results suggest that the IGF-1/PI3 K/Akt pathway plays an important role in cardiomyocyte survival. Un-stimulated Akt does not convey cardiocyte survival benefits. However, IGF-1 induces Akt-dependent and Akt-independent (ERK1/2) downstream effectors for enhancing cardiocyte survival. ANG II survival benefits are due to its hypertrophic effects and not to anti-apoptotic ones. However, IGF-1 survival benefits are due to the activation of both hypertrophic and anti-apoptotic pathways.

## 2341/T/Poster Board #890

**Genetic Counselling for Inherited Cardiovascular Conditions: A Comparison between Wales (UK), North American Practice and Australia.** *M.C. Nicol[1], D. Kumar[1], A. Clarke[1], M. Care[2], C. Morel[2], I. Macciocca[3].* 1) Institute of Medical Genetics, University Hospital of Wales, CF14 4XW, Wales, United Kingdom; 2) Department of Medicine, The Fred Litwin Family Centre in Genetic Medicine, University Health Network & Mount Sinai Hospital Toronto Canada; 3) Victoria Clinical Genetics Services, Murdoch Children's Research Hospital Melbourne Australia.

Abstract The National Service Framework (NSF) for Coronary Heart Disease in England sets out quality requirements, criteria and systems needed to provide diagnosis, treatment, information and support to families affected by sudden cardiac death (SCD). The chapter 8 of NSF (Standard 5 for Wales NSF) includes recommendations for inherited cardiovascular conditions (ICCs) presenting with SCD. In Wales and other parts of the UK there is evidence of a fragmented approach in meeting the NSF recommendations. Continuing progress in cardiac genetics research and increased awareness among clinicians of the possibilities of genetic testing are leading to rapidly increasing rates of clinical referral and requests for molecular diagnostic testing. Such demands prevent the stabilisation of care pathways, especially in the context of inadequate funding and underdeveloped service infrastructure. In addition to health professionals, Police and Coroners, are often the first people dealing with the bereaved family coping with SCD. This often causes delay in referring families to cardiology and genetic services. A comparison of services in Wales, Melbourne and Toronto has identified aspects of good clinical practice that can guide the development of cardiovascular genetic services. Several of these must be implemented through the coroners' system: * Establishing a cardiac genetics service led by cardiologists and supported by clinical geneticists and genetic counsellors; * Referral of retained organs for specialist cardiac pathology from a coroner's autopsy when no diagnosis has emerged and sudden cardiac death is a possibility; * Information given by the coroner to the bereaved family about the possibility of referral to cardiac genetics; * Information shared by the coroner to the clinical geneticist for autopsy review which can guide differential diagnosis; * Storage of frozen blood by coroner's office to facilitate genetic testing on the proband once the autopsy has been reviewed; * Information given to the family physician of the bereaved, suggesting referral for cardiac genetic assessment; This should enable direct referral of SCD families into a multi-disciplinary cardiovascular genetic network intended to support families and answer their questions, avoid delay in screening 'at-risk' relatives and to coordinate the management of family members in the most effective manner.

## 2342/T/Poster Board #891

**Oligogenic inheritance and clinical variability in long QT syndrome.** *J. Dean[1], D. Walker[1], C. Clark[1], K. Kelly[1], C. Brown[1], W. Lam[2], V. Murday[3], D. Goudie[4], A. Choy[4].* 1) Department of Medical Genetics, Argyll House, Aberdeen, United Kingdom; 2) South East Scotland Regional Genetics Service, Western General Hospital, Edinburgh; 3) West of Scotland Regional Genetics Service, Yorkhill Hospital, Glasgow; 4) University of Dundee, Ninewells Hospital, Dundee.

In Scotland, patients with a personal or family history of familial arrhythmia (e.g. Long QT syndrome (LQTS), Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)) access clinical risk assessment, genetic counselling and genetic testing through the Familial Arrhythmia Network Scotland, a managed clinical network of cardiologists, pathologists and clinical and laboratory geneticists set up in 2008. In probands with suspected LQTS, KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2 are screened in Aberdeen. For Brugada syndrome, SCN5A is screened, and in ARVC, PKP2. 123 probands with suspected LQTS, 12 with suspected Brugada syndrome and 24 with ARVC were assessed. Mutations were detected in 43% of LQTS, 16% of Brugada, and 16% of ARVC cases. In LQTS, 24% had KCNQ1, 17% KCNH2 and 2% SCN5A mutations. No mutations in KCNE1 or KCNE2 were detected. Ten LQTS probands had 2 mutations - six had Jervell Lange Neilsen syndrome clinically. The other 4, (3 with 2 mutations in KCNQ1 and 1 with a KCNQ1 and a KCNH2 mutation) had normal hearing. Probands with two mutations had longer QTc's on resting ECG and earlier age of onset than probands with one mutation. Initially, our genetic screening program prioritised the gene to be tested based on clinical features (T wave morphology, precipitants of arrhythmia). The high frequency of multiple mutation detection in apparently autosomal dominant LQTS (4/54, 7%) suggests that parental ECGs do not reliably disclose the parental origin of apparently dominant LQTS, and that all five LQTS genes should be screened in all probands, regardless of clinical presentation. This unexpected oligogenic aetiology of more severely affected probands from apparently dominant LQTS families who have more mildly affected heterozygous relatives may provide one explanation for the well known clinical variability of LQTS.

## 2343/T/Poster Board #892

**Digenic mutations in *FBN1* and *TGFBR2* contribute to Marfan syndrome.** *Y. Fan[1], M. Steinraths[2], B.A. Westerfield[1], J.J. Varela[1], L.S. Pena[1], M. Vatta[1], R.A. Friedman[1].* 1) John Welsh Cardiovascular Diagnostic Laboratory, Section of Cardiology, Department of Pediatrics, Texas Children's Hospital, Baylor College of Medicine, Houston, TX; 2) Vancouver Island Medical Genetics, Victoria General Hospital, 1 Hospital Way, Victoria BC, Canada.

Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder characterized by variable manifestations mainly in three major organ systems including skeletal, ocular, and cardiovascular involvement. Multiple mutations in the genes encoding fibrillin-1 (FBN1) at 15q21.1 and transforming growth factor beta receptor type II (TGFBR2) at 3p22 were found to cause MFS type 1 (MFS1) in 1991 and type 2 (MFS2) in 2004 respectively. FBN1 is an extracellular matrix glycoprotein that is the main component of the calcium-binding microfibrils while TGFBR2 is a transmembrane protein that forms a heterodimeric complex with TGFBR1 and binds TGF-beta. However, digenic MFS has not previously been reported. We report a European Caucasian family in which the 15-year-old female proband presented with tall stature, arachnodactyly, scoliosis, dilatation of the aortic root, mitral valve prolapse and superior lens subluxations. She is developmentally normal with a broad, single uvula and her interpupillary distance measures at the 75th percentile for her age. A previously reported heterozygous missense mutation 4096G>A (E1366K) in exon 33 of the *FBN1* gene and a new heterozygous missense variant of unknown significance 310C>A (P104T) in exon 3 of the *TGFBR2* gene were identified in the proband through direct sequencing. Parental testing demonstrated both sequence variations to be de novo in the proband. Bioinformatics analysis of the TGFBR2 sequence variation using BlastP, ClustalW 1.8, PANTHER and PolyPhen revealed that the proline at amino acid position 104 is highly conserved across species from human to *Xenopus laevis* and the variant P104T is predicted to be pathogenic. Most importantly, this variant was not detected in 188 normal ethnically-matched control chromosomes. Based on the family study, in silico analysis and the control study, we conclude that the *TGFBR2* variant P104T identified in this individual is a disease causing mutation. Identification of digenic mutations in MFS provides direct evidence of the functional association between FBN1 and TGF-beta signaling in extracellular matrix in humans, supporting a synergistic effect of FBN1 and TGFBR2 in the pathogenesis of MFS, and indicating that the presence of mutations in one gene should not exclude screening for further genetic variation. This finding suggests that innovative anti-TGF-beta therapeutic strategies such as losartan might play an important role in the treatment of MFS and related disorders.

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## 2344/T/Poster Board #893

**Next-Generation-Diagnostics of heterogeneous monogenic disorders using High-Throughput-Sequencing: the promising example of Hypertrophic Cardiomyopathy.** *S. Fokstuen[1], C. Iseli[2,3], D. Robyr[4], A. Munoz[4], S.E. Antonarakis[1,4], J.L. Blouin[1].* 1) Genetic Medicine, University Hospitals Geneva, Geneva, Switzerland; 2) Ludwig Institute for Cancer Research, Lausanne, Switzerland; 3) Swiss Institute of Bioinformatics, Lausanne, Switzerland; 4) Genetic Medicine and Development, University of Geneva School of Medicine, Geneva, Switzerland.

With a prevalence of 1/500, Hypertrophic Cardiomyopathy (HCM) represents the most common monogenic cardiac disorder (autosomal dominant inheritance), and is also considered the primary cause of sudden cardiac death in young adults. HCM shows a remarkable genetic and allelic heterogeneity (> 550 mutations in at least 22 genes identified so far). Molecular testing for HCM has a growing impact on the medical management of patients/families. To overcome the extensive genetic heterogeneity we have developed a 30 Kbp microarray to resequence all exons (n=160), splice-sites and 5'-UTR of 12 HCM genes (HCM-RA, Fokstuen et al. 2008). This method is now used as a diagnostic tool in our clinical practice. It is very rapid and cost-effective but does not yet detect small indels (~14% of all known HCM mutations). Moreover, HCM-RA lacks flexibility as the addition of new genes requires a new design. In order to solve these shortcomings, we now analyze the 12 HCM array genes (targeted by multi-exonic amplicons) by short single and paired-end reads of High-Throughput-Sequencing (HTS) and a newly developed downstream data analysis pipeline. Mutations/variants are confirmed by classical sequencing. We initially reanalyzed the DNA of 19 patients previously hybridized by the HCM-RA (11 without identified mutations, 8 positive-controls as a composite-pool) in one lane of Illumina Genome-Analyzer flow cell. All 8 known positive control mutations and SNPs previously identified by the HCM-RA were also found by HTS. Furthermore we identified novel variants: two indels and a nonsense mutation in MYBPC3. In order to further explore the practical feasibility of HTS for diagnostic mutation analysis, we tried different options for optimal cost-effectiveness in clinical practice : resequencing of a single patient or pooling of 2-5 patients in one lane. Additional known mutations, like p.Tyr842X in MYBPC3, were found as well as further potentially pathogenic variants, which are still in evaluation. Although improvements are still needed in target enrichment, reduction of false positive variants and data analysis, HTS holds considerable promises for mutation/variant analysis of highly heterogeneous monogenic disorders in clinical practice.

## 2345/T/Poster Board #894

**Akt-dependent regulation of potassium channels by IGF-1 in cardiomyocytes.** *G.E. HADDAD, Z. Alvin, R. Duan, A. Zhao.* Physiology & Biophysics, Howard University, Washington, DC.

Pathological dysregulation of the repolarization of the cardiac action potential is associated with ventricular dysfunction and cardiac arrhythmias. The delayed outward rectifier current (IK), the major ion channel responsible for the ventricular repolarization, can be modulated by inotropic and trophic hormones, such as IGF-1. Due to its known anti-apoptotic effects, IGF-1 can play a critical survival role during heart failure. The aim of this work was to assess the regulation of IK by the cell-survival pathway, IGF-1/PI3 K/Akt, in freshly isolated neonatal rat ventricular myocytes, using patch-clamp technique in whole-cell configuration. Adenoviral vectors were used to express wild-type and mutant-forms of specific signaling molecules in cardiocytes. Cells were stimulated from a holding potential of -40mV, by 500msec, 10-mV step voltages to +40mV. IGF-1 reduced the functional density of IK from 4.1 ± 0.5 pA/pF to 2.6 ± 0.3 pA/pF. IGF-1 effect was eliminated by the PI3 Kinase inhibitor, LY294002 (3μM). Adenoviral transfection of cardiocytes with a constitutively activated PI3 Kinase, Ad.BD110, mimicked the IGF-1 effect on IK (2.5 ± 0.4 pA/pF), which was abrogated by LY 294002. Similar to Ad.BD110, transfection of the cardiac myocytes with wild-type Akt reduced IK (2.4 ± 0.3 pA/pF), which was completely reversed by LY 294002. Inhibition of Akt by dominant-negative Akt (Ad.Akt179M) abrogated IGF-1 effect on IK. In addition, Ad.Akt179M co-transfection with Ad.BD110 showed a yin-yang effect on IK. We conclude that IK is negatively regulated by IGF-1 through downstream activation of PI3 Kinase and Akt. Such a mechanism could play an anti-arrhythmic role during cardiac events that are associated with the activation of the PI3 K/Akt signaling, such as cardiac hypertrophy.

## 2346/T/Poster Board #895

**The ARVD/C variome.** *J.D.H. Jongbloed[1], P.A. van der Zwaag[1], L.G. Boven[1], A. Boesjes[1], A.C.P. Wiesfeld[2], I.C. van Gelder[2], M.P. van den Berg[2], R.M.W. Hofstra[1], E. Buskens[3], J.P. van Tintelen[1], R.J. Sinke[1].* 1) Department of Genetics, University Medical Center Groningen, Groningen, Netherlands; 2) Department of Cardiology, University Medical Center Groningen, Groningen, Netherlands; 3) Department of Epidemiology, University Medical Center Groningen, Groningen, Netherlands.

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is a disorder characterized by segmental fibro-fatty replacement of the right ventricular myocardium leading to electrical instability predisposing to ventricular arrhythmias and sudden cardiac death. In 30-50% of cases the disease is familial with an autosomal dominant mode of inheritance with reduced penetrance and variable expression. The discovery of high yields of mutations in the plakophilin-2 (PKP2) gene encoding a desmosomal protein, and mutations, although with lower yields, in other desmosomal genes (DSG2, DSC2, DSP, DES and JUP), showed that ARVD/C can be considered a desmosomal disease. In addition, a mutation in the transmembrane protein 43 encoding gene (TMEM43), not (yet) related to the desmosome, was identified in Canadian families with ARVD/C. Analyzing these genes with current DNA diagnostic techniques is laborious, expensive and time consuming. Moreover, several studies revealed that the presence of compound heterozygous and/or bigenic mutations often occurs in ARVD/C patients, showing that the analyses of all genes related to this disease, irrespective of the fact that a mutation was already identified in one of the genes, is very important. For these reasons, methods to cost-effectively screen all candidate genes at once are required. Recently introduced deep sequencing techniques provide an excellent opportunity for such approach. Therefore, we initiated deep sequencing protocols to identify mutations/variants in the above mentioned ARVD/C genes within a single experiment. To validate the applicability of this technique in a diagnostic setting, the genes were also analyzed using currently used techniques (CSCE, DGGE, direct sequencing). First results underscore the results of previous studies that report the occurrence of multiple mutations/variations within patients/families. These results will be integrated in our recently launched, freely accessible online database, providing updated information on mutations in ARVD/C-associated genes (www.arvcdatabase.info).

## 2347/T/Poster Board #896

**Frequency of Deletions and Duplications in Sarcomere Genes in Hypertrophic Cardiomyopathy.** *T. Lee[1], R. Busin[2], B. Kattman[2], J. Wynn[1], J. Compton[2], S. Aradhya[2], W. Chung[1].* 1) Department of Pediatrics, Columbia University Medical Center, New York, NY; 2) GeneDx, Gaithersburg, MD.

Hypertrophic cardiomyopathy (HCM) is the most common monogenetic cardiovascular disease with a prevalence of 1 in 500 individuals and is the most common cause of sudden cardiac death in adolescents and young adults. The disease is genetically heterogeneous with mutations identified in over 17 genes. Currently available clinical genetic testing panels include sequencing of exons for MYBPC3, MYL2, MYL3, MYH6, MYH7, ACTC, ACTN2, TPM1, TNNC1, TNNT2, TNNI3, CAV3, PRKAG2, LAMP2, GLA, TTR, MTTG, MTTI, and MTTK which identifies disease causing mutations in ~ 60% of patients. It is unclear what accounts for the disease in the remaining 40% of patients, but we hypothesized that large deletions or duplications of these genes could account for a proportion of the HCM cases in which no mutation was identified by sequence analysis. Using a custom designed oligonucleotide exon array containing 810 oligonucleotides for 15 genes for hypertrophic cardiomyopathy we tested 56 sequence negative HCM patients for deletions or duplications. The array was designed containing two or more probes in each exon and the flanking 100 bases and three probes in the intronic regions. Hybridization was performed with sex-matched and differentially labeled reference DNA. Data were analyzed by DNA Analytics software. We identified an intragenic deletion of exons 28-40 in MYH7 that was confirmed by dye reversal and quantitative PCR. The patient is a 14 year old male diagnosed with HCM at age 10 when he presented with chest pain. His echocardiogram demonstrated asymmetric hypertrophy of the mid to apical portion of the ventricular septum. On cardiac MRI, the left ventricular mass was quantitatively mildly increased (Z score +2.2) with normal biventricular systolic function. His family history is significant for a mother and maternal grandfather with documented cardiomyopathy and a maternal great aunt and maternal great uncle who died at the ages of 60 and 38 of unspecified cardiac disease. These data suggest that genomic alterations in genes commonly associated with HCM are an infrequent but important genetic cause of HCM in ~2% of patients without identified mutations by sequence analysis. Therefore, deletion/duplication testing should be considered for HCM cases with normal sequencing results.

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## 2348/T/Poster Board #897

**Sequencing Known Genes for Thoracic Aortic Disease in the GENTAC cohort (Genetically Triggered Thoracic Aortic Aneurysms).** *D. Milewicz[1], H. Pannu[1], R. Pyeritz[2], C. Basson[3], H. Dietz[4], C. Maslen[5], K. Eagle[6], B. Kroner[7], the GENTAC consortium.* 1) Dept Internal Med, Univ Texas Med Sch, Houston, TX; 2) Dept Internal Med, Univ of Penn, Phildelphia, PA; 3) Dept Internal Med, Weill Med Col of Cornell Univ, New York, NY; 4) Dept Ped, Johns Hopkins Univ, Baltimore, MD; 5) Dept Mol Med Genet, Oregon HSU, Portland, OR; 6) Dept Internal Med, Univ of Mich. Ann Arbor, MI; 7) RTI International.

Thoracic aortic aneurysms leading to aortic dissections (TAAD) have a significant contribution from single gene mutations with 6 genes identified to date. The NHLBI-supported GenTAC (Genetically-triggered Thoracic Aortic Aneurysms and Related Conditions) registry collects clinical data and DNA/plasma samples on patients at risk for genetically-triggered thoracic aortic disease due to known syndromes (Marfan syndrome (MFS; gene FBN1), Loeys-Dietz syndrome (LDS; TGFBR1 and TGFBR2), vascular Ehlers Danlos syndrome (vEDS, COL3A1), Turner syndrome (TS)) and patients with clinical evidence of genetically triggered aortic disease such as familial TAAD (FTAAD), bicuspid aortic valve and TAAD (BAV/TAAD), and onset of TAAD < 50 years of age. We sought to characterize the gene mutations in the GenTAC cohort. Of the initial 710 GenTAC patients enrolled through December 2008, 103 patients entered with known mutations (56 FBN1 mutations in MFS patients, 4 TGFBR1 and 4 TGFBR2 mutations in LDS, and 4 COL3A1 mutations in vEDS) and 35 patients had TS. 47 patients did not have TAAD. We sequenced the remaining 560 GenTAC patients (35%; MFS, 33%; BAV/TAAD, 16%; FTAAD, 16%; other) for variants in the 6 known genes for TAAD; FBN1 data are pending. Preliminary analysis is presented on variants previously shown to cause disease (19) and possible mutations based on predicted protein truncation or protein structural changes (8). Amongst potential mutations that are in the process of confirmation, there were 1 frameshift and 6 missense variants identified in TGFBR1, and 3 missense changes in TGFBR2. Only one of these patients was diagnosed with LDS at the time of GenTAC enrollment and the others are being reassessed for features of LDS. COL3A1 mutations were identified in 3 vEDS (one patient had 2 mutations) and 4 patients with aortic disease that did not carry an initial diagnosis of vEDS (although diagnoses are being reassessed). Potential mutations also included ACTA2 missense variants in 3 patients with FTAAD and MYH11 variants in 4 patients with FTAAD. The rarity of mutations in the 5 known TAAD genes in the GenTAC patients who do not have MFS indicates further research needs to be done to identify genes predisposing to TAAD. Resequencing services Univ of Washington, Department of Genome Sciences, N01-HV-48194 from NHLBI.

## 2349/T/Poster Board #898

**Nonsense-mediated decay pathway activity modifies disease severity and penetrance among *BMPR2* mutation carriers with Pulmonary Arterial Hypertension.** *J.A. Phillips[1,2,3,4], E.D. Austin[1], J.D. Cogan[1], R. Hamid[1], K.C. Stanton[1], C.A. Phillips[1], L.A. Wheeler[2], I.M. Robbins[2], J.H. Newman[2], J.E. Loyd[2].* 1) Department of Pediatrics, Vanderbilt Univ Sch Med, Nashville, TN; 2) Department of Medicine, Vanderbilt Univ Sch Med, Nashville, TN; 3) Department of Biochemistry, Vanderbilt Univ Sch Med, Nashville, TN; 4) Department of Pathology, Vanderbilt Univ Sch Med, Nashville, TN.

**Background** Autosomal dominant inheritance of germline mutations in the bone morphogenetic protein receptor type 2 (*BMPR2*) gene are a major risk factor for heritable pulmonary arterial hypertension (HPAH). Found in ~80% of HPAH kindreds, *BMPR2* mutations have variable expressivity and reduced penetrance. We hypothesized that: 1) heterogeneity in *BMPR2* mutations contributes to differences in severity and penetrance, 2) *BMPR2* mutation transcripts that are destroyed by nonsense-mediated decay (NMD) (NMDpos) cause haploinsufficiency (HI), and 3) HI mutations are less severe and less penetrant than mutations whose transcripts are not destroyed by NMD (NMDneg) because of the latter's potential to cause dominant negative (DN) effects. **Methods** Testing for *BMPR2* mutations was performed in 169 patients with PAH (125 with HPAH and 44 with sporadic PAH). Of the 106 patients with a detectable *BMPR2* mutation, lymphoblastoid cells were available in 96 to functionally assess their susceptibility to the NMD pathway of RNA surveillance. Phenotypic characteristics were compared between carriers whose *BMPR2* mutations were classified as NMDpos versus NMDneg. **Results** The exonic locations of *BMPR2* mutation carriers did not correlate with differences in age at diagnosis, death, or survival. However, patients with NMDneg mutations had statistically significant younger ages at diagnosis (30.6 vs. 39.9 yrs, p=0.004) and death (32.4 vs. 42.0, p=0.014), as well as shorter survival from diagnosis to death or lung transplantation (5.02 vs. 9.99 yrs, p=0.044) than did the patients who had NMDpos (HI) mutations. Furthermore, 68.3% of NMDneg but only 41.3% of NMDpos mutations were penetrant before age 36 years, p=0.010. As a result, patients with an NMDpos mutation had a 3.0 higher odds of diagnosis after age 35 years than did those with an NMDneg mutation (p=0.010). **Conclusions** First, *BMPR2* mutation carriers with NMDneg mutations have more severe PAH phenotypes than those with NMDpos mutations. This suggests that potentially DN mutations can be more detrimental than mutations that can only cause HI. Second, three fold more NMDneg mutation carriers were diagnosed <35 yrs of age than HI mutation carriers. This suggests that potentially DN mutations are more penetrant that HI mutations. Third, these findings suggest that treatment and prevention strategies for PAH that are directed specifically at *BMPR2* pathway defects may need to vary according to the NMD status of the mutation.

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## 2350/T/Poster Board #899

**Prenatal presentation of Jervell and Lange-Nielsen (LQT1) identifies an extended family of syncope and sudden death segregating with four gene mutations in three long QT syndromes: LQT1, LQT2, and LQT3.** *C.S. Rao[1], D.J. Levy[1], U. Birgersdotter-Green[2], M.E. Nunes[1].* 1) Dept Genetics, Kaiser Permanente, San Diego, CA; 2) Division of Cardiology, Cardiac Electrophysiology, UCSD, San Diego, CA.

We report a case of long QT (LQT) syndrome complicated by the inheritance of LQT1, LQT2, and LQT3 syndrome mutations in a single infant. Fetal bradycardia with heart rate of 90-100 was detected in a third trimester gestation in an otherwise uncomplicated pregnancy to a 36yo G3P1 mother of Afghan ancestry. Parents denied consanguinity. Neonatal ECG of the male infant revealed QTc interval of >500 ms, confirmed on repeat at two weeks. The infant failed the newborn hearing screen and was found to have congenital profound bilateral sensorineural hearing loss. A clinical diagnosis of Jervell and Lange-Nielson (LQT1) was made. Parents deny personal histories of syncope or related symptoms but upon ECG both were found to have long QTc intervals. Pedigree analysis revealed an extensive history of syncope, sudden infant death syndrome, and sudden cardiac death in both the maternal and paternal families. Molecular studies on the affected infant detected homozygosity for the known deleterious mutation, R174H, in the KCNQ1 gene confirming the diagnosis of Jervell and Lange-Nielson (LQT1) syndrome. Molecular studies of the affected infant also revealed additional mutations complicating the diagnosis. In the KCNH2 gene associated with Romano-Ward (LQT2) syndrome a novel class II (possible deleterious) variant of unknown significance, K93R, was detected, in addition to a known K897T polymorphism. In the SCN5A gene associated with LQT3/ Brugada syndromes a novel class I (probable deleterious) mutation, R1739Q, was also detected. Molecular and phenotypic studies of both parents and extended sibship to determine the co-segregation patterns for each of these mutations will be described. The KCNQ1 gene encodes the alpha subunit of the voltage-gated IKs cardiac potassium channel, the slowly activating component of the delayed rectifier current largely responsible for repolarization of the cardiac action potential. The KCNH2 gene encodes the IKr potassium channel and is responsible for the termination of the cardiac action potential. The SCN5A gene encodes the alpha subunit of the voltage-gated INa cardiac sodium channel. Mutations disrupt the inactivation of this channel resulting in prolongation of Na+ influx during depolarization. The family is being evaluated to determine compounding effect of multiple inherited LQT gene mutations. This case emphasizes the importance of detailed family history and use of LQT gene panel in determining multiple coexisting diagnoses.

## 2351/T/Poster Board #900

**CHIP-based sequence analysis of 34 cardiomyopathy genes in 250 patients reveals new genes involved in HCM and DCM and multiple pathogenic mutations in single patients.** *H. Smeets[1,2], W. Van Dijk[1], A. Stassen[1], P. Lindsey[1,2], Y. Arens[1], P. Helderman[1], C. Marcelis[3], J. Van der Smagt[4], S. Heymans[2,5], P. Volders[2,5], R. Jongbloed[1,2], A. van den Wijngaard[1].* 1) Dept Clinic Genet, Maastricht UMC, Maastricht, The Netherlands; 2) Research School CARIM, Univ Maastricht, Maastricht, The Netherlands; 3) Dept Clinic Genet, UMC St. Radboud, Nijmegen, The Netherlands; 4) Dept Clinic Genet, Utrecht UMC, Utrecht, The Netherlands; 5) Dept Cardiol, Maastricht UMC, Maastricht, The Netherlands.

Inherited cardiomyopathy is a frequent cardiac disease with a prevalence of 1:500 (HCM) to 1: 2500 (DCM). Due to the large amount of genes involved, the clinical heterogeneity and the laborious screening methods, it is difficult to unravel rapidly the genetic cause in all cases. Current diagnostic screening solves only 60-70% of the families by testing a limited number of genes in an often time-consuming sequential fashion and usually stops when a pathogenic mutation has been identified. However, double pathogenic mutations seem to be present in 5-10% of the familial cases. In order to create a fast, parallel genetic screening pipeline for inherited cardiomyopathy, we designed a resequencing array (CardioCHIP) covering 34 genes in duplicate (300Kb). We included genes known to be involved in DCM, HCM, LVNC and LGMD and candidate genes based on their presence in the sarcomere and Z-disc. All exons and flanking introns (38bp) were included on the CardioCHIP, covering the heart- and muscle-specific RNA-isoforms. The 5'UTR and 3'UTR regions and, for a selection of genes, the promoter regions were included as well. The genes, which were interrogated by the CardioCHIP, were amplified in 152 LR-PCR covering 395 exons, resulting in a sequence of 146.541 nucleotides. So far, 250 patients were sequenced for all 34 cardiomyopathy genes. The mutation detection rate is around 99% and about 98% of the novel exonic variants can be confirmed by conventional sequence analysis. In addition to mutations detected in the 13 genes routinely tested for HCM or DCM, we identified mutations in the 21 additional genes, some of which were the first for those new candidate genes. We identified in several patients up to 4 pathogenic mutations, involved in different pathogenic processes. Our data indicate that parallel analysis of multiple genes is a prerequisite for genetic testing in HCM and DCM to identify rapidly the genetic cause. Due to the potential presence of multiple mutations and to prevent misinterpretation, it is essential to test all genes in all patients and not proceed solely on the first genetic defect identified, as is the current practice. This is also the case for the relatives at risk, which would imply that for proper prognosis and preventive treatment all candidate genes should be tested in parallel for each individual.

## 2352/T/Poster Board #901

**Barth syndrome is not a common cause of dilated cardiomyopathy in adults.** *M. Taylor[1], D. Slavov[1], X. Zhu[1], D. Ferguson[1], J. Jirikowic[1], A. Di Lenarda[2], G. Sinagra[2], L. Mestroni[1].* 1) Adult Med Gen Prog, Univ Colorado Hlth Sci Ctr, Aurora, CO; 2) Ospedale Maggiore and University of Trieste, Trieste, Italy.

**Introduction:** Barth syndrome is a rare, X-linked genetic condition with neutropenia, skeletal myopathy, and dilated cardiomyopathy. Tafazzin (Xq28) mutations cause Barth syndrome, which has been mostly described in boys, although cardiomyopathy has been rarely described in adults. Some data suggest that certain boys with Barth syndrome may experience improvement of their cardiomyopathy in childhood, with recurrence of dilated cardiomyopathy as adults. Since the typical ascertainment of Barth syndrome has been in pediatric patients, whether tafazzin mutations contribute to dilated cardiomyopathy in adult populations, including women is unknown. **Methods:** We screened 295 families with dilated cardiomyopathy (210 male and 84 female probands) for mutations in tafazzin by mutation screening using denaturing high-performance liquid chromatography and sequence analysis. Putative mutations were evaluated for being causative of dilated cardiomyopathy based on standard criteria including screening in available relatives and in healthy controls and for effects on splicing efficiency in the case of one intronic mutation. **Results:** We found minimal variation in the coding sequence of tafazzin in our study. Four novel variants were identified in single individuals from four separate families (IVS2 -27 G/A; Exon 5 F128S, Exon 6 L169L, and exon 11 +114 G/A). Although these mutations were unique among over 700 case and control chromosomes, none of these mutations met all our criteria for pathogenic mutations and most likely represent rare polymorphisms. Furthermore, we showed that the exon 5 mutation (F128S) previously reported as pathogenic in the Barth Syndrome Foundation database is most likely a rare polymorphism restricted to African American populations. The F128F variant, therefore, is the only nonsynonymous change (excluding the initiation codon) that is tolerated in the amino acid sequence of the tafazzin protein. **Conclusion:** Our screening of a large, well-phenotyped cohort suggests that tafazzin mutations are not common causes of dilated cardiomyopathy in adults.

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## 2353/T/Poster Board #902

**The burden of living with the risk of inherited arrhythmia for patients coming to genetic counselling.- A study of health status in patients with the risk of Long QT syndrome and Hypertrophic Cardiomyopathy compared to patients with the risk of inherited Breast/Ovarian and Colorectal cancer.** *A. Hamang[1,2], G.E. Eide[1,3], K. Nordin[1,4], B. Rokne[1], C. Bjorvatn[1,2], N. Øyen[1,2].* 1) Department of Public Health and Primary Care, University of Bergen, Trondheim, Norway; 2) Center of Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway; 3) Centre for Clinical Research, Haukeland University Hospital, Bergen, Norway; 4) Department of Public Health and Caring Sciences, Uppsala University, Sweden.

Background: Today it is possible to identify genetic vulnerability for a number of different diseases, including hereditary cancer and serious inherited arrhythmias, through family history and direct mutational testing. Systematic knowledge about how it is to live with the risk of hereditary cancer and how people perceive their health is growing, but the literature is very limited with regards to health status when being at risk of inherited arrhythmias, where the manifestations of symptoms can range from asymptomatic to sudden death. In Norway both patient groups and their family members receive genetic counselling and are followed up with the same routine through predictive genetic testing. Aim: To measure the impact of being at risk of inherited arrhythmia compared to inherited cancer on the functioning and wellbeing of the patients coming to genetic counselling. Patients and methods: The study used a two-group cross-sectional comparative design. Two hundred and fourteen patients with a genetically based risk of cancer, either breastovarian or colorectal cancer, aged 18-80, and 127 patients with an increased risk of inherited arrhythmia, either Long QT syndrome or Hypertrophic cardiomyopathy aged 18-83 and, coming to genetic counselling between 2002-2004 and 2005-2007 were asked to participate in a study. Health status was measured by the Short Form Health Survey [SF-36] on eight domains with values in the range 0 (worst) to 100 (best). The patient SF-36 scores were compared with independent samples t-tests. Results The arrhythmia risk group reported lower SF-36 scores as compared to the cancer risk group on all domains; Significant differences were found in general health (mean difference 6.2 (95% confidence interval 1.6 to 10.8)) and in social functioning (mean difference 6 (95% confidence interval 1.3 to 10.8)), The difference in mental health (3.4 (-0.5 to 7.4)) were borderline significant. Discussion: It may look like the patients with a genetic risk of arrhythmia have a different health profile as compared to patients coming to genetic counseling because of inherited cancer risk. Conclusion: Genetic counselling services must continue to develop and give attention to the differences between patient groups in the further work of development of guidelines and tailored information in the genetic counselling.

## 2354/T/Poster Board #903

**Mutation screening for vascular type of Ehlers-Danlos Syndrome using high resolution melting curve analysis (hrMCA).** *A. Watanabe[1,2], B.T. Naing[1], T. Shimada[1,2].* 1) Dept Biochem & Molec Biol, Nippon Med Sch, Tokyo, Japan; 2) Div Clinical Genetics, Nippon Medical School Hospital, Tokyo, JAPAN.

Vascular type of Ehlers-Danlos syndrome (vEDS), also known as EDS type IV (MIM#130050) is a life-threatening autosomal dominant inherited disorder caused by mutations of type III collagen, *Col3A1*, and clinically characterized by spontaneous rupture of bowel and large arteries. Two-thirds of the mutations in the *Col3A1* gene are a single nucleotide substitutions which cause changes from glycine to other amino acids in the triple helix region composed of (Gly(glycine)-X-Y)$_{343}$ repeats and most of the rest are splicing mutations. The *COL3A1* gene is located at 2q31-q32, contains 52 exons distributed over 44 kb. Since there is no hot spot, mutation detection of *Col3A1* gene is complicated and time consuming, In most cases, mutation search was done using total RNA isolated from patient's fibroblasts. A high resolution melting analysis (hrMCA) is based on monitoring of the fluorescence released during the melting of double-stranded DNA labeled with specifically developed saturation dye, such as LC-Green and has been developed as a promising pre-screening and economical post-PCR mutation scanning method. We applied hcMCA to establish *Col3A1* mutation screening using genomic DNA. PCR primers pairs for *Col3A*1 (52 amplicons in 52 exons) to cover the complete coding region and splice sites were designed. We analyzed genomic DNA samples from patients who have been diagnosed as vEDS. All gene mutations including known three Gly substitution mutations and three splicing mutations of *Col3A1* gene revealed different melting curves in hrMCA. In addition, we found one new nonsense mutation (c.2491C>T; Gln831Ter) in exon 42 which could not be detected by analysis of total RNA because of nonsense mediated mRNA decay. Our results demonstrated that hrMCA of genomic DNA is a powerful and inexpensive scanning method for mutation detection of *Col3A1* gene. Genome scanning by hrMCA should be useful for screening of genetic diseases caused by heterogeneous mutations of the gene composed of many exons like *Col3A1*.

## 2355/T/Poster Board #904

**A multi-stage / multi-design strategy identifies a new QTL for VWF on chromosome 6 - a possible link with VTE?** *G. Antoni[1,2,3], N. Saut[4], Y. Luo[3], G. Burgos[4], C. Biron-Andreani[5], J.F. Schved[5], G. Pernod[6], I. Juhan-Vague[4], M.C. Alessi[4], L. Drouet[1,2], S. Visvikis-Siest[7], P.S. Wells[8], J. Emmerich[9], D.A. Tregouet[1,2], P.E. Morange[4].* 1) INSERM, UMR_S 937, F-75013, Paris, France; 2) UPMC Univ Paris 06, UMR_S 937, F-75013, Paris, France; 3) Dalla Lana School of Public Health, University of Toronto, Ontario, M5T 3M7, Canada; 4) INSERM, UMR_S 626, F-13385, Marseille, France; Université de la Méditerranée, Marseille, F-13385 France; 5) Laboratoire d'Hématologie, CHU Montpellier, France; 6) Service de Médecine Vasculaire, CHU Grenoble, France; 7) Equipe INSERM "Génétique Cardiovasculaire" CIC 9501, Nancy, France; 8) Ottawa Health Research Institute, Ottawa, Ontario, K1Y 4E9, Canada; 9) INSERM U765,Université Paris-Descartes, France.

Factor VIII (FVIII) and von Willebrand Factor (VWF) are two known quantitative risk factors for venous thromboembolism (VTE). In this work, we employed a multi-stage / multi-design strategy to identify new loci that would contribute to VTE susceptibility by modulating FVIII and/or VWF levels. For this purpose, a pedigree linkage analysis was first performed on five extended French-Canadian families including 261 individuals genotyped for 1079 microsatellites and identified four putative regions linked to FVIII and vWF levels. The strongest linkage signal was observed on chromosome 9q34 overlapping the ABO locus, a known susceptibility loci to FVIII and VWF levels. The other three regions, located on chromosomes 2, 6 and 12, were then evaluated by in silico association analysis of a genome-wide association study composed of 419 VTE patients and 1288 controls with the aim of narrowing the linkage signals by focusing on candidate region for VTE. Four SNPs in the chromosome 6 region were in silico associated with VTE at $p<10^{-4}$ and were then investigated for association with FVIII and VWF in a sample of 123 healthy French nuclear families (2 parents and 2 offspring). One SNP was found to explain up to 3% (p=0.0018) of VWF variability in these families and also explained 1% (p=0.009) of VWF variability in an independent cohort of 823 patients with VTE at young age (<50 years). Besides, the allele that has been found associated with decreased VWF levels tended to be associated with a lower risk of VTE (OR=0.70 [0.47-1.04], p=0.081) in an independent French case-control study for VTE of 607 cases and 607 controls. In conclusion, using a multi-stage approach including pedigree linkage analysis, association analysis in nuclear families and case-control data, we obtained some strong arguments about the role of a new locus on chromosome 6 that could be involved in the susceptibility to VTE by controlling plasma VWF levels.

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## 2356/T/Poster Board #905

**Genome-wide association study of P-selectin in European ancestry individuals from Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium.** *M. Barbalic[1], J. Dupuis[2,3], A. Dehghan[4], J.C. Bis[5], R.B. Schnabel[2], W. Koenig[6], N.S. Jenny[7], J.C. Witteman[4], C.M. Ballantyne[8], E.J. Benjamin[2].* 1) Human Genetics Center and Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX; 2) National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, MA; 3) Department of Biostatistics, Boston University School of Public Health, Boston, MA; 4) Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands. Member of the Netherlands Consortium on Healthy Aging (NCHA); 5) Cardiovascular Health Resaearch Unit, Department of Medicine, Epidemiology and Health Services, University of Washington, Seattle, WA; 6) Department of Internal Medicine II - Cardiology, University of Ulm Medical Center, Ulm, Germany; 7) Department of Pathology, University of Vermont College of Medicine, Burlington, VT; 8) Department of Medicine, Baylor College of Medicine and Center for Cardiovascular Prevention, Methodist DeBakey Heart and Vascular Center, Houston, TX.

P-selectin participates in the inflammatory process by promoting adhesion of leukocytes to vascular wall endothelium. Its levels have been shown to be highly heritable and, importantly, associated with various cardiovascular disorders. Soluble P-selectin is readily measurable in large cohort studies but the more biologically active molecule is thought to be the membrane bound form. To identify loci affecting soluble levels of P-selectin, we performed a genome-wide association study (GWAS) in a sample of 4,115 individuals of European ancestry in 3 cohorts (Atherosclerosis Risk in Communities (ARIC), Framingham Heart Study (FHS) and Rotterdam Study (RS)) as a part of The Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) consortium. We tested the association with ~2 million imputed SNPs using linear regression under an additive genetic assumption. We obtained three genome-wide significant hits nearby the SELP (p=4.05x10-61), ABO (p=1.86 x10-41) and SCAP (p=3.72x10-7) genes. To verify if the same genes are responsible for variation in membrane bound P-selectin levels, we sought to replicate the top findings in a sample of 1,088 individuals from ARIC study with the measures of platelet bound P-selectin. Only the variant in the SELP gene remained significant (p=0.02) when platelet bound P-selectin levels were analyzed. Our results identified genes influencing P-selectin levels and suggested different genetic determinants of soluble and platelet bound P-selectin. They contribute to the knowledge of the adhesion molecules physiology that is necessary to understand the link between the inflammation and atherosclerotic process.

## 2357/T/Poster Board #906

**Molecular genetic analysis of gene expression in tissues from infants with idiopathic conotruncal defects.** *D.C. Bittel[1], N. Kibiryeva[1], J.A. Swihart[2], M.G. Butler[3], G.K. Lofland[2], J.E. O'Brien Jr[2].* 1) Section Medical Geneteics, Children's Mercy Hosps, Kansas City, MO; 2) Section of Cardiovascular and Thoracic Surgery, Children's Mercy Hosps, Kansas City, MO; 3) Departments of Psychiatry & Behavioral Sciences and Pediatrics Kansas University Medical Center.

Children with conotruncal heart defects, decreased pulmonary blood flow from pulmonary artery stenosis or atresia and right ventricular hyperplasia present with a complex set of health problems. Tetralogy of Fallot (TOF) is the most common defect. Treatment of these patients has evolved dramatically in the last few decades, yet investigation into the underlying genetic basis has not provided a molecular mechanism for the failure of proper cardiac development for the majority of children with TOF. A clear delineation of genetic aspects of TOF may allow development of tailored therapeutic approaches to medical and surgical intervention while improving outcomes for these difficult to manage patients. Hence, we have examined cardiovascular tissue from the right ventricle, pulmonary valve and pulmonary artery obtained at the time of reconstructive surgery from 19 children with Tetralogy of Fallot (3 with 22q11.2 chromosomal deletion and 16 nonsyndromic children with normal chromosome studies) and applied whole genome expression microarray technology to characterize gene expression patterns relative to tissues from 5 normally developing comparison subjects. We detected a signal from approximately 20,000 probes (ranging from 39% to 35% of array probes in the three tissues examined). More than 1000 genes had a 2-fold change in expression in the right ventricle (RV) of children with TOF compared to RV from matched control infants. Most of these genes were involved in compensatory functions (e.g., hypertrophy, cardiac fibrosis and cardiac dilation); however, several canonical pathways were implicated as having altered expression patterns. Pattern formation pathways (i.e., WNT, Notch and Hedgehog) were generally suppressed, especially in the RV. This may reflect a general malfunction of these regulatory pathways leading to inaccurate boundary formation and improper structural development in the embryonic heart. Pathway suppression was not ubiquitous but appeared to be confined to these signaling pathways. Routine cytogenetic tests on the majority of these children did not reveal cytogenetic anomalies associated with congenital heart disease. We suggest that small structural or functional disturbances or tissue specific genomic and/or epigenetic fluctuations could lead to gene expression fluctuation causing regulatory network disruption and thus failure of proper cardiac development in the affected individuals requiring more testing.

## 2358/T/Poster Board #907

**A Genome-Wide Study of Common and Rare Variants for Myocardial Infarction and Related Traits in South Asians: The INTERHEART study.** *R. Do[1], C. Xie[2], A. Montpetit[3], G. Pare[4], S. Yusuf[2], J.C. Engert[1], S.S. Anand[2] on behalf of the INTERHEART investigators.* 1) Department of Human Genetics, McGill University, Montréal, QC, Canada; 2) Population Health Research Institute, Hamilton Health Science, McMaster University, Hamilton, ON, Canada; 3) McGill University and Genome Quebec Innovation Center, Montreal, QC, Canada; 4) Center for Cardiovascular Disease Prevention, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.

Genome-wide association (GWA) studies have been successful at identifying common SNPs with weak effects for myocardial infarction (MI) in Europeans. However, these studies have not been performed in many other ethnicities and the role of rare variants with strong effects is unknown. We investigated both common SNPs and rare variants, with MI in 600 South Asians, a population known to have higher rates of MI, from the INTERHEART study. We genotyped 353,202 SNPs using the Illumina HapCNV370 in 300 early-onset (mean 39.1 years) cases and 300 aged controls (mean 60.6 years). A GWA scan of common SNPs and MI identified a SNP in the 4q35.2 region (p=7.71x10-6) as the strongest result. 38 other associations were observed (p<0.0001). Scans for 10 risk factors of MI were performed and we observed putative associations for each trait (ie. for p<1x10-5, 13 SNPs for LDL-C, 17 for HDL-C, 18 for TG, 20 for BMI, 23 for waist c., 37 for hip c., 2 for waist/hip, 6 for apoB, 2 for apoA1, and 20 for hbA1c). We will follow-up these results in a second stage consisting of an additional 1267 South Asians. In addition, we investigated rare variants using the segmental sharing procedure in PLINK, a method for using whole genome SNP data in population-based samples to identify loci containing multiple rare variants associated with disease. This method scans the genome for extended segments that display excessive identical by descent sharing among case-case pairs. Six linkage peaks (2q36.3, 6p22.2, 8p23.1, 9q31.2, 13q21.2, 15q13.2, p< 0.001) were identified, and the second strongest result (8p23.1, p=0.00028) had been previously identified by our group in a genome-wide linkage study of coronary heart disease in Saguenay Lac St. Jean families. We investigated the role of common SNPs for MI within these regions and observed the strongest association in the 9q31.2 region (p=1.4x10-5). In conclusion, we have completed stage one of a two stage genome-wide study of genetic loci predisposing to MI in South Asians.

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## 2359/T/Poster Board #908

**CGH Micro-Array analysis of children with left-side congenital heart defects.** *H. Gill[1], G. Sandor[2], S. Adam[3], M. Thomas[3], T. Tucker[3], J.M. Friedman[3].* 1) Provincial Medical Genetics Program, Children's Hospital, Vancouver, BC, Canada; 2) Paediatric Cardiology Program, Children's Hospital, 4500 Oak St, Vancouver, BC, Canada; 3) University of British Columbia, Vancouver, Canada.

Congenital heart defects (CHD) are the commonest human malformation. In the majority of affected individuals no underlying cause can be clearly identified. Genetic factors are thought to play an important part and recent studies have implicated submicroscopic chromosomal imbalances or pathogenic copy number variants (CNV) in 5- 25% of cases. We sought to investigate the contribution of pathogenic CNV in our population of children with left-sided CHD (LCHD). Since LCHD has been shown to cluster in families, we hypothesized that a genetic etiology would be evident in this group. We recruited children (and their parents) with LCHD from our regional pediatric cardiology service. Cases were excluded if the cause of the LCHD was known, if both parents were not available for testing, if the child was deceased, or if the family lived outside of the province of British Columbia, Canada. Microarray CGH was performed using the Nimblegen 12x135K whole-genome tiling microarrays. The data were analyzed using NimbleScan software, and CNVs were identified using Nexus software (Biodiscovery, Inc.) Of 24 cases of LCHD analysed to date, 20 had aortic coarctation (CoA). In 5 of these cases CoA was the primary lesion of a more complex cardiac defect. 4 cases had aortic stenosis (AS). In 1 of these AS was part of a more complex defect. The male:female ratio was 16:8. The age range was 30 months to 21 years. All were seen by a clinical geneticist and were confirmed as isolated CHD with no other malformations or dysmorphic features. No de novo pathogenic CNVs were found in our cases. 3 published studies have investigated the contibution of CNVs in the pathogenesis of CHD. None focused on isolated LCHD. Erdogan et al 2008 investigated 105 cases of isolated CHD and found 18 pathogenic CNVs (17%). Of 18 cases with isolated LCHD, 1 infant with CoA had a possibly pathogenic de novo CNV. Richards et al (2008) investigated 20 cases of isolated CHD and 20 cases of non-isolated CHD. 5 pathogenic CNVs were identified in the non-isolated group(20%), 1 of which had CoA. However, no de novo CNVs were identified in the isolated group of which 4 had LCHD. Thienpont et al (2007) investigated 60 patients with CHD and other malformations or mental retardation and found 10 (17%) to have a pathogenic CNV. In conclusion, it is likely that de novo pathogenic CNV are not a frequent cause of isolated LCHD.

## 2360/T/Poster Board #909

**Whole genome SNP and CNV analysis of atrioventricular septal defects in the Down syndrome population.** *A.E. Locke[1,6], D.J. Cutler[1], E. Feingold[2], C. Maslen[3], G. Capone[4], R.H. Reeves[5], S.L. Sherman[1], M.E. Zwick[1].* 1) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Departments of Human Genetics and Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 3) Department of Cardiovascular Medicine, Oregon Health & Science University, Portland, OR; 4) Division of Neurology & Developmental Medicine, Kennedy Krieger Institute, Baltimore, MD; 5) Department of Physiology & McKusick-Nathans Institute for Genomic Medicine, Johns Hopkins University, School of Medicine, Baltimore, MD; 6) Program in Genetics & Molecular Biology, Emory University, Atlanta, GA.

Trisomy 21, the chromosomal abnormality responsible for Down syndrome (DS), is a complex condition associated with a constellation of abnormalities affecting multiple organ systems. The combination of these anomalies is severe, with as few as 20% of conceptuses with trisomy 21 surviving to term. Congenital heart defects (CHD) represent the most common malformation in children born at term. Trisomy 21 is the leading cause of CHD. Nearly half of all DS births are affected by some form of CHD. Complete atrioventricular septal defects (AVSD) occur at a rate of nearly 1/5 and are one of the most common forms of CHD in individuals with DS. When contrasted with the 1/10,000 rate of AVSD in the general population, the 2000-fold greater risk among those with DS strongly suggests that the increased dosage of chromosome 21 (chr21) genes contributes to the occurrence of CHD, and especially AVSD. Through a multi-site recruitment effort, we have ascertained individuals with DS who have complete AVSD (cases) and those who have structurally normal hearts (controls) and their parents. Maternal questionnaires have been completed to obtain demographic information and maternal health history during pregnancy. Medical records on probands have been abstracted to document heart status and other birth defects. Here we present results from the first 120 case-parent trios and the 120 controls based on Affymetrix Genome Wide SNP 6.0 array. In addition to genome wide SNP association, we use common strategies to identify copy number variable regions throughout the genome in this population. We present annotation of the genetic variants we identified and validated, including comprehensive high-resolution aCGH of chromosome 21. Finally, we test all SNPs and CNVs for association with AVSD under both a case/control framework and a case-parent trio (TDT) framework.

## 2361/T/Poster Board #910

**Genome-wide association study on HDL cholesterol level in the Marshfield Personalized Medicine Research Project as part of the eMERGE network.** *S.D. Turner[1], C.A. McCarty[2], Y. Bradford[1], D. Berg[3], P. Peissig[3], J. Linneman[3], J. Starren[3], R.A. Wilke[4], M.D. Ritchie[1].* 1) Center Human Genetics Research, Vanderbilt University, Nashville, TN; 2) The Center for Human Genetics , Marshfield Clinic Research Foundation; 3) Biomedical Informatics Research Center, Marshfield Clinic Research Foundation; 4) Department of Medicine and Department of Pharmacology and Toxicology, Medical College of Wisconsin.

The Electronic Medical Records and Genomics (eMERGE) network is an NHGRI funded initiative with the goal of testing whether electronic medical record (EMR) systems can serve as a resource for collecting phenotypic information for genomic studies. To test this hypothesis, five groups around the United States are using natural language processing algorithms to derive phenotypes in patients who have DNA samples in the institution's biobank. The Marshfield Clinic in Central Wisconsin represents one such institution and our phenotypes are cataracts and high-density lipoprotein (HDL) cholesterol (U01-HG004608). Here we will discuss our genome-wide association study for HDL cholesterol. Individuals having below-normal levels of HDL cholesterol are at increased risk for cardiovascular disease. In order to more thoroughly understand the genetic basis of this complex trait, we have conducted an initial GWAS in 3900 participants in the Marshfield Clinic Personalized Medicine Research Project (PMRP). Self reported race within the PMRP cohort (using U.S. Census classification at the time of study entry) indicates the population is 98.2% white Caucasian of central and northern European descent, making it ideal for a genetic association study since risk of population stratification is minimized. PMRP contains data from >20,000 participants. Participants are ≥ 18 years of age (median 49 years). The database contains HDL levels for >15,000 of these individuals. Clinical lab HDL cholesterol levels were electronically harvested from subject EMRs in 3900 individuals. All samples were genotyped with the Illumina 660-Quad platform. Each SNP passing rigorous quality control measures was tested for association with HDL cholesterol level using linear regression, assuming an additive model. Here we report several highly significant findings, shedding more light on the genetic contribution to a complex lipid phenotype of substantial public health interest. In addition, we compare and contrast these results with the many already published SNPs associated with lipid levels. Finally, we demonstrate the success of using an EMR-derived phenotype to perform genetic analysis of complex disease. This study along with the other eMERGE projects provide evidence supporting the use of biobanks linked to EMRs for genomic studies.

## 2362/T/Poster Board #911

**Genome-wide copy number analysis to identify candidate genes for Thoracic Aortic Aneurysms and Dissections.** *S. Prakash[1], D. Guo[2], M. Bray[1], S. Lemaire[1], D. Milewicz[2], J. Belmont[1].* 1) Baylor College of Medicine, Houston, TX; 2) University of Texas Health Science Center at Houston, Houston, TX.

Thoracic Aortic Aneurysms and Dissections (TAAD) cause 15,000 deaths each year due to acute complications such as aortic rupture, myocardial infarction or stroke. In a genome-wide copy number analysis of 786 sporadic TAAD cases, we identified copy number variants (CNVs) of 29 novel candidate genes that are significantly associated with TAAD. The cohort for this study was derived from the Specialized Center for Clinically Oriented Research (SCCOR) in TAAD and includes patients who underwent surgical repair of thoracic aneurysms at St. Luke's Episcopal Hospital and Baylor College of Medicine (100% Caucasian, 35% female, average age 63, 36% Stanford Type A dissections). Genomic DNA from whole blood was hybridized to Illumina 370 Quad Beadchips. We extracted the intensity and allele frequency data into three copy-calling algorithms (CNVPartition, PennCNV and DNAcopy), and the results were filtered to select CNVs that intersect with known genes. We identified 3189 CNVs in 2289 genes, including 24 patients with large (> one megabase) events. Most of these were copy-neutral events that appear consistent with regions of mosaic uniparental isodisomy. We are currently obtaining additional samples from these patients in order to confirm these events on Illumina 610 Quad Beadchips. We limited our subsequent analysis to CNVs that are statistically associated with TAAD in comparison with control databases representing 3500 unaffected individuals. Each putative CNV was directly confirmed by the first author. Interactome analysis of the candidate genes showed that many participate in cytoskeletal remodeling or TGF-β signaling pathways in vascular smooth muscle cells. Since mutations in smooth muscle contractile proteins and TGF-β receptors cause familial predisposition to TAAD, these novel CNVs are likely to be pathogenic variants that predispose to thoracic aneurysms. To prove a causal relationship of several promising candidate genes with TAAD, we are currently obtaining independent lines of evidence, including replication in other patient cohorts, detection of additional mutant alleles in these genes and functional studies.

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## 2363/T/Poster Board #912

**Identification of ZNF366 and PTPRD as Novel Determinants of Plasma Homocysteine in a Family-based Genome-wide Association Study.** *A. Buil[1], A. Mälarsting[2], JC. Souto[3], R. Clarke[4], F. Blanco-Vaca[5], J. Fontcuberta[3], J. Peden[6], M. Andersen[2], A. Silveira[2], S. Barlera[7], U. Seedorf[8], H. Watkins[6], L. Almasy[9], A. Hamsten[2], JM. Soria[1].* 1) Genomics Complex Diseases, Hosp de Sant Pau, Barcelona, Spain; 2) Atherosclerosis Research Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden,; 3) Department of Hematology and 5Department of Biochemistry Hospital de la Santa Creu i Sant Pau, Barcelona, Spain,; 4) Clinical Trials Service and Epidemiological Studies Unit (CTSU); 5) Department of Biochemistry Hospital de la Santa Creu i Sant Pau, Barcelona, Spain,; 6) Department of Cardiovascular Medicine, University of Oxford, Oxford, UK,; 7) Department of Cardiovascular Research, 'MarioNegri' Institute for Pharmacological Research, Milano, Italy,; 8) Leibniz-Institut für Arterioskleroseforschung an der Universität Münster, Münster, Germany; 9) Department of Population Genetics, Southwest Foundation for Biomedical Research, San Antonio, Texas, USA.

Total plasma homocysteine concentration (tHcy) is an established biomarker for atherothrombotic diseases and a highly heritable trait. Polymorphisms in genes involved in methionine metabolism only account for a minor proportion of the variation in tHcy in the population. We searched for novel genetic determinants of plasma tHcy using a genome-wide single nucleotide polymorphism (SNP) association study approach. First 283,437 SNPs were tested for association with tHcy in 21 large Spanish families (n=387). Replication analyses of 18 SNPs meeting predefined criteria for association were then performed in patients with premature myocardial infarction (n=1238). Novel associations were found for SNPs near the ZNF366 gene (lead SNP rs7445013, adjusted beta= -0.13, p=0.0004) and the PTPRD gene (rs973117, adjusted beta= -0.10, p=0.005). The genetic contribution to tHcy variation attributable to SNPs at ten loci was estimated to be 10.3% in the family study and 4.0% in the replication study, after adjustment for effects of non-genetic factors. Our findings implicate novel pathways in homocysteine metabolism, and the roles of the associated genes in the etiology of vascular diseases warrant further investigation.

## 2364/T/Poster Board #913

**Multiethnic genetic association study of carotid intima-media thickness using a targeted cardiovascular SNP microarray.** *M.B. Lanktree[1], S. Yusuf[2,3], R.A. Hegele[1], S.S. Anand[2,3], the SHARE investigators.* 1) Departments of Medicine and Biochemistry, Robarts Research Insitute, University of Western Ontario, London, Ontario, Canada; 2) Population Health Research Institute, Hamilton Health Sciences, Hamilton, Ontario, Canada; 3) Departments of Medicine and Clinical Epidemiology, McMaster University, Hamilton, Ontario, Canada.

Identification of subclinical atherosclerosis by ultrasonographic measurement of carotid intima-media thickness (cIMT) is a validated tool, in conjunction with traditional risk factors, for assessment and prediction of cardiovascular disease (CVD) risk. Many previous association studies have, with variable success, identified variants in candidate genes associated cIMT. We evaluated the association between cIMT and ~50,000 SNPs, densely mapping ~2,100 genes, found on the gene-centric Illumina (University of Pennsylvania) CVD beadchip in a multiethnic sample. B-mode ultrasound was performed and DNA was collected from a population-based sample of South Asian (n = 328), Chinese (n = 302), and Caucasian (n = 268) participants. The most robust association with cIMT was observed for rs3791398 in *HDAC4* encoding histone deacetylase 4 (P = 1.8e-5). Given recent associations reported between polymorphisms in natriuretic peptide precursors and hypertension, it is interesting that our third strongest association was with rs10082235 in *NPR1* encoding natriuretic peptide receptor A/guanylate cyclase A (P = 5.4e-5). We also identified marginally significant replication of associations reported in 7 of 15 functional candidate genes ($0.01 \le P \le 0.05$). A model that included SNP genotypes associated with plasma total cholesterol was significantly associated with cIMT (P = 6.0e-4, $r^2 = 0.368$), but did not substantially improve the extent of cIMT variation that could be explained versus traditional risk factors. To our knowledge, this is the first array-based high-density genetic study of associations with cIMT in a multiethnic sample. The results indicate that careful phenotypic evaluation of subclinical atherosclerosis and larger-sized samples, including subjects from multiple ethnicities, will be required to identify variants that are reliably associated with cIMT.

## 2365/T/Poster Board #914

**Genome-wide association study of coronary heart disease and its main risk factors in 8,367 African-Americans from the ARIC, CARDIA, CFS, JHS, and MESA population-based cohorts: The CARe Project.** *G. Lettre[1], K. Ejebe[2], D. Farlow[2], T. Young[2], N. Patterson[2], R. Fabsitz[3], A. Folsom[4], M. Fornage[5], E. Fox[6], E. Larkin[7], G. Papanicolaou[3], M. Reilly[8], J.I. Rotter[9], D. Siscovick[10], H. Taylor[6], R.P. Tracy[11], J.N. Hirschhorn[2], S. Gabriel[2], S. Kathiresan[2], E. Boerwinkle[5], The CARe Project.* 1) Montreal Heart Institute, Montreal, Quebec, Canada; 2) Broad Institute, Cambridge, MA, USA; 3) National Heart, Lung, and Blood Institute, Bethesda, MD, USA; 4) University of Minnesota, Minneapolis, MN, USA; 5) University of Texas, Houston, TX, USA; 6) University of Mississippi, Jackson, MS, USA; 7) Case Western Reserve University, Cleveland, OH, USA; 8) University of Pennsylvania, Philadelphia, PA, USA; 9) University of California in Los Angeles, Los Angeles, CA, USA; 10) University of Washington, Seattle, WA, USA; 11) University of Vermont, Burlington, VT, USA.

In populations of European ancestry, genome-wide association studies (GWAS) have mapped many genetic loci associated with coronary heart disease (CHD) and its main risk factors, including plasma low-density lipoprotein cholesterol (LDL-C), plasma high-density lipoprotein cholesterol (HDL-C), cigarette smoking, type-2 diabetes, and hypertension. To date, however, no large-scale GWAS have focused on CHD in African-Americans. To identify genetic determinants of CHD and its risk factors in this population, the NHLBI-funded Candidate-gene Association Resource (CARe) Project genotyped 8,367 African-Americans from five population-based cohorts (ARIC, CARDIA, CFS, JHS, MESA) using the Affymetrix 6.0 platform.

Appropriate quality-control criteria, including principal component analyses, were applied to remove poorly performing SNPs and samples, as well as population outliers. We tested the association between >800,000 SNPs and circulating HDL-C and LDL-C levels, cigarette smoking, type-2 diabetes status, hypertension status, and prevalent CHD in each cohort. HDL-C, LDL-C, and cigarette smoking were analyzed as quantitative traits using linear regression, whereas type-2 diabetes, hypertension, and CHD were analyzed as dichotomous phenotypes using logistic regression. Association results were combined across studies using meta-analysis. To address the issue of admixture, we developed a new test statistic that combines evidence of association from both genotype and ancestry information. Using this approach, we replicated several associations originally discovered in populations of European descent, for instance SNPs near *CETP*, *LPL*, and *LIPC* with HDL-C levels (P-values $<5x10^{-8}$), SNPs near *APOE*, *CELSR2/PSRC1/SORT1*, and *PCSK9* with LDL-C levels (P-values $<5x10^{-8}$), SNPs near *TCF7L2* with type-2 diabetes (P-values $<5x10^{-6}$), and SNPs in the 9p21 region with CHD (P-values $<5x10^{-4}$). Taking into account local ancestry also allowed us to identify large admixture signals for type-2 diabetes over regions on chromosome 1p32 and 15q21 (P-values $<1x10^{-6}$) and for hypertension over a region on chromosome 1q24 (P-values $<5x10^{-5}$). Importantly, these signals would have been missed using only genotype information. We are currently conducting a replication study in additional African-Americans to test these and other new loci associated with CHD or its main risk factors.

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## 2366/T/Poster Board #915

**Longitudinal Genome-wide Association of Cardiovascular Disease Risk Factors in The Bogalusa Heart Study.** *E.N. Smith[1], M. Shaw[2], R. Salem[2], W. Chen[3], S. Srinivasan[3], E.J. Topol[1], N.J. Schork[1], G.S. Berenson[3], S.S. Murray[1].* 1) Scripps Genomic Med, Scripps Translational Sci, La Jolla, CA; 2) University of California, San Diego, CA; 3) Tulane University, New Orleans, LA.

A large benefit of using longitudinal data as opposed to cross-sectional data is that one can discriminate genetically-mediated developmental or aging-related profiles. The study of such profiles can lead to the identification of risk profiles of susceptible individuals before disease onset. For example, cross-sectional data results may only determine variation associated with BMI (i.e., genotype-specific main effect), but may not be able to determine variants associated with the development of obesity over time (i.e., SNP by age interaction effect). The discovery of variants associated with SNP by age effects could be used to screen asymptomatic individuals and provide the opportunity for implementation of preventive measures before disease onset. Previous genome-wide association (GWA) studies have found numerous associations with CVD and its risk factors in cross-sectional data. We are currently performing a GWA study on CVD risk factors using longitudinal data from >1200 individuals in the Bogalusa Heart Study (BHS), a population-based bi-racial cohort with some individuals having as many as 15 CVD risk factor screenings from childhood to adulthood, spanning over 30 years. We have genotyped these individuals using Illumina's Human610 and HumanCVDv2.0 genotyping BeadChips and have used previously associated markers to develop quantitative predictors of risk factor profiles. We show that for many traits, these scores are significant predictors of the traits over time in European-American individuals, with generally consistent effects from childhood through adulthood. In African-American (AA) individuals, scores could be less predictive, indicating a need for more genetic studies in AA individuals. In an effort to find loci that vary in effect over time, we performed longitudinal GWA using mixed models and describe loci associated with traits over time (SNP main effects) and those with effects that vary over time (SNP by age interaction effects). We reproduce a number of previously characterized associations and show that in addition to a SNP main effect on triglyceride levels, we also see a SNP by age interaction at the 11q23.3 locus (rs12280753, $p = 2 \times 10^{-8}$) containing the APOA1/C3/A4/A5 genes, which has previously been associated with triglyceride levels. Overall, our GWA study on longitudinal data provides insight into the genetic basis of how individuals develop disease over time, from childhood into adulthood.

## 2367/T/Poster Board #916

**Pathway-based genome-wide association analysis of coronary heart disease identifies biologically important gene-sets.** *W. Yang[1], L. de las Fuentes[2], V.G. Dávila-Román[2], C.C. Gu[1,3].* 1) Department of Biostatistics, Washington University, St Louis, MO; 2) Department of Medicine, Cardiovascular Division, Washington University, St Louis, MO; 3) Department of Genetics, Washington University, St Louis, MO.

Genome-wide association (GWAS) studies of complex diseases such as coronary heart disease challenge investigators on how to select relevant genetic variants among hundreds of thousands of markers being tested. While a selection strategy purely based on statistical significance of association tests will result in many false positives in GWAS, integrated analysis using information on learnt genetic pathways might help single out biologically important findings. In the present study, we apply a customized gene-set enrichment analysis (GSEA) method to the GWAS data from the Framingham Heart Study (500K SNPs, n=6,421) to evaluate enrichment of genetic association in a collection of 1,395 gene-sets for their contribution to coronary heart disease (CHD). In our method, the most significant SNP within a gene is selected as deputy and its association statistics is normalized to adjust for varying gene size; the gene-sets collection was constructed form known pathways in public-domain databases (KEGG, GO etc.). Among the 1,395 gene-sets, we found 25 with nominal p-values under 0.01, four of which are known for their role in CHD, including vascular genesis (GO:0001570), fatty acid biosynthetic process (GO:0006633), fatty acid metabolic process (GO:0006631) and glycerolipid metabolic process (GO:0046486). Interestingly, the 4 gene-sets involve 170 genes, but only 3 of the genes contain any SNP ranked among the top 100 from 404,467 SNPs in the single-SNP scan. We also detected novel gene-sets less known for their importance to CHD. Particularly, the RAC1 cell motility signaling pathway and the sulfur amino acid metabolic process (GO:0000096) showed most significant enrichment in association with CHD ($p<0.001$). Many genes in the RAC1 signaling pathway are actually important in diabetes and vascular diseases, including myosin and its targets (MYL2, MLCK, PIK3CB and KCNH2), and RAC1 was reported critical for hypertrophic response in the heart. On the other hand, many genes involved in the sulfur amino acid process are related to methylation (e.g., GCLC, GCLM, MTHFR, MTHFD1, MTR and the well-known CVD-associated homocysteine (BHMT)), which may reflect the potential role of epigenetics in the development of CHD. In summary, we showed that the pathway-based analysis can help prioritize association signals in GWAS studies; and the identified gene-sets point out plausible directions for down-stream search of genetic variants important to CHD.

## 2368/T/Poster Board #917

**Follow up genome-wide association study of intracranial aneurysms identifies novel risk loci and confirms previous findings.** *K. Yasuno[1,2], R.P. Lifton[3,4,5], M. Gunel[1,2,3,6], Genetics of Intracranial Aneurysm Trial Investigators.* 1) Neurosurgery, Yale University School of Medicine, New Haven, CT; 2) Program on Neurogenetics, Yale University School of Medicine, New Haven, CT; 3) Genetics, Yale University School of Medicine, New Haven, CT; 4) Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT; 5) Internal Medicine, Yale University School of Medicine, New Haven, CT; 6) Neurobiology, Yale University School of Medicine, New Haven, CT.

Intracranial aneurysms (IA) affect approximately 2% of the population and cause 500,000 hemorrhagic strokes annually in relatively young patients (median age 50), resulting in death and severe neurological impairment. The pathogenesis of aneurysm formation and rupture is unknown, and premorbid identification is essential to prevent catastrophic hemorrhage. We have recently completed a genome-wide association (GWA) study using 2,100 IA cases and 8,000 controls from Finland, the Netherlands and Japan, and identified 3 IA risk loci on chromosomes 2, 8 and 9 (Bilguvar et al. Nat Genet 40, 1472-1477, 2008). In order to increase the power to detect modest risk loci, we have conducted additional whole-genome genotyping for 1,000 German and 640 European IA cases using Illumina Human610 BeadChip, and enrolled >12,000 whole-genome genotyped controls from Germany (PopGen and KORA-gen), Finland (Health 2000 and NFBC1966) and iControlDB. As a replication cohort, we also involved an additional Japanese cohort (2,200 cases and 3,300 controls). As a discovery cohort, we analyzed the Finnish cohort and other European cohorts separately because of the clear ancestry difference. We then matched genetic ancestry between cases and controls utilizing the spectral clustering method (Lee et al. Genet Epidemiol, 2009). The association analysis was performed for each SNP genotyped across all the cohorts that passed the QC filters (> 260,000) by applying the conditional logistic regression for ancestry-matched data. Two new loci showed genomic-control corrected p-values < 5x10-7 and were replicated in two Japanese cohorts.

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## 2369/T/Poster Board #918

**Genome wide association(GWA) meta-analyses identify loci affecting risk of young onset type 2 diabetes(T2D) and age of onset of the disease.** *M. van Hoek[1], E. Zeggini[2, 3], A.D. Morris[2], R. Welch[4], V. Steinthorsdottir[5], K. Strassburger[6], S. Cauchi[7], V. Lyssenko[8], C. Dina[7], E.J.G. Sijbrands[1], G. Thorleifsson[5], Y.S. Aulchenko[9], L.J. Scott[4], P. Almgren[8], C.M. van Duijn[9], T.M. Frayling[10, 11], M. Boehnke[4], M.I. McCarthy[2, 12] for the DIAGRAM+ consortium.* 1) Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, Netherlands; 2) The Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford, UK; 3) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK; 4) Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan, USA; 5) deCODE genetics, 101 Reykjavík, Iceland; 6) Institute of Biometrics and Epidemiology, German Diabetes Center, Leibniz Institute at Heinrich-Heine-University, Düsseldorf, Germany; 7) CNRS-8090-Institute of Biology, Pasteur Institute, Lille, France; 8) Department of Clinical Sciences, Diabetes and Endocrinology, Lund University, University Hospital Malmo, Malmo, Sweden; 9) Department of Epidemiology and Biostatistics, Erasmus University Medical Center, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands; 10) Genetics of Complex Traits, Peninsula Medical School, Exeter EX1 2LU, UK; 11) Diabetes Genetics, Institute of Biomedical and Clinical Science, Peninsula Medical School, Barrack Road, Exeter EX2 5DW, UK; 12) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Oxford OX3 7LJ, UK.

Genome Wide Association (GWA) studies have resulted in a rapid increase of replicated genetic association signals for T2D. However, recognition of disease subtypes provides opportunities towards better prediction and clinical management. A number of studies have shown that strong family history predisposes to earlier age of onset. This may reflect heterogeneity in the genetic background of T2D. We hypothesized that different genetic association patterns operate in early onset T2D. Furthermore, we hypothesized that genetic loci act as modifiers of age of onset. To investigate these associations, we performed meta-analyses on GWA data of the DIAGRAM+ consortium. We analyzed GWA data of 7 studies of European descent(deCODE, DGDG, DGI, FUSION, KORA, the Rotterdam Study and WTCCC). First, we performed a meta-analysis on young onset T2D in 1,317 cases with ages of diagnosis<45 (excluding type 1 diabetes and monogenic forms) and 29,470 controls.(effective sample size 5,088) Secondly, we performed a meta-analysis on age of diagnosis as a continuous trait in 7,773 cases. The strongest meta-analysis signal for young onset T2D was *TCF7L2*(p= $6.3x10^{-27}$) and another 7 of the 17 previously known T2D susceptibility loci had p-values<$10^{-5}$. Excluding previously-proven associations, we identified five novel loci putatively associated with young onset T2D(p-values from $6.0x10^{-7}$ to $2.6x10^{-6}$.) The top finding was located on chromosome 13q33 (near *LOC728215*, p=$6.0x10^{-7}$) and four further loci were found on chromosomes 4q24, 6p12,10q22, and 12q12. For the continuous trait meta-analysis, none of the previously found T2D associated loci had P<$10^{-5}$. The five top continuous trait signals exhibited pvalues ranging from $1.7x10^{-6}$ to $4.9x10^{-6}$. The top result was located on chromosome 1p(near *AMPD1*, p=$1.7x10^{-6}$), and four other loci were found on chromosomes 5q15,11p14, 12q21 and 13q14. BMI adjustment did not change the results. In conclusion, we found 10 new loci associated with young onset T2D and age of onset.(meta-analysis p-value<$10^{-5}$ in at least five studies). Replication must reveal the robustness of the associations. The first replication set will consist of approximately 1,100 young onset cases (age of diagnosis<45) versus 1,100 controls and the second set will consist of approximately 8,000 cases with a wide range of ages of onset. Eventually, our findings are expected to make a valuable contribution to our understanding of the disease and its phenotypic appearances.

## 2370/T/Poster Board #919

**Coronary ARtery DIsease Genome-wide Replication And Meta-Analysis (CARDIoGRAM) - Design of a prospective meta-analysis of 14 genome-wide association studies.** *I.R. König on behalf of CARDIoGRAM.* Institut für Medizinische Biometrie und Statistik, Universität zu Lübeck, Germany.

Coronary artery disease (CAD) is the leading cause of death in U.S. and Europe and is a heritable trait. Classical genome-wide association studies (GWAS) have uncovered at least 13 common alleles associated with CAD. However, each variant confers a modest effect, and together, the variants explain a small fraction of inter-individual variation only. These observations suggest that additional loci harboring CAD-associated variants might be discoverable with larger samples and improved statistical power. To accomplish this, we assembled the CARDIoGRAM Consortium that pools GWAS data from ADVANCE, CADomics, CHARGE, deCode, GerMIFS I-III (KORA), LURIC/AtheroRemo, MedStar/PennCath, MIGen, OHGS, and the WTCCC. In total, the consortium comprises more than 22,000 well-characterized cases with CAD and more than 60,000 controls mostly from population-based studies. In each individual study, genome-wide genotyping was carried out on either Affymetrix or Illumina platforms, and imputation was conducted to generate genotypes for ~2.2 million SNPs in each study. With the aim of conducting a type II meta-analysis using GWAS results from the single studies and consortia, standard operating procedures were generated in order to harmonize the data analyses. Extensive quality control was performed both study-wise and centrally so as to provide standardized data formats of high quality. With the assembled sample size, the estimated power to detect modest effects is substantially increased. For instance, even for genome-wide significance, the power is about 80% for an odds ratio of 1.1, provided that the minor allele frequency is at least 10%. Meta-analyses for the CAD phenotype as well as for important subgroups including myocardial infarction and early-onset CAD will be carried out. Following the initial evaluations, wet lab replication genotyping of top results will be sought in > 15,000 additional cases and 15,000 controls. CARDIoGRAM brings together an enormous wealth of GWAs data on CAD and MI, thus representing the largest study to date to uncover the inherited basis for the leading public health problem in the industrialized world.

## 2371/T/Poster Board #920

**Genome-Wide Association Study of Anatomic Coronary Artery Disease.** *G.W. Beecham[1], D. Seo[2], K. Hamilton[1], I. Konadari[1], J.R. Gilbert[1], P. Goldschmidt-Clermont[2], M.A. Pericak-Vance[1].* 1) Miller School of Medicine, Institute of Human Genomics, University of Miami, Miami, FL; 2) Miller School of Medicine, University of Miami, Miami, FL.

Coronary heart disease is the leading cause of death in the United States, with atherosclerosis of the coronary arteries being the major underlying etiology. Patients with atherosclerotic coronary artery disease (CAD) are more likely to suffer a myocardial infarction or sudden cardiac death. CAD is highly heritable, but there have been few genetic studies of anatomic CAD and the knowledge of CAD genetics is incomplete. To further determine the genetic etiology of anatomic CAD, we have performed the first genome-wide association study of anatomic coronary artery disease, using our dataset of 900 cardiac catheterization patients. Each patient received cardiac catheterization and was assigned a CAD burden index which measures their overall anatomic CAD burden based on size and location of coronary lesions. Data were generating using the Affymetrix SNP array 6.0 platform for 900,000 SNPs. Extensive quality control tests were performed to ensure the integrity of the data, including Eigenstrat methods to correct for population substructure. We tested for association with linear regression using the PLINK software package, and included smoking, cholesterol, blood pressure, age, sex, and two vectors describing the population substructure of the sample. We detected strong association ($p < 0.00001$) on 14 different chromosomes, in 10 different genes. Among these are the largely uncharacterized PRUNE homologue gene on chromosome 1 (p-value = 8 x 10-7) and the citron (rho-interacting, serine/threonin kinase 21) gene on chromosome 12. These signals are being validated in an independent dataset of 1100 CAD patients. These data provide promising results that may lead to further insights into the genetics of anatomic coronary artery disease.

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## 2372/T/Poster Board #921

**A Genome-wide association study identifies new loci for ACE activity: potential implications for response to ACE inhibitor.** *C. Chung[1,2], R. Wang[3], J. Chen[4], C. Fann[1], H. Leu[5], H. Ho[6], C. Ting[6], T. Lin[7], S. Sheu[7], W. Tsa[8], J. Chen[8], Y. Jong[9], S. Lin[5], Y. Chen[1], W. Pan[1].* 1) Inst Biomedical Sci, Academia Sinica, Taipei, Taiwan; 2) Division of Molecular Medicine, Institute of Public Health, School of Medicine, National Yang-Ming University, Taipei, Taiwan; 3) Department of Public Health, China Medical University, Taichung, Taiwan; 4) Cardiovascular Research Center, National Yang-Ming University, Taipei, Taiwan; 5) Taipei Veteran General Hospital, Taipei, Taiwan; 6) Taichung Veteran General Hospital, Taichung, Taiwan; 7) Kaohsiung Medical Center, Kaohsiung, Taiwan; 8) College of Medicine, National Cheng Kung University, Tainan, Taiwan; 9) Department of Cardiology, Tao-Yuan General Hospital Department of Health, Taiwan.

Introduction: Angiotensin converting enzyme (ACE) is a key enzyme of the famous renin-angiotensin-aldosterone system and pivotal in blood pressure regulation and other physiological conditions. Because ACE activity is implicated widely in biological systems, we aimed to identify its novel quantitative trait loci for the purposes of understanding ACE activity regulation and pharmacogenetics relating to ACE inhibitor (ACEI). Methods: We performed a two-stage genome-wide association study using Illumina HumanHap550 SNP Chip information from 400 young-onset hypertension (YOH) subjects in the first stage, confirming the findings with an additional 623 YOH subjects in the second stage, and replicating the results in a separate YOH family studies (428 hypertension pedigrees). Results: Eight SNPs of the ACE structural gene were significantly associated with ACE activity (-log p value range, 8.9-19.0). SNP rs4343 in exon17 near the well-known Insertion/deletion polymorphism had the strongest association. The second-stage confirmatory study verified significantly four of these SNPs: rs4343 in ACE gene (-log p =24.52), rs495828 (-log p =7.45) and rs8176746 (-log p =3.42) in ABO gene and rs1015870 in PCDH9 gene (-log p = 2.11). We further replicated the association between ABO blood types and ACE activity in the independent family study, and demonstrated a potential differential blood pressure response to ACEI in subjects with varied numbers of ACE activity-raising alleles. Conclusion: These findings may broaden our understanding of the mechanisms controlling ACE activity and advance our pharmacogenetic knowledge on ACEI.

## 2373/T/Poster Board #922

**A genome-wide association study identifies a potential locus near PKHD1 that might increase aldosterone renin ratio in Taiwanese young-onset hypertension patients.** *TY. Kuo[1], KM. Chiang[1], HY. Ho[2], CT. Ting[2], TH. Lin[3], SH. Sheu[3], WC. Tsai[4], JH. Chen[4], HB. Leu[5], WH. Yin[6], TY. Chiu[7], CI. Chen[7], SJ. Lin[5], JW. Chen[5], WH. Pan[1].* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Taichung Veterans General Hospital, Taichung, Taiwan; 3) Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan; 4) National Cheng Kung University Hospital, Tainan, Taiwan; 5) National Yang-Ming University School of Medicine and Taipei Veterans General Hospital, Taipei, Taiwan; 6) Cheng Hsin Rehabilitation Medical Center, Taipei, Taiwan; 7) Min Sheng General Hospital, Taoyuan, Taiwan.

Primary aldosteronism is the most common secondary cause of hypertension and has much higher prevalence rate among hypertension patients than previously thought. High plasma aldosterone and low plasma renin concentrations are the main characteristic of primary aldosteronism. Therefore the ratio of plasma aldosterone concentration and plasma renin activity is a widely accepted screening test for primary aldosteronism. We performed a genome-wide association study for identifying genetic variants that might influence Aldosterone-renin ratio (ARR) We genotyped 560,184 SNPs using Illumina 550K SNP Array in 400 hypertensive patients aged between 20 and 50 from Taiwanese population. ARR was calculated for each patient with plasma aldosterone concentration (PAC) as the numerator and plasma renin activity (PRA) as the denominator. Of these patients, individuals with ARR>=40 (ng/dl)/(ng/ml/hr) were considered as cases (n=75) and those with ARR<25 and were considered as controls (n=285). Single SNP tests and haplotype-based tests of association were used for statistical analysis. We identified two SNPs, RS9473992 (P=1.91x10-8) and RS6928877 (P= 2.73x10-7) on 6p12.2 near PKHD1, that were significantly associated with the increase of ARR. A common haplotype that contains the risk alleles of the two SNPs was also identified. Fisher exact probability test for this haplotype shows significant association by haplotype analysis before Bonferroni correction (P=2.61x10-7). However, this association was no longer significant if we considered aldosterone or renin concentrations alone in our study. In addition to PKHD1 gene that is associated with polycystic kidney and hepatic disease, a coding sequence similar to Wilms tumour 1-associating protein was also nearby. For verifying the two SNPs, we are going to use another independent set of samples with 150 cases (high ARR) and 353 controls (low ARR) based on the same criteria. As far as we know, this study is the first genome-wide association study using ARR as a quantitative trait for hypertension.

## 2374/T/Poster Board #923

**Genome-wide association scan identifies multiple loci for plasma levels of protein C in the ARIC cohort.** *W. Tang[1], S. Basu[2], A.R. Folsom[1], X. Kong[2], J.S. Pankow[1], N. Aleksic[3], E. Boerwinkle[4].* 1) Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN; 2) Division of Biostatistics, University of Minnesota, Minneapolis, MN; 3) DNA Laboratory, University of Texas Health Science Center at Houston, Houston, Texas; 4) Human Genetics Center, University of Texas Health Science Center at Houston, Houston, Texas.

Protein C is a vitamin K-dependent plasma protein zymogen that, upon activation, functions as an important anticoagulant and also exerts anti-inflammatory, anti-apoptotic, and endothelial barrier stabilization effects. Genetic deficiencies in protein C are associated with increased risk of venous thromboembolism. To map DNA sequence variants influencing plasma levels of protein C, we performed a large-scale, genome-wide association study among 8,048 participants of European ancestry in the Atherosclerosis Risk in Communities (ARIC) Study. A genome-wide scan was conducted with the Affymetrix SNP array 6.0 and imputed to ~2.5 million HapMap SNPs. A linear regression was conducted and adjusted for age, sex, and field center in an additive genetic model. Dosage information for imputed SNPs was incorporated into the regression analysis. The genomic control coefficient was 1.04, suggesting negligible test statistic inflation. A total of 513 SNPs exceeded the genome-wide significance threshold of 5.0E-8 and marked 3 loci on chromosomes 2 and 20: 2p23 (spanning 208K bp, smallest p-value 2.0E-17), 2q13-q14 (spanning 435K bp, 3.8E-36), and 20q11 (spanning 3.3M bp, 2.7E-203). At the 2p23 region, 5 genes were revealed and the SNP with the smallest p-value was from the glucokinase (hexokinase 4) regulator (GCKR) gene, a novel locus for protein C; at the 2q13-q14 region, 6 genes were revealed and the SNP with the smallest p-value was from the protein C structure gene (PROC); at the 20q11 region, 38 genes were revealed and the SNP with the smallest p-value was near the activated protein C receptor gene (PROCR), also called endothelial cell protein C receptor (EPCR); this SNP is in high linkage disequilibrium with a PROCR SNP (missense variant) that showed similar p-value (2.0E-200). The protein C receptor serves as a receptor for activated protein C and further enhances the activation of protein C. After adjustment for the PROCR SNP, 4 genes at the 20q11 region remained genome-wide significant. In summary, our study identified both new and known genetic loci influencing plasma levels of protein C in the general population. These findings may provide important information for prevention and treatment of disorders in which activated protein C is implicated.

## 2375/T/Poster Board #924

**Genome-wide association analysis identifies loci of Sudden Unexplained Death Syndrome (SUDS).** *P. Teekakirikul[1], S. Sangwatanaroj[2], S.R. DePalma[1], J.M. Gorham[1], B. McDonough[1,3], J.G. Seidman[1], C.E. Seidman[1,3,4].* 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Division of Cardiovascular Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; 3) Cardiovascular Division, Brigham and Women's Hospital, Boston, MA; 4) Howard Hughes Medical Institute, Boston, MA.

Sudden Unexplained Death Syndrome (SUDS), a leading natural cause of death in young Southeast Asian men, has been shown with the significant familial aggregation; however, genetic etiology has not been clarified. This fatal arrhythmic syndrome shares the same ECG pattern as Brugada Syndrome, an inherited heart disorder with autosomal dominant pattern. To date, Brugada syndrome has been related to cardiac sodium channel gene (*SCN5A*) mutation. To identify the genetic variants of SUDS, we used both candidate gene and genome-wide approaches in this study. We sequenced *SCN5A* in 20 Southeast Asian SUDS survivors and detected no genetic variants that account for SUDS. We further performed genome-wide association study using Affymetrix 250K Sty SNP Chip based on these Southeast Asian SUDS cases (n=20) and the Asian population frequency data from the Hapmap (n=90). Of 238,304 SNPs, only two significant SNPs clustered on Chromosome 1 (1p13.3), rs6697697 and rs6686425, demonstrated marked variation in genotype frequency between cases and controls (Bonferroni-corrected *P* value: 0.003 and 0.006, respectively). The risk alleles of two correlated SNPs were strongly associated with SUDS: 1) rs6697697 with odds ratio of 8.06 (3.649-17.78) and 2) rs6686425 with odds ratio 7.82 (3.583-17.06), respectively. These two polymorphisms were then successfully validated using re-sequencing and Sequenom iPLEX Genotyping. We therefore focused on this first putative region. To refine the association, we proceeded to genotype additional 49 SNPs in the 222-kilobase region and detected a single haplotype across this region. Haplotype analysis showed the significant association with SUDS (*P* value = $1.68 \times 10^{-7}$). All SNPs in this genomic region were under Hardy-Weinberg Equilibrium. This linkage disequilibrium block contains two spliced expressed sequence tags (ESTs) that are expressed in human testicular tissues. In summary, we reported a single haplotype in the 1p13.3 region revealed by genome-wide scan that is associated with SUDS and warrants further investigation.

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## 2376/T/Poster Board #925

**Replication of Caucasian GWAS lipid signals in Mexicans.** *D. Weissglas[1], A. Huertas-Vazquez[1], L. Riba[2], T. Tusie-Luna[2], C. Aguilar-Salinas[3], P. Pajukanta[1]*. 1) Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA; 2) Molecular Biology and Genomic Medicine Unit, Instituto de Investigaciones Biomédicas de la UNAM, INCMNSZ, Mexico City, Mexico; 3) Department of Endocrinology and Metabolism, INCMNSZ, Mexico City, Mexico.

**Background:** Cardiovascular risk factors such as unfavorable serum lipid levels are highly common among Mexicans and increasing at alarming rates. Previous epidemiological studies have specifically demonstrated an increased predisposition to low high-density lipoprotein cholesterol (HDL-C) and high serum triglyceride (TG) levels in the Mexican population. However, no genome-wide association studies (GWAS) for lipids have been performed in Mexicans. **Methods and Results:** We genotyped 15 SNPs associated with plasma HDL-C and/or TG levels in recent Caucasian GWAS in Mexican familial combined hyperlipidemia families and hypertriglyceridemia case-control study samples. These variants were within or near the genes ANGPTL3, GALNT2, GCKR, APOB, LPL (2), XKR6-AMAC1L2, TRIB1, ABCA1, MTNR1B, NCAN, APOA1/C3/A4/A5, MMAB/MVK, LIPC and LCAT. We performed a combined analysis of the family-based and case-control studies (n = 2,348) using the Fisher's method for combining p-values. We observed significant association with $P < 0.01$ at four of the 15 loci (APOA1/C3/A4/A5, GCKR, GALNT2 and LIPC). These signals were for the same trait and risk allele as in Caucasians. Interestingly, our strongest signal was obtained for TGs with the rare allele of rs964184 ($P = 1.7 \times 10^{-10}$) in the APOA1/C3/A4/A5 cluster that was more common in Mexicans (34%) when compared to Caucasians (14%). Furthermore, the common risk allele of rs10468017, near the LIPC gene, was also more prevalent in Mexicans (85%) than in Caucasians (70%). **Conclusions:** We show replication of four Caucasian GWAS lipid signals in Mexicans. The remaining loci will require a comprehensive investigation to exclude or verify their significance in Mexicans. We also demonstrate that two of these susceptibility variants are more prevalent in the Mexican population and may confer to the increased predisposition to dyslipidemias in Mexicans.

## 2377/T/Poster Board #926

**Genome-wide association study on systolic blood pressure measures in the Framingham Heart Study.** *B. Kerner[1], J. Kong[1], B.O. Muthén[2].* 1) Ctr Neurobehavioral Gen, Univ California, Los Angeles, Los Angeles, CA; 2) Professor Emeritus, University California, Los Angeles, Los Angeles, CA.

The physiology and patho-physiology of blood pressure regulation is highly complex. In order to better understand this regulatory network researchers have undertaking the task to identify genomic variants that might be associated with the variance in blood pressure measures in the general population using genome-wide association studies in ten thousands of individual. Given the complexities of blood pressure regulation it is highly likely, however, that population samples are heterogeneous. Genetic factors that carry risk for blood pressure elevation early in life might be different from those influencing high blood pressure later in life. Physiologic blood pressure variation in normal individuals might have different genetic contributions than those found in high risk individuals. Growth mixture modeling is a less explored method in genetic research to address unobserved heterogeneity in population samples. Here, we applied this technique to longitudinal data of the Framingham Heart Study. We examined systolic blood pressure measures in 1060 males in 692 families and detected three subclasses, which varied significantly in their developmental trajectories over time. The first class consisted of 60 high-risk individuals with elevated blood pressure early in life and a steep increase over time. The second group of 131 individuals displayed first normal blood pressure, but reached high blood pressure values late in their life time. The largest group of 869 individuals could be considered a normative group with normal blood pressure on all exams. In order to identify genetic modulators for this phenotype we tested for genome-wide association between class membership probability and single nucleotide polymorphisms using the Affymetrix 500k array. Class 1 membership probability was significantly associated with SNPs in 23 genes ($p<5 \times 10^{-8}$). Six genes contained multiple significant associations including KANK1 (9p24.3), AGPAT5 (8p23.1), CREB5 (7p15.1), GULP (2q32.3-33), CAPN13 (2p22-p21), and LIPN (10q23.31). Class 2 membership probability was significantly associated with an intronic SNP in SGPL1 (10q21). A limitation of our study is the small sample size of the high risk group. These results await replication; however, they suggest that analyzing genetic data stratified for high-risk subgroups defined by a unique development over time could be useful for the detection of rare mutations in common multi-factorial diseases.

## 2378/T/Poster Board #927

**The Genetics, Exercise and Research(GEAR)Study: A new paradigm for targeting fitness through genetics.** *J. Farmer, D. Tekin, T. Musto, D. Vance, M. Slifer, D. Seo, P. Goldschmidt-Clermont, P. Vance, E. Rampersaud.* University of Miami, Miller School of Medicine, Miami, FL.

Research approaches to better understand the role of individual genetic variation in response to physical activity is a crucial step towards development of personalized clinical medicine. By revealing how genetics and physical activity are associated with health related outcomes, clinicians can develop preventative approaches for the treatment of at-risk asymptomatic individuals. The potential value of physical activity has been demonstrated in prevention of cardiovascular diseases, depression, and dementia. Although some studies have examined the modifying effects of genetics on exercise response in clinical patient populations, very little is known about the relationship between genetics and physical activity among healthy sedentary individuals. To better characterize this relationship we must develop new paradigms for exploring the role of genetics as a component of medical exercise prescriptions. The goal of this project is to define genetic profiles that are associated with varying levels of response to physical activity in the general population. Thus we assembled a multidisciplinary research team consisting of exercise trainers and physiologists, cardiologists, geneticists, molecular biologists, epidemiologists and explored the use of a university wellness center program to answer these questions. Specifically, we have developed the Genetics, Exercise and Research (GEAR) study, a unique, comprehensive approach to understanding the genetics of fitness. GEAR enrolls healthy sedentary individuals to participate in a 12-week exercise training program who undergo a baseline health related fitness (HRF) assessment including (1) cardio-respiratory fitness (CR) measured by the modified Balke treadmill protocol, (2) musculoskeletal fitness (MSK) measured by upper and lower body 1RM testing, and (3) body composition (BC) measured by a skin fold test. Each participant will provide a blood sample (fasting) at baseline and at the end of training for genetic analyses. Lipid and related biomarker profiles of cardiovascular health will also be measured. Questionnaires will be administered to collect information on demographics, family history, medical history, and lifestyle risk factors including dietary information. Physical activity over a 12-week period of time will be documented using a self-report log book with follow-up fitness assessments to evaluate the changes in HRF. Study design and implementation protocol along with baseline data will be presented.

## 2379/T/Poster Board #928

**Epistasis and the Genetic Architecture of Arterial Thrombosis in West Africans.** *N.M. Penrod[1], F.W. Asselbergs[2], K. Poku[3], J.H. Moore[1], S.M. Williams[4].* 1) Department of Genetics, Dartmouth College, Lebanon, NH; 2) University of Groningen, The Netherlends; 3) University of Ghana, Accra; 4) Vanderbilt University, Nashville, TN.

The enzymes, tissue plasminogen activator (t-PA) and plasminogen activator inhibitor 1 (PAI-1) act in concert to counterbalance thrombus formation and degradation in a homeostatic defense against the development of arterial thrombosis and excessive bleeding. PAI-1 production is upregulated by the renin-angiotensin system (RAS), specifically by angiotensin II the product of the angiotensin converting enzyme (ACE). Angiotensinogen (AGT) is the key substrate of this system being cleaved by renin (REN) to initiate these downstream enzymatic reactions. Upon inhibition, ACE indirectly stimulates the release of t-PA which, in turn, activates the corresponding fibrinolytic system. Polymorphisms in these pathways have been shown to significantly impact the plasma levels of t-PA and PAI-1 differentially between males and females in a large-scale population-based sample from urban West Africa. Here we explore the involvement of epistatic interactions between the same polymorphisms in central genes of the RAS and fibrinolytic systems on plasma t-PA and PAI-1 levels within the same population (n=992). Statistical modeling of epistatic interactions between polymorphisms in the ETNK2, REN, ACE, PAI-1, t-PA, and AGT genes was carried out using two-way ANOVA to assess their interaction effects on plasma levels of t-PA and PAI-1. We found the strongest interactions between polymorphisms of REN with the TPA I/D variant (p=0.001) for females and the PAI 4G/5G variant (p=0.009) for males, indicative of PAI-1 levels. Similarly, t-PA levels associated with ETNK2 variations in association with the PAI 4G/5G variant (p=0.029) for females and the REN G/T polymorphism (p=0.024) for males. These results represent only a few of the many significant interactions detected in this analysis. Strikingly, many more interactions were found in this African population than in a previously published study of Caucasians from the Netherlands. The outcome of this analysis confirms the notion that the underlying genetic architecture of cardiovascular disease is complex and, as such, it is necessary to consider the relationship between interacting polymorphisms of the pathway specific genes that predict t-PA and PAI-1 levels.

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## 2380/T/Poster Board #929

**Diet Modifies the Myocardial Infarction Risk of Chromosome 9p21 Variants Globally.** *J.C. Engert[1,4], R. Do[1], X. Zhang[2], S. islam[2], S.D. Bailey[2], S. Ragarajan[2], M.J. McQueen[2], X. Wang[3], S. Yusuf[2], S.S. Anand[2] on behalf of the INTERHEART investigators.* 1) Department of Human Genetics, McGill University, Montréal, QC, Canada; 2) Population Health Research Institute, Hamilton Health Sciences and Departments of Medicine and Clinical Epidemiology, McMaster University, Hamilton, ON, Canada; 3) Laboratory of Human Genetics, Beijing Hypertension League Institute, Beijing, China; 4) Department of Medicine, McGill University, Montréal, QC, Canada.

Background: Multiple studies have confirmed that genetic variants in the chromosome 9p21 locus are associated with coronary heart disease (CHD) in several populations. However, interactions of these variants with environmental factors have not been extensively explored. Methods: We genotyped four SNPs at the 9p21 locus in 1,744 Europeans, 1,867 South Asians, 2,292 Chinese, 1,100 Latin Americans and 1,172 Arabs (n=8,175 total) from the INTERHEART study. We assessed the association of 9p21 with myocardial infarction (MI), and tested for interaction with environmental factors including three dietary pattern scores derived from a 19 food item dietary questionnaire. Results: All four chromosome 9p21 variants were in linkage disequilibrium and associated with MI in the combined sample that included all ethnicities (1.18?OR?1.20, 7.42x10-9?P?2.83x10-7). All four SNPs were associated in Europeans (1.17?OR?1.18, 0.016?P?0.024), South Asians (1.22?OR?1.27, 0.0003?P?0.0025), and Chinese (1.18?OR?1.22, 0.0009?P?0.0056) and three were associated in Latin Americans (1.22?OR?1.32, 0.0066?P?0.029)_ while Arabs displayed a non-significant trend for all four SNPs (1.04?OR?1.12). A significant interaction was observed with a prudent diet pattern score for all 4 SNPs, with rs2383206 having the strongest interaction on MI (P=0.0005). A pronounced effect of the G allele of rs2383206 on MI was observed in the group with a low prudent diet score (OR=1.34, P=$7.38x10^{-8}$), while the effect was diminished in a step-wise fashion in the medium (OR=1.17, P=0.0046) and high scoring groups (OR=1.03, P=0.57). The combination of a low prudent diet score and two copies of the risk allele was associated with a two-fold increase in risk for MI (OR=2.04, P=$2.45x10^{-10}$). Similar interactions were found with the level of raw vegetable intake, a major component of the prudent diet score. Conclusions: We demonstrate that diet modifies the risk of 9p21 on acute MI across multiple ethnic groups. These results enhance our understanding of the underlying pathophysiology of chromosome 9p21 variation and highlight the important interplay of genes and environment in the etiology of CHD.

## 2381/T/Poster Board #930

**Synergistic effect of polymorphisms of paraoxonase gene cluster and arsenic exposure on electrocardiogram abnormality.** *Y. Liao[1,2], W. Li[2], C. Chen[1,3], R. Prineas[4], W. Chen[1,5], Z. Zhang[4], C. Sun[2], S. Wang[2,6].* 1) Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, Taiwan; 2) Division of Environmental Health and Occupational Medicine, National Health Research Institutes, Taiwan; 3) Genomics Research Center, Academia Sinica, Taiwan; 4) Epidemiologic Cardiology Research Center, Department of Epidemiology and Prevention, Division of Public Health Sciences, Wake Forest University School of Medicine, NC, USA; 5) Genetic Epidemiology Core Laboratory, National Taiwan University Center for Genomic Medicine, Taiwan; 6) Institute of Environmental Medicine, College of Public Health, China Medical University Hospital, Taichung, Taiwan.

Arsenic has been linked to increased prevalence of cancer and cardiovascular disease (CVD), but the long term impact of arsenic exposure remains unclear. Human paraoxonase (PON1) is a high-density lipoprotein associated antioxidant enzyme which hydrolyzes oxidized lipids and is thought to be protective against atherosclerosis, but evidence remains limited to case-control studies. Only recently have genes encoding enzymes responsible for arsenic metabolism, such as AS3MT and GSTO, been cloned and characterized. This study was designed to evaluate the synergistic interaction of genetic factors and arsenic exposure on electrocardiogram abnormality. A total of 216 residents from three tap water implemented villages of previous arseniasis-hyperendemic regions in Taiwan were prospectively followed for an average of 8 years. For each resident, a 12-lead conventional electrocardiogram (ECG) was recorded and coded by Minnesota Code standard criteria. Eight functional polymorphisms of PON1, PON2, AS3MT, GSTO1, and GSTO2 were examined for genetic susceptibility to ECG abnormality. Among 42 incident cases with ECG deterioration identified among 121 baseline-normal subjects, arsenic exposure was significantly correlated with incidence of ECG abnormality. In addition, polymorphisms in two paraoxonase genes were also found associated with the incidence of ECG abnormality. A haplotype R-C-S constituted by polymorphisms of PON1 Q192R, -108C/T and PON2 C311S was linked to the increased risk. Subjects exposed to high levels of As (cumulative As exposure N14.7 ppm-year or drinking artesian well water N21 years) and carrying the R-C-S haplotype had significantly increased risks for ECG abnormality over those with only one risk factor. Results of this study showed a long term arsenic effect on ECG abnormality and significant gene-gene and gene-environment interactions linked to the incidence of CVD. This finding might have important implications for a novel and potentially useful biomarker of arsenic risk.

## 2382/T/Poster Board #931

**Apolipoproteinn AI and CIII gene polymorphisms among a sample of Healthy Kuwaiti's and CHD patients.** *S. Al-Bustan[1], M. Alnaqeeb[1], B. Annice[1], I. Al-Rashdan[2,3].* 1) Biological Sci, Faculty Sci, Kuwait Univ, Khalidya, Kuwait; 2) Department of Medicine, Faculty of Medicine, Kuwait Univ, Jabriya, Kuwait; 3) Chest Hospital, Al-Sabah Hospital Complex, Shuwaikh, Kuwait.

Apolipoproteins are responsible for the clearance of lipoproteins, failure of which may lead to the development of coronary heart disease (CHD. APOCIII and APOAI genes, components of the APOAI/CIII/IV/V gene cluster on chromosome 11q23-24, encode protein constituent of VLDL and chylomicron remnants, and HDL, respectively. Single nucleotide polymorphisms (SNP's) may alter their function affecting the transport and metabolism of lipoproteins possibly leading to atherosclerosis. In the last few decades CHD has become the leading cause of death in Kuwaitis, with a 33% increase in the mortality rate. Therefore, the objective of this study was to characterize genotypes and minor alleles and their potential association to CHD in the Kuwaiti population. APOCIII 3238C-G and the APOAI -2500C-T SNP's introducing an *Sst*I and *Xmn*I restriction sites, respectively were analyzed in 174 Kuwaiti CHD patients and 234 healthy controls most of which are of Arab ethnicity. Genotyping DNA samples was determined by PCR-RFLP of the *Sst*I and *Xmn*I restriction sites. Genotype and allele frequencies were determined by simple gene counting methods. Hardy-Weinberg Equilibrium and Linkage disequilibrium among the two polymorphisms were estimated for both groups using GENEPOP (Version 3.4). Preliminary analysis of genotype and allele frequencies at both loci in both the CHD (S1:0.834,S2:0.166; X1:0.828, X2:0.172) and healthy controls (S1:0.811,S2:0.189; X1:0.788,X2:0.212) were comparable to those reported in other populations and were found to be in Hardy-Weinberg equilibrium (p>0.05) indicating no direct association to the increased risk of developing CHD in the Kuwaiti population. However, the presence of a strong family history of heart disease in the Kuwaiti patients in addition to the fact that the two polymorphisms were found to be in strong linkage disequilibrium (p=0.006) in the normal controls but not in the patient group (p=0.281) may suggest a genetic component interacting with plasma lipid transport and metabolism in increasing the risk of CHD in the Kuwaiti population. Investigating potential direct or indirect involvement of minor alleles in patients could reflect the interaction of the the highly prevalent dyslipidemia, obesity, sedentary life-style with the diverse ethnicity in Kuwait to promote CHD. Further analysis of other relevant variables such as plasma lipid levels, BMI, ethnicity and a larger sample pool will be required and is currently being analyzed.

## 2383/T/Poster Board #932

**Natural history of the Danon disease phenotype in a large affected population.** *D. Boucek, J. Jirikowic, C. Wells, L. Mestroni, M. Taylor.* University of Colorado Denver, Aurora, CO.

**Background:** Danon disease (OMIM#300257) is a rare X-linked disorder that was originally described in boys affected with cardiomyopathy, skeletal myopathy, and intellectual dysfunction. Subsequent reports of affected females with cardiomyopathy and cardiac conduction problems showed that the disease has X-linked dominant inheritance although males are clearly affected earlier and with greater severity. To date, the majority of reports have focused on the severe nature of the cardiomyopathy in single families or small case series (largest previous case series = 13 families). **Methods:** We developed a Danon disease registry and a patient-information website (www.danondisease.org) under an IRB protocol in order to identify multiple families affected by Danon disease. Family members are evaluated in person or interviewed by telephone. Reported medical data and family history data are collected and reviewed and we ask about cardiac and non-cardiac signs and symptoms of Danon disease. **Results:** To date we have been contacted by 40 families with confirmed or suspected Danon disease with the largest pedigree having 14 affected individuals. Based on our analysis of available clinical data, the average age of first symptom development is 8.2 years and 24.4 years for males and females, respectively. The average age of diagnosis is 11.1 years and 33.7 years for males and females, respectively. Cardiomyopathy in boys is predominantly hypertrophic (75%), but dilated disease does occur (8%); in females 46% and 15% have hypertrophic or dilated cardiomyopathy, respectively. Cardiac conduction abnormalities occur in 75% of males and 61.5% of females. Muscle weakness is reported in 82% of males and, somewhat surprisingly, in 62% of females, suggesting that myopathy in females has been under-recognized previously. Extra-cardiac problems include: retinal pigmentary changes (52%), mild cognitive disability (100% males; 38% females), and abdominal complaints (~25%; including pain, diarrhea/constipation). **Conclusions:** Here we describe the broad phenotype of Danon disease based on data from 40 families, the largest cohort reported to date. Both males and females develop a phenotype with females being diagnosed approximately 2 decades after males. We also noted prevalent skeletal myopathy complaints and mild cognitive concerns in females that have not been reported previously.

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## 2384/T/Poster Board #933

**A cardiovascular lifestyle change program for heart disease reversal changes patterns of gene expression in peripheral blood.** *D. Ellsworth[1], J. Weyandt[1], B. Love[2], A. Burke[3], M. Haberkorn[3], H. Patney[1], R. Jordan[1], M. Vernalis[4].* 1) Integrative Cardiac Health, Windber Research Institute, Windber, PA; 2) Invitrogen, Carlsbad, CA; 3) Integrative Cardiac Health, Windber Medical Center, Windber, PA; 4) Integrative Cardiac Health, Walter Reed Army Medical Center, Washington, DC.

Heart disease is a leading cause of death, disability, and healthcare burden. Many cardiac patients receive surgical interventions and lipid-lowering drug therapy as the main component of their treatment, but interventions involving risk factor modification through comprehensive lifestyle changes also are effective in reducing traditional risk factors for cardiovascular disease and in slowing or reversing the progression of coronary atherosclerosis. Although lifestyle change is effective in improving traditional risk profiles, little is known about molecular responses that may form the basis for disease regression. The study population consisted of 74 individuals, 37 Caucasian participants who completed a prospective, nonrandomized intervention designed to stabilize or reverse progression of coronary artery disease through dietary changes, exercise, stress management, and group support and an equal number of nonintervention controls matched to patients based on age, gender, heart disease status, and diabetes status. Risk factors and risk for future coronary events were assessed over the course of one year. Global gene expression profiling was conducted on peripheral blood samples using Affymetrix U133A 2.0 arrays containing ~14,500 genes. Most patients showed significant (P<0.05) improvements in body mass index, lipids, blood pressure, and exercise capacity, as well as long-term cardiovascular risk compared to the control group. Over the course of one year, 1,418 genes showed significant changes in expression (P<0.05 and fold change >1.2) in lifestyle participants compared to only 7 genes among controls. Up-regulated genes outnumbered down-regulated genes by approximately two-fold. Functional ontologies of genes showing the most significant up-regulation included nucleic acid and protein binding, transcription regulation, and cellular assembly. Fundamental molecular changes occur during intensive cardiovascular lifestyle modification. Differentially expressed genes may contribute to improved vascular health through biological processes such as lipid metabolism, plaque stability, and fatty acid transport. Defining molecular responses to lifestyle modification programs may improve our understanding of heart disease and aid in the development of more effective treatments.

## 2385/T/Poster Board #934

**Refining molecular pathways leading to calcific aortic valve stenosis by studying gene expression profile of normal and calcified stenotic human aortic valves.** *Y. Bosse[1,2], D. Fournier[1], A. Pépin[1], P. Pibarot[1], P. Mathieu[1].* 1) Institut universitaire de cardiologie et de pneumologie de Québec, Laval University, Quebec, PQ, Canada; 2) Laval University Hospital Research Center (CRCHUL), Quebec, PQ, Canada.

Calcific aortic valve stenosis (AS) is a major societal and economic burden that is rising following the current shift toward an older population. Understanding the pathobiology of AS is crucial to implement better preventive and therapeutic options. Research conducted during the past decade clearly point to active molecular and cellular processes involved in disease pathogenesis. However, no genomic approaches were used to identify genes and pathways that are differentially regulated in aortic valves of patients with and without AS. A large-scale quantitative measurement of gene expression was performed on five normal and five AS valves using Affymetrix GeneChips. A total of 409 and 306 genes were significantly up- and down-regulated in AS valves, respectively. The two most highly up-regulated genes were matrix metalloproteinase 12 (MMP12) and chitinase 3-like 1 (CHI3L1). The up-regulation of these two biological relevant genes in AS was validated by real-time PCR in 38 aortic valves (12 normal and 26 AS). To provide a global biological validation of the whole-genome gene expression analysis, the microarray experiment was repeated in a second set of aortic valves with (n = 5) or without (n = 5) AS. There was an overrepresentation of small p values among genes claim significant in the first microarray experiment. A total of 223 genes were replicated (p value < 0.05 and fold change > 1.2), including MMP12 and CHI3L1. This study reveals many unrecognized genes potentially implicated in the pathogenesis of AS. These new genes were overlaid on known pathological pathways leading to AS in order to refine our molecular understanding of this disease.

## 2386/T/Poster Board #935

**Apolipoprotein C-III Deficiency Reduces Dyslipidemia in Subjects at Risk for Type 2 Diabetes.** *T.I. Pollin, C.M. Damcott, B.D. Mitchell, M. Miller, A.R. Shuldiner.* University of Maryland School of Medicine, Baltimore, MD.

We recently reported the first known null mutation, R19X, in the human *APOC3* gene, which encodes apolipoprotein C-III (apoC-III). ApoC-III delays triglyceride (TG) hydrolysis and hepatic uptake of TG-rich lipoprotein remnants. The mutation is found in ~5% of the Lancaster Old Order Amish, and carriers have low fasting and postprandial triglyceride (TG) and high HDL cholesterol levels as well as reduced risk of coronary artery disease as measured by coronary artery calcification scores.

A dyslipidemic lipid profile consisting of high TG levels and low HDL-C levels is a frequent complication of diabetes, and apoC-III production is dysregulated in the presence of insulin resistance. Therefore, we sought to determine whether the *APOC3* R19X mutation maintains its favorable effects on lipid levels in the presence of impaired glucose tolerance (IGT) or type 2 diabetes (T2DM) in 886 individuals in the Amish Family Diabetes Study. The frequency of the mutation was similar among individuals with T2DM (10/132, 7.6%), IGT (10/148, 6.8%) and normal glucose tolerance (NGT, 38/606, 6.3%). Although TG levels were higher among those with IGT and T2DM compared to those with NGT, the *APOC3* null mutation was associated with decreased TG levels in all three groups (NGT: median 34 vs. 66 mg/dl in carriers vs. noncarriers, p < 0.0001; IGT: 44 vs. 81 mg/dl, p < 0.0001; and T2DM: 75 vs. 111 mg/dl, p = 0.11). Similarly, HDL-C levels were increased in *APOC3* R19X carriers in all three groups (NGT: mean 63 vs. 51 mg/dl, p < 0.0001; IGT: 61 vs. 49 mg/dl, p = 0.008; and T2DM: 52 vs. 48 mg/dl, p = 0.22). In summary, heterozygous apoC-III deficiency improves dyslipidemia in the presence of an insulin resistant state, raising the possibility that apoC-III lowering might be a potentially useful treatment for reducing CVD risk in patients at risk for T2DM.

## 2387/T/Poster Board #936

**Survival- and age-variant genes in coronary artery disease.** *J. Dungan[1], E. Hauser[2], S. Shah[3], W. Kraus[3].* 1) School of Nursing, Duke University, Durham, NC; 2) Division of Medical Genetics, Deptartment of Medicine, Duke University, Durham, NC; 3) Division of Cardiology, Department of Medicine, Duke University, Durham, NC.

The American Heart Association reports that sudden death is the initial presentation of coronary artery disease (CAD) in 50-60% of people. CAD prevalence and mortality increase with age. We hypothesize that candidate genes for CAD may vary by survival and age, potentially biasing gene associations with CAD. We aimed to characterize survival bias and age effects for selected CAD candidate genes in the Duke CATHeterization GENetics Study and to determine if controlling for survival and age biases clarifies the strength of association between genes and CAD. We analyzed 1,885 subjects in the Duke CATHGEN cohort dataset. Thirty-six single nucleotide polymorphisms (SNPs) from 6 CAD candidate genes were selected to explore survival and age biases in CAD positive cases. To assess survival effects, traditional survival analyses were performed. Survival curves were illustrated using Kaplan-Meier rates with models censored on days to death or last follow up. Log-rank Chi-square tests and Cox proportional hazards were performed for comparison of survival by alleles (dominant model) and genotypes (additive model). To characterize age effects in gene variants, trends for minor allele frequencies (MAFs) by age categories (modified deciles) were evaluated. Logistic regression models were fit for CAD diagnosis (dependent variable) and each gene (independent variables), controlling for age, sex, race, and death (survival). Alpha was set at .05 for two-sided tests. Three SNPs corresponding to the limbic system associated membrane protein gene (LSAMP; RS6788787, RS1915585, and RS1462845) and two kalirin gene SNPS (KALRN; RS7623685 and RS1867647) showed significant differences in allele and/or genotype frequencies by survival. There were trends for lower MAFs in deceased subjects and varying MAF by age categories for the 5 SNPs. Three LSAMP SNPs had significant interactions with survival status: RS6788787 ($p$ = .02), RS1915585 ($p$ = .005) and RS9847048 ($p$ = .02). SNPs in the LSAMP and KALRN genes vary by survival and show trends for varying by age, providing evidence of survival and age effects in gene associations with CAD. Significant SNP by survival status interactions suggest that the effect of genotype on CAD is significantly mediated by survival. Controlling for survival and age in gene associations may be important to refining the genetic contribution to CAD. Our future work includes analyzing data for 5,571 more genotyped subjects from the CATHGEN cohort.

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## 2388/T/Poster Board #937

**Altered Body Composition and Elevated Levels of Circulating Plasma Leptin in The Vascular form of Ehlers Danlos syndrome.** *B. Griswold[1], J. Lehman[2], J. Napora[1], L. Sloper[2, 3], O. Carlson[1], N. McDonnell[2].* 1) Laboratory of Clinical Investigation, NIA, Baltimore, MD; 2) Clinical Research Branch, NIA, Baltimore, MD; 3) MedStar Research Institute, NIA, Baltimore, MD.

Vascular Ehlers- Danlos Syndrome (VEDS) is a hereditary disorder of connective tissue caused by mutations in procollagen III. Patients have reduced life expectancy due to arterial or organ rupture. In addition to well-described phenotypic manifestations such as joint laxity, severe scarring and characteristic facial features, we observed that persons with VEDS have an increase in abdominal fat despite normal or low body weight. Since leptin is a marker for increased abdominal fat, we hypothesized that circulating leptin levels in VEDS would be higher than in controls. We also obtained quantitative body composition data utilizing Dual energy X-Ray absorptiometry (DEXA). Plasma samples from 20 subjects with molecularly confirmed VEDS (Age 39 ± 10; 6 males and 14 females) and 20 age, sex and BMI matched controls were assayed for fasting plasma leptin levels utilizing an ELISA platform. Plasma leptin (mean ± SEM) in VEDS was significantly higher than in controls (VEDS: 19 ± 4.3 ng/dL ; controls: 9.1 ± 2.0 ng/dL; p< 0.01). Analysis of body composition data showed that trunk fat was significantly increased in the VEDS subjects as compared to controls (p <0.01) and limb fat/trunk fat ratio was significantly decreased (p < 0.001). Leptin is adipokine with complex interactions in the endocrine system and in the extracellular matrix, and is thought to be involved in the pathogenesis of vascular disease and to promote vascular inflammation in the normal population. Why VEDS subjects have increased truncal fat is not clear but it likely results in exacerbation of vascular damage in this group of patients who have underlying abnormalities of the arterial wall. Targeting the abdominal fat accumulation and increased leptin in VEDS may result in improved outcomes in this disorder for which no effective treatment is yet known.

## 2389/T/Poster Board #938

**Genetic Predisposition for coronary artery disease: A case control study in North Indian population.** *R. Tripathi[1], S. Agarwal[1], S. Tewari[2], P. Singh[3].* 1) Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sc, Lucknow, Uttar Pradesh, India; 2) Department of Cardiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India; 3) Department of Anaesthesiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India.

Background: The coronary artery disease (CAD) is a multifactorial disorder. The manifestation of the disease depends on interactions between environmental and genetic risk factors. Thus, the identification of gene polymorphisms relating to atherosclerosis may contribute towards developing early diagnostic methods and guiding preventive procedures. Objective: Aim of the present study is to find out the association of gene polymorphisms (MTHFR C677T, ACE I/D, LPL N291S, E-selectin S128R, OLR1 501G>C, IVS4-73C>T and 3'UTR 188C>T) with coronary artery disease. Method: 329 angiographically proven CAD patients as cases and 331 individuals with normal treadmill stress test having no history of CAD were included in the study. Genotype analysis was done by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). Result: We found MTHFR T allele frequency 7.3% in controls and 11.0% in patients (p=0.004, OR 1.75, 95% CI 1.16-2.55), the D allele is prevalent as 46.6% in control and 54.4% in patients for ACE I/D polymorphism (p=0.003, OR 1.38, 95% CI 1.10-1.72). Whereas, for LPL N291S gene the S allele frequencies were 2.2% and 5.1% in control and patients respectively (p=0.003, OR 2.52, 95% CI 1.29-4.98), the distribution of R allele for E-selectin gene was found 5.6% in controls and 9.6% in patients (p=0.031 OR 1.57 95% CI 1.21-2.91). The OLR1 (501G>C) C allele frequency was 9.8% in controls and 17.8% in patient group (p=0.00 OR 1.99 95%CI 1.42-2.78). Rest of the two polymorphism of OLR1 gene (IVS4-73C>T and 'UTR 188C>T) were insignificantly associated with coronary artery disease (p=0.66 OR 1.05 95% CI 0.84-1.32 and p=0.30 OR 1.13, (0.89-1.43). Interpretation: Our findings suggests that MTHFR C677T, ACE I/D LPL N291S, E-selectin S128R, OLR1 501G>C are significantly associated with CAD however the 3'UTR 188C>T and IVS4-73C>T is insignificant in association.

## 2390/T/Poster Board #939

**Association between Interleukin-10 Gene Polymorphism and Carotid artery Intima-media Thickness, Plaque.** *D. Shin[1,3], GI. Yu[1,3], SY. Lee[1,3], JJ. Lee[1,3], HK. Son[1,3], MY. Lee[1,3], CW. Nam[2,3], IS. Chung[1,3].* 1) PREVENTIVE MEDICINE, KEIMYUNG UIVERSITY SCHOOL OF MEDICINE, TAEGU, TAEGU, KOREA; 2) INTERNAL MEDICINE, KEIMYUNG UNIVERSITY SCHOOL OF MEDICINE, TAEGU, TAEGU, Korea; 3) INSTITUTE FOR MEDICAL GENOME RESEARCH KEIMYUNG UNIVERSITY TAEGU, KOREA.

This study was conducted to examine the association between the carotid artery intima-media thickness (IMT), plaque presence and the interleukin-10(IL-10) gene polymorphism at position -1082, -819, and -592. The data used for this study were obtained from 135 subjects (76 men, 59 women), aged 31-82 years. Ultrasonography was used to measure the carotid artery IMT and plaque. IMT measurement were performed at 4 sites, including both common carotid arteries, and both bulbs. The data were analyzed in two categories: plaque present and plaque absent. Gender, age, uric acid, and homocysteine showed significant differences in plaque present and absent groups(p<0.05). single nucleotide polymorphism(SNP) at position -819 and -592 of IL-10 gene were statistically associated with the right carotid bulb (p>0.005). The association between SNP at position -1082, -819, and -592 of IL-10 gene polymorphism and the IMT (Right and Left Carotid artery and carotid bulb) was studied. SNP at position -819 was significantly correlated with right carotid bulb IMT (p=0.008). SNP at position -592 was also significantly correlated with right carotid bulb IMT(p=0.009). These results should be taken into the consideration that genetic SNP can affect carotid plaque formation and thus, atherosclerosis. Selective approaches of different SNP positions with various genes can be performed to find a closer relationship with carotid plaque formation.

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## 2391/T/Poster Board #940

**Maternal Use of a Folate Containing Supplement Protects Against Atrioventricular Septal Defects in Males with Down Syndrome: A Report from the National Down Syndrome Project.** *L.J.H. Bean[1], E.G. Allen[1], S.W. Tinker[1], N. Hollis[1], A.E. Locke[1], K.J. Dooley[2], S.B. Freeman[1], S.L. Sherman[1].* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Sibley Heart Center, Dept Pediatrics, Emory Univ, Atlanta, GA.

Maternal folate deficiency has been associated with neural tube defects, congenital heart defects, and other birth defects. Individuals with Down syndrome (DS) are at high risk for congenital heart defects and have been shown to have abnormal folate metabolism. Epidemiological data from the National Down Syndrome Project was analyzed to determine the effect of maternal use of a folate containing supplement on the risk of congenital heart defects in probands with Down syndrome (DS). A total of 1012 mothers (511 white, 111 black, and 390 Hispanic) reported race / ethnicity and use of folate containing supplements. Cardiac records on each individual with DS were obtained. The incidence of heart defects in probands was compared between those whose mothers started taking folate before the fourth week of pregnancy (when heart development begins) and those whose mothers took no supplementation before the eighth week of pregnancy (when heart development is complete). Logistic regression was used with the outcome variable being presence/absence of a heart defect in the proband and the predictor variables being maternal folate supplementation use, race/ethnicity and sex of the proband. The most common type of heart defect observed in individuals with DS is atrioventicular septal defect (AVSD); thus, we focused on this specific defect for the analyses presented here. We found a significantly increased risk of AVSD to probands with DS born to mothers who did not take a folate containing supplement (OR = 1.56; 95%CI 1.02-2.40) adjusting for proband sex and race/ethnicity. Because of the nearly two-fold excess of female probands (OR 1.93 (1.40, 2.67)) among DS cases with AVSDs that we previously reported, we stratified our analyses by proband sex. We observed that lack of maternal folate supplementation significantly increased the risk of AVSD in males (OR = 2.22; 95%CI 1.12-4.40), but not females (OR = 1.28; 95%CI 0.73-2.24), with DS. In addition, despite an overall lower incidence of AVSDs in probands with DS born to Hispanic mothers, the effect of folate use was most pronounced in this group. Population-specific effects of folate supplementation may reflect underlying differences in genetic and environmental risk factors between populations. Overall, these results indicate that maternal folate use protects males with DS from AVSDs more so than females. These results possibly explain the higher female risk of AVSD, and suggest an epigenetic mechanism for AVSD.

## 2392/T/Poster Board #941

**Alternative splicing of genes expressed during early embryonic development.** *T. Revil[1], D. Gaffney[1], C. Dias[1,2], L.A. Jerome-Majewska[1,3], J. Majewski[1,2].* 1) Dept. of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Genome Quebec Innovation Centre, Montreal, Quebec, Canada; 3) Dept. of Pediatrics, Montreal Children's Hospital, Montreal, Quebec, Canada.

A number of genes that are required for normal morphogenesis of developing embryos, including Fgf8, have been shown to have isoform-specific functions. To date, these genes have been identified in isolated experiments and by fortuitous splicing events associated with gene targeting. We predicted that alternative splicing, the process by which a single gene can produce several functionally distinct isoform, is a common event required for establishment of the normal body plan. In this work, we present a whole genome approach, using exon microarrays, to profile the variation in pre-mRNA splicing, transcription initiation, and polyadenylation site choice during early murine development. We show that variation in isoform expression is widespread, and affects many genes with known and predicted function during embryonic development. We use RT-PCR and in-situ hybridization to further validate and investigate spatial and temporal expression patterns of specific isoforms. We propose that alternative isoform expression is a common mechanism controlling the diversity of developmental process, and that techniques such as isoform-specific knockouts, should be utilized to truly dissect the complexity of developmental pathways.

## 2393/T/Poster Board #942

**A Novel Mouse Model Recapitulates Defects Seen in 1p36 Deletion Syndrome.** *B. Kim, H. Zaveri, O. Shchelochkov, SH. Kang, M. Justice, B. Lee, D. Scott.* Human Molec Gen, Baylor College Med, Houston, TX.

Children with monosomy 1p36the most common terminal deletion syndrome with an incidence of 1 in 5,000have a constellation of symptoms that can include by cardiovascular defects, mental retardation, postnatal growth deficiency, hearing loss and CNS abnormalities. Although most features of monosomy 1p36 have been attributed to genes located within 6.2 Mb of the telomere, Kang et al. has reported a cohort of patients with similar findings caused by interstitial deletions of 1p36 located proximal to this region. The smallest deletion in this cohort involved a 2.9Mb region containing Atrophin 2 (ATR2), which encodes a nuclear receptor co-repressor. Mice that are homozygous for the Atr2 null mutation openmind (op) die at E9.5 with open neural tubes and cardiac defects which limit their usefulness as a disease model. However, using a newly identified hypomorphic allele of Atr2 known as eye3, we have generated an allelic series of mice with partial Atr2 function. A portion of $Atr2^{op/eye3}$ mice live into adulthood and have many of the characteristics seen in 1p36 deletion patients. $Atr2^{op/eye3}$ mice have postnatal growth retardation, hearing loss, and a decreased brain size compared to wild type from E17.5 until adulthood. The cerebellums of $Atr2^{op/eye3}$ are abnormal with limited fissure formation at P0 and abnormal foliation of the anterior lobe during the postnatal development. $Atr2^{op/eye3}$ mice also fared poorly in tests requiring normal balance and coordination. At E17.5 the dentate gyri of $Atr2^{op/eye3}$ mice was significantly smaller than controls and a decrease of ganglion cell number was observed in the retina. Since cardiovascular defects, post natal growth deficiency, hearing loss, and CNS abnormalities are found in children with 1p36 deletion syndrome, it is likely that haploinsufficiency of ATR2 contributes to this disorder. We are presently using our Atr2 mouse models to identify the molecular interactions between Arophin 2 and specific nuclear receptors that are responsible for these defects.

## 2394/T/Poster Board #943

**Novel BBsome independent phenotypes in a mouse model of Bardet-Biedl syndrome 3.** *T. Vogel[1], D.Y. Nishimura[2], C.C. Searby[2,6], R.E. Swiderski[2], R.F. Mullins[3], P.R. Pretorius[2,6], S. Seo[2,6], Q. Zhang[2,4], K. Bugge[2,6], D.R. Thedens[3], M.D. Cassell[4], J.A. Wemmie[5], V.C. Sheffield[2,4,6].* 1) Dept Neurosurgery, Univ Iowa, Iowa City, IA 52242, USA; 2) Dept. Pediatrics, Univ Iowa, Iowa City, IA 52242, USA; 3) Dept. Ophthalmology, Univ Iowa, Iowa City, IA 52242, USA; 4) Dept. Anatomy, Univ Iowa, Iowa City, IA 52242, USA; 5) Dept. Psychiatry, Univ Iowa, Iowa City, IA 52242, USA; 6) Howard Hughes Medical Institute, University of Iowa Carver College of Medicine, Iowa, IA 52242, USA.

Bardet-Biedl syndrome (BBS) is a pleiotropic, autosomal recessive, heterogeneous disorder caused by mutations in a group of genes that contribute to ciliary function. As many as fourteen genes are reported to cause BBS. Individuals with BBS have the features of obesity, retinal degeneration, cognitive delay, and congenital anomalies including polydactyly, heart defects and renal abnormalities. Of the genes known to cause BBS, seven encode proteins which form a complex known as the BBsome which is proposed to play a role in vesicular transport to the cilium and plasma membrane. BBS3, a member of the Ras family of small GTP-binding proteins, is also postulated to play a role in vesicular transport, but is not part of the BBsome. To better understand the function of BBS3 and its relationship to components of the BBsome, we created a *BBs3* knockout mouse model. While the *Bbs3* knockout mouse develops retinal degeneration, some features of this model differ from other mutant BBS mice. Notably, *Bbs3* knockout mice do not develop early onset obesity and morphological analysis of the central nervous system reveals an early and severe form of ventriculomegaly when compared to a knockin model of *BBs1* (M390R/M390R) and knockout mouse models of other components of the BBsome developed in our laboratory. Behavioral studies have also identified differences in locomotor function, intracranial pressure, balance and coordination, and memory when compared to wild type mice. Together these results indicate that the *BBs3* mouse model will offer unique insights into the developmental mechanisms underlying BBSsome independent phenotypes.

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## 2395/T/Poster Board #944

**Identification of five α-actinin genes in *Danio rerio* and evidence for functional differences between sarcomeric isoforms.** *V. Gupta, R. Darnall, A.H. Beggs.* Genomics Program and Division of Genetics, The Manton Center for Orphan Disease Research, Children's Hospital Boston, Harvard Medical School, Boston, MA.

The α-actinins are an ancient protein family sharing a common evolutionary origin with other members of the spectrin-gene superfamily of actin binding proteins. Structurally and functionally, α-actinins can be divided in to Ca2+-sensitive cytoskeletal isoforms that organize the actin cytoskeleton, and Ca2+-insensitive sarcomeric isoforms that constitutively bind actin filaments at muscle Z-lines. Loss of function of different α-actinins results in distinct phenotypes *in vivo*, related in part to different patterns of expression, however, the isoforms largely show indistinguishable activities in most *in vitro* assays. To determine the structural basis for functional differences between isoforms, we are utilizing zebrafish as a model system to study the different sarcomeric α-actinin isoforms *in vivo*. Although most vertebrates have four α-actinin genes, we find that the zebrafish genome includes five. Three appear to encode for sarcomeric forms, while two are cytoskeletal. Each isoform exhibits a distinct and unique pattern of gene expression as assessed by mRNA in situ hybridization. Morpholino-based knock-down of the sarcomeric isoform, *actn2*, leads to impaired development of zebrafish brain and skeletal muscles. Coinjection of *actn2* mRNA, but not *actn3* mRNA, leads to rescue of the phenotype. These studies, for the first time, show *in vivo* evidence of functional differences among sarcomeric α-actinins. Future studies utilizing the zebrafish model system will lead to a better understanding of α-actinin-dependent pathways in development and function of different organs.

## 2396/T/Poster Board #945

**MicroRNAs as Regulators of Adrenal Cortical Development.** *J.K. Mazilu, Y. Zhao, S. Lin, E.R.B. McCabe.* University of California Los Angeles, Los Angeles, CA.

The molecular mechanisms involved in adrenal gland development remain poorly understood. Though mutations in SF1 and DAX1 have been linked to adrenal hypoplasia congenita (AHC, MIM #300200), approximately 40% of patients with AHC referred to our group and others for testing do not have mutations in either of these genes. While the role of microRNA (miRNA) in development has been established, its role in adrenal development has been largely unexplored. To investigate this role, we choose to study miRNA in adrenal cortical development in the zebrafish, Danio rerio, due to its ease of genetic and molecular manipulation. Dicer1 is a protein essential for miRNA processing. Using a transgenic line of zebrafish expressing GFP in the interrenal organ (adrenal cortex equivalent), we have evidence that dicer1 morphant embryos, which lacked mature, functioning miRNA, have disrupted adrenal development and a greater incidence of adrenal arrest when compared to wild type (WT) and mismatched morpholino (mmMO) embryos. Specifically, ≤10% of WT and mmMO embryos showed two sites of GFP expression, indicating putative adrenal arrest; 30-40% of dicer1 morphants showed this adrenal arrest phenotype, indicated by two or more sites of GFP expression. Moreover, dicer1 morphants retained these multiple sites of GFP expression while WT and mmMO embryos do not. Other features of dicer1 morphants included underdeveloped heads and eyes, absence of a swim bladder, developmental delay, and a "curly tail" phenotype. Co-injection of dicer1-morpholino (MO) and miRNA extracted from embryos at 8 hpf best rescued adrenal development while moderately rescuing other dicer1 morphant features. Since miRNA has been reported to regulate many developmental pathways, we are interested in examining its role in modulating adrenocortical primordial cells' responses to signaling pathways, such as the TGF-β signaling pathway, that participate in adrenal development and function. The medical implications include identifying specific roles and mechanisms of action for miRNA in adrenal development. Such information will have applications in cell-based therapeutics, such as adrenal cortical differentiation of induced pluripotent stem cells (iPS), and will provide insight into a variety of adrenal pathologies, including, not only AHC, but also adrenal aplasia and adrenal tumorigenesis, as well as therapies for these and related disorders, such as congenital adrenal hyperplasia (CAH).

## 2397/T/Poster Board #946

**DAX1: Earliest Marker in Zebrafish Tooth Development is a Marker for Human Tooth and Ameloblastoma Tumors.** *J. Powers[1], Y. Zhaou[2], S. Lin[2], E.R.B. McCabe[2].* 1) Dept Neonatology, Univ California, Los Angeles, Los Angeles, CA; 2) UCLA, Los Angeles, CA.

Background Zebrafish teeth develop on pharyngeal jaws in the fifth branchial arch, but have a pattern of early tooth development remarkably similar to mammals (1). Recently, eve1 has been shown to be involved in initiation of primary tooth (4V1) and early ameloblast development (2). dax1 is initially expressed in the fifth branchial arch in zebrafish at approximately 26 hpf and co-localizes with eve1 expression at ~48hpf. Our previous studies showed embryos injected with dax1 morpholino (MO) exhibit down-regulation of eve1 expression. Based on the zebrafish observation, we hypothesized that DAX1 might be involved in tooth development and ameloblast differentiation in humans. Objective The purpose of this study was to identify whether DAX1 is expressed in normal human dental tissue and ameloblastoma tumors. Design/Methods We obtained samples of previously resected formalin-fixed paraffin embedded normal human dental tissue, and benign and malignant ameloblastoma tumor tissue. We performed in situ hybridization on the de-paraffinized samples using normal adrenal cortex tissue as control. The samples were stained with BCIP/NBT and visualized for DAX1 expression. We also performed western blot analysis on the tissue samples to detect the levels of DAX1 expression in normal dental tissue compared to benign and malignant ameloblastoma tumor tissue. Results Our results showed expression of DAX1 in normal human dental tissue in the regions corresponding to the enamel organ where ameloblasts are housed. DAX1 expression was increased in malignant ameloblastoma tissue compared to normal tissue and benign tumor. Conclusions Prior to our studies, DAX1 has not been shown to be associated with tooth development and ameloblastoma tumors. We were able to use our observations in a zebrafish animal model and translate our findings to humans. Despite ~450 million years since the last common ancestors of zebrafish and humans, identification of orthologous genes and gene functions can lead to novel insights into normal and abnormal developmental processes. 1. Borday-Birraux V, Van der Heyden C, Debiais-Thibaud M, Verreijdt L, Stock DW, Huysseune A, Sire, Evol Dev 8, 130 (2006). 2. Laurenti P, Thaeron C, Allizard F, Huyseeune A, Sire JY, Dev Dyn 230, 727 (2004).

## 2398/T/Poster Board #947

**Expression of Trps1 in Secretory Odontoblasts Results in Dentinogenesis Imperfecta-Like Phenotype in Transgenic Mice.** *D. Napierala[1], I. Maciejewska[2], B. Dawson[1,3], E. Munivez[1], R. D'Souza[2], B. Lee[1,3].* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Baylor College of Dentistry, Texas A&M Health Science Center, Dallas, TX; 3) Howard Hughes Medical Institute.

Odontoblasts are neural crest-derived cells that that differentiate from the dental papilla cells. The terminal differentiation of odontoblasts requires reciprocal cell-matrix interactions and is characterized by withdrawal from the cell cycle, polarization of the cells and secretion of dentin matrix. Hereditary defects in dentin formation in humans are classified as dentinogenesis imperfecta (DGI) or dentin dysplasia (DD). While mutations in the collagen type I (*COL1A1*) and dentin sialophosphoprotein (*DSPP*) genes are responsible for the majority of DGI and DD cases, the etiology of isolated hereditary dentin disorders in many affected families is still unknown. Our recent studies on the role of the Trps1 transcriptional repressor in the mineralization process revealed its novel and important function in odontoblasts. Trps1 is a GATA-type transcription factor involved in craniofacial development and endochondral ossification. During early tooth development *Trps1* is expressed in dental mesenchyme and, after cell differentiation, in preodontoblasts and dental papilla. However, its expression is turned off in differentiated secretory odontoblasts. We generated transgenic mice over-expressing *Trps1* under control of 2.3kb fragment of collagen 1a1 promoter (col1a1-Trps1 mice), which drives expression of a transgene specifically in osteoblasts and odontoblasts. Col1a1-Trps1 mice demonstrate growth retardation after weaning and most die within one week after weaning due to feeding problems. Histological and micro-CT analyses revealed dramatic decrease of dentin mineralization and abnormal dentine structure in transgenic mice. Additionally, expression of major non-collagenous proteins of dentin matrix, dentin sialoprotein (Dsp) and dentin matrix protein 1 (Dmp1), was reduced in *Trps1* transgenic mice. In summary, we have demonstrated that continuous expression of *Trps1* in differentiated odontoblasts results in severe abnormalities in dentin formation and mineralization, which resemble human DGI phenotype. The severity of the dentin abnormalities in col1a1-Trps1 transgenic mice suggests that Trps1 regulates multiple genes involved in dentin formation.

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## 2399/T/Poster Board #948

**Identification of X-linked myotubular myopathy in Labrador retrievers.**

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Mutations in the MTM1 gene, encoding myotubularin, cause X-linked myotubular myopathy, a well-defined subgroup of centronuclear myopathies in humans. Six male Labrador retrievers, from 3 to 4 months of age, from three apparently unrelated litters, presented to the Western College of Veterinary Medicine in Saskatchewan with generalized weakness and muscle atrophy. The first documented clinical signs were recorded during vaccinations around 7 weeks of age, with decreased weight, dropped jaw, generalized weakness, and hind limb muscle atrophy. The clinical course was progressive, leading to comprehensive veterinary assessments around 14 weeks and eventual euthanasia due to severe generalized weakness around 4 months of life (range 3.5 to ~ 6 mo). Laboratory workup was generally noncontributory; serum CK was either normal or only very slightly elevated. Muscle biopsies revealed variability in fiber size, centrally placed nuclei and subsarcolemmal ringed and central dense areas. The subsarcolemmal and central dense areas stained darkly positive with mitochondrial specific reactions for SDH and COX allowing histologic differentiation from Labrador retrievers with CNM. All dogs tested for the known centronuclear myopathy (CNM) mutation in the PTPLA gene were negative. Immunoblot analysis showed that myotubularin was absent in muscle extracts from affected dogs but present in muscle from a control littermate. Ultrastructural studies confirmed the centrally located nuclei, abnormal perinuclear structure and mitochondrial accumulations. Triads were infrequent with an abnormal orientation of T tubules. Immunofluorescence staining of frozen muscle sections using antibodies to label T tubules (DHPRa1) and adjacent sarcoplasmic reticulum (RYR1) confirmed an abnormal distribution of these structures. DNA analysis of the MTM1 gene in all six affected males revealed a unique variant in exon 7 causing the non-conservative missense change N155K in the linker region between the GRAM-PH and phosphatase domains of myotubularin. Three proven carrier dams from two of these kindreds were shown to be heterozygous for the variant and a healthy male littermate was shown not to carry the variant. Analysis of a world-wide panel of 237 unrelated and unaffected Labrador retrievers, and 59 additional control dogs from 25 breeds, failed to identify this variant. Altogether these results suggest that the N155K variant is the pathogenic mutation responsible for the myopathy in these dogs.

## 2400/T/Poster Board #949

**Modification of *C. elegans bbs* mutant phenotypes by guanylate cyclases implicates a perturbation in cGMP levels.**

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Bardet-Biedl syndrome (BBS) is an autosomal recessive, genetically heterogeneous, pleiotropic disorder. Cardinal features include photoreceptor degeneration, obesity, digit and cystic renal anomalies, cognitive impairment and hypogonadism. Most BBS genes encode ciliary proteins related to the intraflagellar transport (IFT) system. Consistently, *C. elegans bbs* mutants have defects in ciliary morphology and function (Blacque et al., 2004, Tan et al., 2007). We determined common phenotypes shared by all *bbs* mutants and noticed that all *bbs* mutants share a statistically significant loss of 15-20% in mean body length and a moderate decrease in body width. Neurosensory defects in *C. elegans* have previously been associated with decreased body length (Fujiwara et al., 2002). The transforming growth factor beta (TGF-β) superfamily of proteins encompasses those in a pathway that also controls body size (Patterson and Padgett, 2000). Our genetic analyses between *bbs* and TGF-β mutants (*dbl-1* and *lon-2*) however suggested that these two pathways work in parallel as their body size defects are additive. To understand how BBS proteins modulate body size, we identified a genetic suppressor that restored the body size of *bbs* mutants to normal length. This encodes the guanylate cyclase GCY-35. While capable of suppressing *bbs* body size defects, mutations in *gcy-35* do not rescue the structure and function defects of the amphid sensory neurons in bbs mutants. To regulate body size GCY-35 is required in a subset of the O2 sensory neurons (Cheung et al., 2004, Gray et al., 2004, Chang et al., 2006, Zimmer et al., 2009), and its localisation appears unaffected in *bbs-7* mutants. BBS proteins, on the other hand, are required in the non-overlapping sensory neurons to regulate body size. The body size defects of *bbs* mutants are thus likely due to a defect in cGMP regulation. Consistently, the body size change induced by both loss and gain of function mutants in the cGMP-dependent kinase EGL-4 (Fujiwara et al., 2002) appear genetically epistatic to the body defects in *bbs* mutants, suggesting it as the downstream effector of the BBS complex in sensory cilia. The non-cell autonomous control of body size through guanylate cyclases in a small subset of ciliated neurons identifies an interesting direction for the further dissection of body size defects in *C. elegans* and later study of our human BBS cohorts.

## †2401/T/Poster Board #950

**Ocular defects in *sall3* deficient zebrafish embryos: insights into human disease.**

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The study of chromosomal anomalies represents a fruitful means of determining the function of previously uncharacterized genes. We investigated a patient with a telomeric deletion [46, XX, del (18)(q23)] who exhibited unilateral colobomatous microphthalmia, as well as systemic defects that included cleft palate, cardiac septal defects, dysmorphic facial appearance and developmental delay. Customized array CGH was used to accurately define the deletion's extent as a first step in identifying genes contributing to 18q deletion syndrome (a disorder characterized by facial dysmorphism, ocular and neurological defects, and mental retardation). The 2.9Mbp deleted region, the smallest reported 18q deletion syndrome interval, likely defines the critical region for the phenotypes seen in this patient. Bioinformatic analyses of the 10 deleted genes identified three that are ocularly expressed, *ADNP2, CTDP1* and *SALL3*. Morpholino inhibition of *adnp2* in zebrafish revealed no ocular phenotype, and as *CTDP1* had been previously characterized, we concentrated on the uncharacterized gene *SALL3*, a member of the *Spalt-like (Sall)* family of transcription factors. Mutation in paralogs results in Townes-Brocks (*SALL1*), and Okihiro or Duane-radial ray (*SALL4*) syndromes, of which the latter phenotype includes ocular motility defects.

*In situ* hybridization of *sall3* in zebrafish at four early stages (15.5, 18, 20, and 24hpf) showed ubiquitous expression, with particularly high levels in developing motor neurons. Morpholino inhibition of *sall3* function recapitulates the patient's colobomatous microphthalmia with extensive retinal laminar patterning defects evident on histological analysis, consistent with a key role for *sall3* in retinal formation. Furthermore, analysis of gene expression shows a decrease of *epha3* and *epha4b* expression in the *sall3* deficient embryos, indicating improper patterning in the eye. Our ongoing characterization of the broader phenotypes of *sall3* morphants, combined with screening microphthalmia and colobomata patients for mutations, is anticipated to elucidate *SALL3*'s contribution to 18q deletion syndrome and ocular malformations.

† Trainee Award Finalist

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## 2402/T/Poster Board #951

**The neural crest basis for craniofacial abnormalities associated with Down syndrome.** *R. Roper[1], M. Stanley[2], N. Duvall[1], S. Stone[1], C. Davis[2], C. Billingsley[1], J. Blazek[1].* 1) Dept Biol, Indiana Univ Purdue Univ, Indianapolis, IN; 2) Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN.

Trisomy 21 occurs in approximately 1 in 750 live births and results in phenotypes collectively referred to as Down syndrome (DS). Individuals with DS present with a wide range of phenotypes which may include cognitive impairment, facial dysmorphology, congenital heart defects, and gastrointestinal tract abnormalities. Craniofacial anomalies, including a shortened midface, small mandible and small oral cavity, may combine with other clinical traits to affect the breathing, feeding and hearing of individuals with DS. Many of the tissues affected in DS, including craniofacial skeleton, have a neural crest (NC) component and we have demonstrated developmental deficits in generation, migration and mitosis of trisomic NC in a DS mouse model. To understand the molecular origin and comorbidity of NC-related phenotypes, we are using DS mouse models and clinical information from infants with DS evaluated by Developmental Pediatrics at the Riley Hospital for Children in Indianapolis, IN. Morphometric analyses of DS mouse models show that the size of the mandibular precursor is altered throughout development and the macroglossia associated with DS may be relative with a normal tongue and small oral cavity. Our phenotypic evaluation of infants with DS shows that NC-related clinical traits tend to cluster together and may be the result of a generalized NC deficit related to trisomy. Because parts of cardiac, GI, and craniofacial structures are derived from NC and may be affected by trisomy 21, the diagnosis of a heart or GI problem may be indicative of secondary sequelae related to a NC deficit, including feeding and airway issues associated with DS. We hypothesize that an understanding of the cellular and developmental bases of craniofacial tissue in mice as well as the comorbidity of clinical features in infants with DS will suggest preventative measures that can be taken during early development to increase the quality of life for individuals with DS and their families.

## 2403/T/Poster Board #952

**ANALYSIS OF FUNCTION OF BMP SIGNALING INHIBITOR - MAB21L2 IN ENTERIC NERVOUS SYSTEM DEVELOPMENT AND IDENTIFICATION OF MAB21L2 ENHANCERS USING ZEBRAFISH.** *G.M. Burzynski[1], L. Petrova[1], J. Osinga[2], R.M.W. Hofstra[2], I.T. Shepherd[1].* 1) Department of Biology, Emory University, Atlanta, GA; 2) Department of Genetics, University Medical Center Groningen, Groningen, The Netherlands.

The enteric nervous system (ENS) is derived from neural crest cells (NCCs). Reciprocal interactions between the NCCs, gut endoderm and gut mesenchyme regulate the proliferation, migration, and differentiation of ENS NCCs. One important signaling pathway involved in this process is the bone morphogenetic protein (BMP) pathway. Gene expression profiling analysis of the zebrafish ENS mutant lessen revealed that the BMP signaling pathway component, Mab21l2 is down regulated in this mutant. Hirschsprung disease (HSCR) is a pediatric condition characterized by the absence of the enteric ganglia along a variable length of the intestine. A recent genome wide linkage analysis performed on a family with isolated HSCR showed that the 4q31.3-q32.3 region is linked to the disease. Within this region, the human orthologue of zebrafish Mab21l2 is located. We have investigated the expression and function of Mab21l2 during zebrafish development to determine its role in ENS development. Mab21l2 is expressed in the anterior CNS, retina, optic tectum, cranial ganglia, pharyngeal arches, hindbrain, neural tube and in the mesenchyme adjacent to the intestine. Knock down of Mab21l2 causes defects in pharyngeal arches and intestinal smooth muscle development. Critically, morphants also display a significant reduction in the number of enteric neurons. Sequencing of the Mab21l2 locus in patients revealed no mutation in the coding region of the gene. As a result we hypothesized that there is a regulatory mutation that causes the disease phenotype. To identify enhancer regions responsible for driving conserved expression of Mab21l2 in vertebrates, which are potentially perturbed in patients we have utilized Tol2 transposon-mediated transgenesis in zebrafish and sequencing of the regualtory regions in patients. Human and zebrafish genomic fragments of Mab21l2 regulatory region have been cloned into Tol2-gfp reporter constructs. These have been injected into zebrafish embryos and the pattern of GFP expression has been determined. The analysis of polymorphisms detected in patients and evaluation of enhancers' activity in transgenic assay will be presented. Together our data suggests that Mab21l2 is a gene important for ENS development, contributing to HSCR phenotype.

## 2404/T/Poster Board #953

**Variants in the trifunctional MTHFD1 gene as risk factors for spina bifida in the Amish population Khan T, Patel H, Crosby A, Patton MA Medical Genetics, St Georges Hospital, London SW17 0RE, United Kingdom.** *T. Khan, H. Patel, A. Crosby, MA. Patton.* Med Gen, St Georges Hosp, London, United Kingdom.

Neural tube defects are common congenital birth defects, affecting approximately 1 in 1000 pregnancies in American Caucasians. These are extremely complex disorders, and many genetic and environmental factors have been implicated in the aetiology, but as yet no single cause has been identified. Previous work at the Regional Genetics Service at St Georges Hospital, London, has highlighted the trifunctional gene, methylenetetrahydrofolate dehydrogenase 1 (MTHFD1), which resides at the locus 14q24, to be in linkage disequilibrium with spina bifida in the Amish population of the USA. During the current project this gene was sequenced in four affected individuals and three interesting sequence variants were found; R293H, R653Q and R134K. These sequence variants were further investigated in more affected individuals and two control groups (non-affected Amish controls and non-Amish non-affected controls). The R293H variant was found in one affected individual with spina bifida and two of his siblings with spina bifida occulta, and although it was found in three Amish controls, it was not found in one hundred non-Amish control chromosomes. The R653Q variant was present at a frequency of 73% in the affected individuals and at a frequency of 57.6% in the non-Amish controls, although on further analysis, t-test analysis did not confirm this to be statistically significant. Finally, the R134K variant was present in 71.4% of the affected individuals and 60% of the individuals with spina bifida occulta, however due to time constraints this variant was not analysed further. Consequently, although further investigation is required, the results of this study indicate that these variants could act as possible risk factors for spina bifida in the Amish population, either as an accumulative effect, or as an additive effect with other genes.

## 2405/T/Poster Board #954

**The CUB domain protein Neto1 is a molecular switch that modulates Plexin D1 binding specificity for semaphorin axon guidance cues.** *J.R. Gingrich[1], D. Ng[1], B. Gore[2], M. Tessier-Lavigne[2], R.R. McInnes[1].* 1) Research Institute, Hospital For Sick Children, Toronto, Ontario, Canada; 2) Department of Research Drug Discovery, Genentech, Inc., San Francisco, CA, U.S.A.

The CUB domains of the neuronal transmembrane protein Neto1 (Ng *et al.*, PloS Biology **7**: 278-300, 2009) are most similar to the CUB domains of neuropilins (Npns), a family of axon guidance molecules. We have determined that Neto1, like the Npns, is essential for axon guidance of neurons in the developing mouse central nervous system since (i) Neto1 is obligatory for midline crossing of commissural interneurons in the E12.5 embryonic neural tube, and (ii) adult Neto1$^{-/-}$ mice display defects in axon projections in the corticospinal and subiculo-mammillary tracts of the brain. Because secreted semaphorins bind to the extracellular CUB domains of the Npns, we reasoned that a secreted semaphorin may be a ligand for Neto1. We examined the *in vitro* binding of various semaphorins to cells co-expressing Npn-1, Npn-2, or Neto1 and individual plexins. First, we confirmed that Npn-1 and Npn-2 bind semaphorins in the absence of a plexin co-receptor. Neto1, in contrast, bound semaphorin 3F (Sema3F) only in the presence of Plexin D1 and co-immunoprecipitated Plexin D1 only in the presence of Sema3F, indicating that Neto1 and Plexin D1 form a co-receptor for Sema3F. Because Plexin D1 can bind Sema3E in the absence of Neto1, these findings suggest that Neto1 switches the ligand binding specificity of Plexin D1 from Sema3E to Sema3F. Application of Sema3F to Cos7 cells co-expressing Neto1 and Plexin D1 led to cell retraction (mean cell area 1373±93 $\mu m^2$ vs. 1766±44 $\mu m^2$ for controls; n=4 studies, p<0.05), indicating that Sema3F/Neto1/Plexin D1 signalling results in cytoskeletal remodelling, consistent with a role in axon guidance. Moreover, explants of subicular neurons from Neto1$^{-/-}$ embryos fail to respond to Sema3F, and application of the Neto1 ectodomain was sufficient to restore the response of Neto1$^{-/-}$ neurons to Sema3F. We conclude that Neto1 modulates the specificity of Plexin D1 ligand binding, potentially altering and refining the response of neurons to semaphorin guidance cues during forebrain development. Mutations in genes encoding axon guidance receptors that result in human disease are uncommon; but we note that the *NETO1* gene is located on human chromosome 18q22-23, within a mental retardation deletion interval and a bipolar disorder locus.

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## 2406/T/Poster Board #955

**Molecular mechanisms of brain malformations in congenital muscular dystrophies.** *H. Hu, Y. Yang, C. Gagen, P. Zhang.* SUNY Upstate Medical University, Syracuse, NY.

Congenital muscular dystrophies with brain malformations are a group of diseases that exhibit overmigration of neurons in the cerebral cortex. At least some are caused by genetic defects of O-mannosyl glycosylation. Earlier studies have demonstrated that disruptions of the pial basement membrane are the underlying causes of overmigration. Hypoglycosylation of $\alpha$-dystroglycan, an extracellular matrix receptor, results in abolished interactions between $\alpha$-dystroglycan and laminin. Using a mouse model of muscle-eye-brain disease, POMGnT1 knockout, mechanisms of basement membrane defect was investigated. While disruptions of the pial basement membrane can theoretically be caused by its reduced strength, increased turnover, or reduced assembly, the results indicated that assembly of the basement membrane was defective due to diminished extracellular matrix-$\alpha$-dystroglycan interactions. For example, laminin assembly at the knockout cell surface is at a much reduced rate. Further, overmigrated neurons disrupted the developing mesenchyme, resulting in ectopic fibroblasts in the cerebral cortex. Evidence suggested that these ectopic fibroblasts induced reactive astrogliosis.

## 2407/T/Poster Board #956

**Chromodomain protein 7 (CHD7) regulates olfactory and inner ear neurogenesis and axonal projections to the vestibular system: implications for sensory impairments in CHARGE Syndrome.** *D.M. Martin[1], W.S. Layman[2], Y. Raphael[3], E.A. Hurd[4].* 1) Pediatrics & Human Gen, Univ Michigan Med Ctr, Ann Arbor, MI; 2) Human Gen, Univ Michigan Med Ctr, Ann Arbor, MI; 3) Otolaryngology, Univ Michigan Med Ctr, Ann Arbor, MI; 4) Pediatrics, Univ Michigan Med Ctr, Ann Arbor, MI.

Neurogenesis and neural innervation of peripheral sensory organs are tightly regulated developmental processes that are critical for normal sensation. CHARGE Syndrome, characterized by multiple sensory impairments, including loss of vision, hearing, balance, and olfaction, is a major cause of deaf-blindness that affects 1 in 10,000 individuals. CHD7, the chromodomain protein mutated in CHARGE, was recently shown in mice to be a positive regulator of neural stem cell proliferation and olfactory sensory neuron formation in the developing olfactory epithelium, but its functions in other sensory tissues and cell types are not well understood. Previous studies also revealed morphological inner ear defects, balance difficulties, and reduced hearing in humans and mice with *Chd7* deficiency, but the cellular and molecular mechanisms leading to these defects have not been explained. Here we tested the hypothesis that CHD7 functions in the inner ear to regulate early neuron formation and axon guidance. We used heterozygous loss of function mice, $Chd7^{Gt/+}$, and a newly generated conditional $Chd7^{flox}$ allele in combination with inner ear specific Cre lines to study *Chd7* global and tissue-specific deficient phenotypes in embryonic and early postnatal ears. Mature $Chd7^{Gt/+}$ mice exhibit selective loss of innervation to the posterior crista, a sensory epithelial structure that regulates angular movement. We also found reduced numbers of delaminating neuroblasts in the *Chd7* heterozygous and homozygous mutant otocyst and cochleovestibular ganglion, and fewer differentiated neurons. There were no measurable defects in embryonic *Chd7* mutant cellular proliferation or survival in the developing *Chd7* deficient otocyst or ganglion, suggesting instead that CHD7 regulates timing of exit from the cell cycle or one or more aspects of neuronal differentiation. Using neuroanatomical tracers, we detected defects in neuronal projections to the posterior cristae in embryonic and postnatal $Chd7^{Gt/+}$ ears. These studies indicate that CHD7 promotes vestibulocochlear neuronal development during early stages of delamination and neurogenesis without major effects on cellular survival. Combined with earlier observations showing that CHD7 promotes neural stem cell proliferation in the olfactory epithelium, these data suggest a general role for CHD7 regulated chromatin remodeling in developing neural progenitors. This work is supported by NICHD.

## 2408/T/Poster Board #957

**Expression of the putative microRNA processing protein, Ars2, in the developing and adult mouse retina.** *R.L. Chow[1], P.E. Nickerson[1], P.L. Howard[1,2].* 1) Biology, University of Victoria, Victoria, BC, Canada; 2) Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, Canada.

Proper development of the central nervous system depends on the coordinated timing of cell-cell interactions and subsequent cell autonomous responses. These cellular responses function, in part, by regulating downstream transcriptional programs and protein expression that mediate cell cycle exit, cell specification and differentiation. There is growing evidence that microRNAs play an important role in developmental programs by regulating the translation and/or stability of transcripts involved in the maintenance of stem and progenitor cells and their differentiation into tissue-specific lineages. A recently identified gene, *Arsenate resistance gene 2 (Ars2)*, is thought to play a role in microRNA processing and RNA metabolism, and is essential for at least two major processes of embryonic development: cellular differentiation and survival. We sought to determine whether *Ars2* functions during retinal development and first asked whether it is expressed in the developing and/or adult retina. Immunocytochemical analysis was performed using antisera generated against either the N or C terminus of human Ars2. Co-labeling of Ars2 and retinal cell type-specific and cell cycle-related markers was performed on mice ranging from P0-P90 in age. Our immunostaining experiments revealed dynamic patterns of Ars2 expression that include: (i) the upregulation of Ars2 in a subset of putative newly-born retinal neurons, and (ii) cell-class specific changes in Ars2 subcellular localization over time. Ars2 expression appears to be specific to neuronal subtypes, and was not detected in Müller glia. The timing and anatomical distribution of these changes in Ars2 expression is consistent with it playing a role in cell cycle exit and in the initiation of cellular differentiation.

## 2409/T/Poster Board #958

**The role of the Prader-Willi syndrome protein necdin in the development of the autonomic nervous system.** *A.A. Tennese, C.B. Gee, R. Wevrick.* Department of Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

Individuals with Prader-Willi syndrome (PWS), a rare neurobehavioural disorder, show pain insensitivity, have reduced salivation, and have abnormalities in feeding, drinking, thermoregulation, intestinal motility and reproduction. These functions are partly regulated by the autonomic nervous system (ANS) and may be caused by a defect in the ANS of PWS individuals. Necdin is one of the genes deleted in PWS and is most highly expressed in murine tissues that are relevant to PWS, including the peripheral and central nervous systems. We previously determined that loss of necdin in mice causes axonal extension, bundling, and branching defects in cultured sympathetic chain ganglia neurons. Therefore, we examined the sympathetic nervous system during prenatal development in necdin-null mouse embryos using immunohistochemistry. We identified a defect in the size and location of the superior cervical ganglion (SCG), the most rostral ganglion in the sympathetic chain, in necdin-null embryos. We determined that the initial migration of neural crest cells into the sympathetic chain is normal. The SCG appears normal at midgestation, but does not migrate towards the head at later stages in development as is normally observed in control embryos. In later stage necdin-null embryos, a decrease in innervation of SCG target tissues and an increase in cell death are also observed. As the survival of neurons requires nerve growth factors produced by target tissues, the reduction in axonal outgrowth likely causes the increased apoptosis in the maturing SCG neurons. The adrenal medulla is also formed by the migration of sympathetic neural crest cells into the adrenal gland. We examined the adrenal medulla in prenatal necdin-null embryos but did not identify any differences in size, location, or staining pattern when compared to control embryos. Further dysregulation of the ANS in necdin-null animals may be caused by a defect in the development and function of the hypothalamus, the master regulator of the ANS.

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## 2410/T/Poster Board #959

**PIAS1: a protein partner of the microphthalmia-associated homeodomain protein CHX10.** *E.A. Ivakine, L. Ploder, A. Huang, R.R. McInnes.* Program in Developmental and Stem Cell Biology, The Hospital for Sick Children Research Institute, Toronto, ON, Canada.

CHX10 is a highly conserved homeodomain-containing transcription factor essential for the development of vertebrate eye. In both humans and mice, homozygous mutations in the *CHX10* gene result in severe microphthalmia, retinal hypoplasia and absence of the optic nerve, but the molecular mechanisms underlying these developmental defects are incompletely understood. To identify CHX10-interacting proteins potentially involved in eye development, we performed a yeast two hybrid screen using a bovine adult retinal cDNA library and identified PIAS1 as a putative interactor. PIAS1 (Protein Inhibitor of Activated Stat1) belongs to a family of PIAS proteins that are broadly defined as transcriptional co-regulators. PIAS proteins function as E3 ligases in the SUMO conjugation pathway, in which the SUMO polypeptide is covalently linked to substrate proteins, but can also regulate the activity of proteins through sumoylation-independent mechanisms. Using immunofluorescence, we demonstrated that Pias1 is expressed in the inner nuclear and ganglion cell layers of the adult mouse retina and is co-expressed with Chx10 in bipolar cells. Analysis of Chx10 expression during eye development revealed the presence of two isoforms differing by 19 amino acids, resulting from differential splicing in exon 5. Expression of the Chx10 -19 isoform predominates throughout eye development from embryonic stages to adulthood. Both the +19 and -19 CHX10 isoforms interacted with Pias1 in a heterologous cell expression system, and analysis of CHX10 protein deletion mutants revealed that the CHX10 homeodomain is essential for the CHX10:Pias1 interaction. We found that both CHX10 isoforms can be sumoylated, both *in vitro* in a cell-free system, and in heterologous cells. Up to 10 possible sumoylation sites are predicted in the CHX10 protein. However, even when the four major and conserved predicted lysine sites were mutated to arginine (K13R, K184R, K241R, K298R) in a single CHX10 protein, we still observed residual CHX10 sumoylation, likely due to sumoylation at secondary sites. We are presently investigating whether PIAS1 directly regulates CHX10 transcriptional activity and/or its sub-localization within the nucleus, and whether this regulation occurs through a sumoylation-dependent or -independent mechanism. Definition of the role of sumoylation in the biology of CHX10 is likely to provide important insight into the mechanisms through which CHX10 regulates vertebrate eye development.

## 2411/T/Poster Board #960

**Notch function in the skeleton is dependent on both canonical and non-canonical Rbpj signaling.** *J. Tao[1], S. Chen[1], T. Yang[1], B. Dawson[1], T. Bertin[1], B. Lee[1,2].* 1) Human and Molecular Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Houston, Texas 77030.

Disruption of evolutionarily conserved Notch signaling causes a wide range of cancers and developmental disorders. Three inherited human diseases result from mutations in genes encoding Notch pathway components: Alagille syndrome, spondylocostal dysostosis, and cerebral autosomal dominant arteriopathy. To understand notch signaling in the skeleton, currently we have developed a series of mouse models for in vivo studies in which tissue specific gain and loss of function mutants were used. We previously generated an osteoblast specific over-expression of the constitutively active Notch 1 Intracellular Domain (NICD) by using collagen type 1 (Col1a1) promoter in committed osteoblasts. These transgenic mice showed a dramatic increase in osteoblast number, proliferation and formation resulting in a severe osteosclerotic phenotype. However, these founder lines exhibited early lethality and variable expression. To gain an insight into potential mechanistic basis for this phenotypic observation, we established a transgenic line with consistent NICD expression and longevity by crossing the $Rosa^{Notch/+}$; with the Col1a1-Cre transgenic mouse. The resulting bigenic mice ($Rosa^{Notch/+}$ ; Col1a1-Cre or Col1-GOF) recapitulated the NICD transgenic mice showing similar phenotypes. Using quantitative RT-PCR, a canonical target of Notch signaling, Hey1, was upregulated more than ten fold in Col1-GOF osteoblasts as would be expected for a NICD gain of function model. To explore the relative contributions of canonical (NICD/Rbpj-dependent) vs. non-canonical (NICD dependent but Rbpj-independent) Notch signaling, we crossed the Col1-GOF mice with a floxed allele of Rbpj. Our analyses showed the addition of the Rbpjf/f allele completely rescued growth retardation and osteosclerosis phenotypes. At the same time, we used a similar approach to study Notch signaling in chondrocytes. The chondrocyte-specific gain-of-Notch-function mice ($Rosa^{Notch/+}$; Col1a2-Cre or Col2-GOF) were stillborn with multiple defects in the skeleton. Significantly, vertebral columns were missing and appendicular long bones were abnormal and short in skeleton preparation. Further, addition of the Rbpjf/f allele to Col2-GOF mice completely rescued long bone phenotypes while vertebral columns were partially rescued. In summary, our data suggest that Notch signaling regulates osteoblasts through the canonical Rbpj-dependent pathway, but for chondrocytes it requires both canonical and non-canonical pathways.

## 2412/T/Poster Board #961

**Sturge-Weber syndrome with CYP1B1 mutations and severe trabecular dysgenesis.** *D. Pathak[1], M. Tanwar[1], T. Dada[2], R. Sihota[2], TK. Das[3], R. Dada[1].* 1) Dept Anatomy, All India Inst Medical Sci, New Delhi, India; 2) Dr. R.P. Centre for Ophthalmic sciences, AIIMS, New Delhi, India; 3) Electron Microscope Facility, AIIMS, New Delhi, India.

Sturge-Weber syndrome (SWS) is a progressive condition of mesodermal phakomatosis. One third cases of SWS have glaucoma. This preliminary study is the first report of CYP1B1 mutation analysis in SWS with congenital glaucoma. Mutations in CYP1B1 gene are major cause of congenital glaucoma.CYP1B1 is involved in metabolism of steroids, melatonin , retinol and other endogenous and exogenous substrates. Mutations in this gene results in diminished or absent metabolism of these key substrates that adversely affect signal transduction pathways and thus impair development and differentiation of anterior segment structures.This results in impaired aqueous outflow and raised intraocular pressure (IOP). CYP1B1 has higher expression in fetal eyes and plays major role in morphogenesis of iris, ciliary body, cornea and anterior chamber angle. Hence we decided to evaluate SWS cases with buphthalmos for CYP1B1 mutations by PCR RFLP followed by sequensing. Three different mutations R390C/H/S were identified at 390 position from earlier studies and all three demolish Hin6 restriction site and DNA sequencing was done to confirm the exact mutation. Trabecular-meshwork (TM) was studied for morphological alterations by scanning electron microscopy (SEM) of trabeculectomy tissue.All patients had normal 46, XY karyotype. PCR-RFLP analyses showed CYP1B1 mutations in 2 of the 5 SWS cases. Case 1 was homozygous for R390H and case 2 was heterozygous for both R390H and E229K mutation. All mutations were confirmed by DNA sequencing. SEM findings were suggestive of severe trabecular-dysgenesis. Mutations in CYP1B1 gene have been reported from different populations in glaucoma patients. No CYP1B1 mutation has been reported in any SWS case till date because syndromic cases were not analysed for mutations in earlier studies. Earlier studies have reported that onset of glaucoma in SWS shows a bimodal pattern. The result from this pilot study show that cases with buphthalmos and early onset glaucoma should be analysed for CYP1B1 mutations. The effect of vascular malformation induced venous engorgement and raised IOP may only be additive and may result in a much more severe phenotype. In conclusion SWS with buphthalmos should undergo CYP1B1 mutations analysis to identify an underlying genetic pathology for glaucoma. This will aid in determining the prognosis and management and will also help to provide comprehensive counseling in such cases.

## 2413/T/Poster Board #962

**Identification of downstream targets for EYA1 in kidney development.** *Y. Fan[1], S. Gisselbrecht[1], B. Busser[4], A.M. Michelson[4], M.L. Bulyk[1,2,3], R.L. Maas[1].* 1) Division of Genetics, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; 2) Department of pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; 3) Harvard-MIT Division of Health Sciences and Technology (HST), Harvard Medical School, Boston, MA 02115; 4) Laboratory of Developmental Systems Biology, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892.

Mutations in human EYA1, a homolog of the Drosophila eyes absent (eya) gene, result in Branchio-Oto-Renal (BOR) syndrome, an autosomal dominant disorder that includes hearing loss, branchial defects and renal abnormalities ranging from mild hypoplasia to agenesis. Eya1 null mice also exhibit renal agenesis, the result of a failure in ureteric bud (UB) outgrowth. Collectively, several studies suggest that the Eya1 protein interacts with the Six1 homeoprotein in intermediate mesoderm to regulate the metanephric mesenchymal (MM) expression of Glial-derived Neurotrophic Factor (GDNF), a morphogen for UB outgrowth. In addition, however, in organ culture, GDNF induces the UB outgrowth in Eya1-/- explants, although outgrowth is attenuated and the bud subsequently fails to branch. Based on these results, we hypothesized that factors other than GDNF might also contribute to UB development, and could be affected in Eya1 mutant mice. To address this question, we used available microarray data from wild type E11.5 mouse embryos to identify genes that are specifically expressed in MM. Next, we compared this gene set with a gene set of Drosophila genes obtained from microarray experiments involving over expression of Drosophila eya in fly mesoderm. We selected, as potential downstream targets of Eya1, MM expressed genes whose fly orthologs were also differentially expressed following eya overexpression. To begin to validate these findings, we examined the expression of these selected genes in Eya1 null mice by in situ hybridization, and by Eya1 knockdown in an E11.5 mouse MM cell line by real-time PCR. In this way, we identified five potential Eya1 targets. In addition, candidate DNA binding sites for the Six1 homeoprotein were identified using the PhylCRM algorithm and data from published protein binding microarrays (PBMs), and similar Six1 binding sites were found in four of the above five genes, consistent with the hypothesis that they might be direct downstream targets of a Eya1/Six1 complex.

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## 2414/T/Poster Board #963

**Comparison between Apert Syndrome Fgfr2+/P253R and Fgfr2+/S252W mice reveal phenotypic and MAPK signaling differences.** *Y. Wang[1], V. Uhlhorn[1], J. Friedenthal[1], D.L. Huso[2], M.B. Beasley[1], E.W. Jabs[1].* 1) Mount Sinai School of Medicine, New York, NY, 10029; 2) Johns Hopkins University, Baltimore, MD, 21205.

Apert syndrome has abnormalities in the skull, limb and viscera organs and is caused by FGFR2 +/S252W and +/P253R mutations in humans. Both mutations result in increased affinity and altered specificity of FGF ligand binding, with the S252W mutation causing greater enhancement in FGFR2c binding to most FGF ligands, relative to the P253R mutation. We generated a Fgfr2 +/P253R mouse model and compared it to our reported Fgfr2 +/S252W mouse on the same C57BL/6J genetic background. Fgfr2 +/S252W mice survived for only 1 day and had more abnormalities in the trachea, thymus, heart, and gastrointestinal tract than Fgfr2 +/P253R mice that lived for up to three weeks. Previously we noted that micro-CT scans of both mutants revealed similar abnormal patterns of calvarial suture fusion. Shortening of the skull length and abnormal osteogenesis and proliferation at the developing intramembranous coronal suture and endochondral long bones were not significantly different between the two mutants. Here we studied the visceral organs of these mice. Both mutants had abnormalities of the lung with atelectasis, collapsed lung structure and thickened mesenchyme. Increased cell proliferation was detected in the lungs of both mutants. The amount of Ki-67 positive cells was increased more significantly in Fgfr2 +/S252W compared to +/P253R and WT littermate lungs. MAPK signaling revealed that phosphorylation of ERK1/2 and P38 proteins was significantly increased in Fgfr2 +/S252W relative to +/P253R and/or WT lungs. Phosphorylated PKCalpha was increased in each mutant, also more significantly in Fgfr2 +/S252W lung. Phosphorylated AKT was significantly decreased in both mutant lungs suggesting its negative feedback crosstalk/modulation of the ERK pathway. The greater activation of ERK1/2, P38, and PKCalpha signaling correlates with the increased cell proliferation in Fgfr2 +/S252W compared to +/P253R lungs. The decreased length of survival may be explained by the molecular pathology of the lungs with subsequent respiratory failure in both mutants with Fgfr2 +/S252W being more affected than +/P253R. These data show a lung phenotype-genotype correlation in Apert mice. Our results demonstrate that although both mutations result in similar phenotypes, the FGFR2 +/S252W and +/P253R mutations have differential effects on various organs and their activation of the MAPK signaling pathway.

## 2415/T/Poster Board #964

**Antisense morpholino studies in *Danio rerio* show that *TXNDC10* is involved in the development of the ventral eye.** *R. Chao[1], L. Nevin[2], P. Agarwal[3], J. Riemer[4], X. Bai[5], A. Delaney[6], M. Akana[7], N. LopezJiminez[1], T. Bardakjian[8], A. Schneider[8], D. FitzPatrick[9], P-Y. Kwok[7], L. Ellgaard[4], D. Gould[5], Y. Zhang[10], J. Malicki[10], H. Baier[2], A. Slavotinek[1].* 1) Dept Pediatrics, Univ California, San Francisco, San Francisco, CA; 2) Dept. of Physiology, UCSF, San Francisco; 3) CVRI, UCSF, San Francisco; 4) Dept. of Biology, University of Copenhagen, Copenhagen; 5) Dept. of Ophthalmology, UCSF, San Francisco; 6) Genome Sciences Center, BC Cancer Research Center, Vancouver; 7) Dept. of Dermatology, UCSF, San Francisco; 8) Clinical Genetics Division, Albert Einstein Medical Center, Philadelphia; 9) MRC Human Genetics Unit, Western General Hospital, Edinburgh; 10) Dept. of Ophthalmology, Harvard Medical School, Boston.

Anophthalmia and microphthalmia are important birth defects, but their pathogenesis is incompletely understood. We had previously studied a patient with severe unilateral microphthalmia who had a 2.7 Mb deletion at chromosome 18q22.1 that was inherited from his phenotypically normal mother. In-situ hybridization showed that one of the deleted genes, *Txndc10,* was expressed in the retinal neuroepithelium and lens epithelium of the developing murine eye. We re-sequenced *TXNDC10* in 66 patients with anophthalmia or microphthalmia, and found one missense substitution in an unrelated patient: c.260G>A, predicting p.Arg39Gln, in a male with unilateral microphthalmia and retinal coloboma. In our most recent studies, we sequenced an additional 24 patients with anophthalmia or microphthalmia that had additional eye anomalies, and found c.456G>A, predicting p.Asp108Asn, in a female with unilateral microphthalmia and severe micrognathia. We used two antisense morpholinos, one targeted against the translational start site and one against the exon two donor splice site, to reduce the expression of the *Txndc10* orthologue in *Danio rerio*, *zgc:110025*. The morphant larvae obtained with both morpholinos had significantly smaller eye sizes at 2 and 4 days post fertilization (dpf) and reduced staining with islet-1 antibody directed against retinal ganglion cells at 2 dpf compared to control-injected larvae. Co-injection of human wild type *TXNDC10* mRNA rescued the small eye phenotype obtained with both morpholinos, whereas co-injection of human *TXNDC10*(p.R39Q) mutant mRNA, resembling the mutation in one of the patients with microphthalmia and coloboma, did not rescue the small eye phenotype. In-situ hybridization showed expanded labeling for two genes involved in ventral eye formation, *Pax2* and *Vax2*, in the ventral eye of the morphants compared to controls injected at 2 dpf, suggesting a delay in ventral eye development in the *Txndc10* morphants. Our results suggest that haploinsufficiency for *TXNDC10* perturbs retinal morphogenesis and represents a novel genetic cause of microphthalmia and coloboma. Future experiments to determine if other thioredoxins are important in eye morphogenesis and to clarify the mechanism of function of *Txndc10* in eye development seem warranted.

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## 2416/T/Poster Board #965

***Extra-toes spotting (Xs)*: Mutations in Eif3c implicate translation initiation complex proteins in Shh signaling.** *E.S Luetkemeier[1], D.E Gildea[1,2], E.J Spaulding[1], S.K Loftus[1], Y. Yang[1], W.J Pavan[1], L.G Biesecker[1].* 1) Genetic Disease Research Branch, NHGRI, National Institutes Hlth, Bethesda, MD; 2) Graduate Program in Genetics, The George Washington University, Washington DC.

The Sonic hedgehog (SHH) pathway regulates many developmental processes, including limb patterning. Dysregulation of this pathway causes malformations, as seen in Greig cephalopolysyndactyly syndrome (GCPS). One cause for GCPS is mutations of *GLI3*, which encodes a transcription factor in the SHH pathway. However, ~15% of patients with GCPS have no *GLI3* mutation. To find other GCPS genes, we used polydactylous mice as animal models. Extra-toes spotting (*Xs*) was selected since the phenotype is similar to Extra-toes ($Gli3^{Xt}$) (a known GCPS animal model). Both mice have preaxial polydactyly and ventral hypopigmentation, along with shortened forelimbs. Previous mapping excluded *Gli3* as the gene mutated in *Xs*. Linkage mapping was performed to narrow the *Xs* interval, followed by sequencing which showed a heterozygous (c.906C>T, p.R303X) variant in *Eukaryotic translation initiation factor 3-subunit C (Eif3c)*. A second *Eif3c* mutation was identified (c.1761_1817del p.L568_L586del) in Extra-toes spotting-like (*Xsl*). Eif3c is a core subunit of Eif3 as it recruits Met-$tRNAi^{Met}$ and mRNA to the 40S ribosome and scans for the start codon during translation initiation. We conclude that mutations in *Eif3c* cause Xs. As $Gli3^{Xt}$ and *Xs* mice show phenotypic overlap, we hypothesized that mutations of *Eif3c* affect Shh/Gli3 signaling. To test this hypothesis, we used *in situ* hybridization in $Xs^{J}$/+ embryos to evaluate the expression of Shh/Gli3 pathway genes. The *Shh, Ptch1, Fgf8, Gli1*, and *Hoxd13* genes were misexpressed, implying that *Eif3c* causes polydactyly by perturbing the Shh/Gli3 pathway. Therefore, *Eif3c* may be a novel candidate for GCPS human patients who lack *GLI3* mutations. Human *EIF3C* is located on chromosome 16p11.2, in a tandem inverted repeat, with four total copies of the gene. This gene is one of only 134 genes that are known to be human-specific duplications. However, the prevalence and orientation of these four copies are unknown. We performed QPCR, aCGH and FISH analyses to show that there is copy number variation of *EIF3C* among humans. We identified a nonsense mutation in *EIF3C* in one patient who has no GLI3 mutation. In summary, mutations in *Eif3c* cause Xs and the human homolog of this gene is a human-specific gene duplication and the locus has both copy number and structural variation in the population. This gene is a candidate for human malformations that overlap with GCPS.

## 2417/T/Poster Board #966

**Upregulation of the Organic Cation/Carnitine Transporter Family in the Pregnant and Lactating Murine Mammary Gland and Implications for the Suckling Infant.** *A. Lamhonwah[1, 2], I. Tein[1, 2].* 1) Dept Pediatrics, Hosp Sick Children, Toronto, ON, Canada; 2) Dept. Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada.

**Background**: Organic cation/carnitine transporters transport carnitine, drugs, and xenobiotics, (e.g. choline, quinidine, verapamil) and are expressed in muscle, heart, blood vessels, brain, placenta, etc. The transport and provision of L-carnitine into breast milk is important for the normal growth and development of the suckling infant, particularly given the very low endogenous L-carnitine biosynthetic capabilities of the infant. Further, organic cationic drugs present in human breast milk may pose a significant exposure risk to the nursing infant. **Methods**: To characterize the expression patterns of mOctn1, -2 and -3 in murine mammary gland, we applied our transporter-specific antibodies to mOctn1,-2 and -3, to murine breast sections of different developmental stages, followed by secondary antibody and the DAB peroxidase detection. Sections were counterstained with hematoxylin. **Results**: We demonstrated the differential expression of mOctn1, -2 and -3 in the epithelial ducts, muscle and lymphoid tissue of murine virginal non-lactating, pregnant, and lactating breast. There was a notable upregulation of expression of all three transporters in both the breast tissue of the pregnant mouse (18 days gestation) and lactating mouse (8 days after giving birth) compared to the virginal 8 week old mouse. There was also very strong expression of peroxisomal Octn3 in the specialized myoepithelial cells of the prelactating breast. **Conclusions**: The upregulation of the Octns is likely related to the increased metabolic energy demand of the actively lactating breast on plasmalemmal Octn2-, mitochondrial Octn1- and peroxisomal Octn3- dependent fatty acid oxidation. This may also be related to an increased nutritional demand by the suckling infant for carnitine and other cationic homologues and xenobiotics from the nursing mother, which would be facilitated by an upregulation of mammary gland ductular epithelial Octn2 transporter.

## 2418/T/Poster Board #967

**TRPS1, a regulator of chondrocyte proliferation and differentiation, interacts with the activator form of GLI3.** *F.J. Kaiser[1], M. Wuelling[2], L.A. Buelens[2], D. Braunholz[1], R. Depping[3], G. Gillessen-Kaesbach[1], A. Vortkamp[2].* 1) Inst. of Human Genetics, University of Luebeck, Germany; 2) Center for Medical Biotechnology (ZMB), Department of Developmental Biology, University Duisburg-Essen, Germany; 3) Department of Physiology, Center for Structural and Cell Biology in Medicine, University of Luebeck, Germany.

The *TRPS1* gene on human chromosome 8q24.1 encodes a multi zinc finger transcription factor protein. Mutations in *TRPS1* cause the tricho-rhino-phalangeal syndrome (TRPS). Besides typical craniofacial anomalies, skeletal malformations are characteristic hallmarks of patients with TRPS. Here we show that TRPS1 interacts with Indian hedgehog (Ihh)/GLI3 signalling and regulates chondrocyte differentiation and proliferation. By immunoprecipitation assays using transiently transfected cells as well as native tissue samples from embryonic mouse limbs, we could demonstrate that TRPS1/Trps1 specifically interacts with the activator form of GLI3/Gli3, whereas a direct binding of the repressor form of GLI3/Gli3 could be excluded. GST pull-down experiments were used to verify the interaction of the isolated GLI3 activator domain with TRPS1. Through the use of different truncated TRPS1 constructs, a domain of 185 aa, containing three predicted zinc fingers, was shown to be sufficient for the interaction with GLI3. Using different mouse models we find that in distal chondrocytes Trps1 and the repressor activity of Gli3 are required to expand distal cells and locate the expression domain of Parathyroid hormone related peptid. In columnar proliferating chondrocytes Trps1 and Ihh/Gli signalling have an activating function. The differentiation of columnar and hypertrophic chondrocytes is supported by Trps1, independent of Gli3. Trps1 seems thus to organize chondrocyte differentiation interacting with different subsets of co-factors in distinct cell types.

## 2419/T/Poster Board #968

**Genetic influences on asthma susceptibility in the developing lung.** *I. Mandeville[1], N. Carpe[1,2], J. Chu[3], L. Ribeiro[1,2], S. Cornejo[1,2], B.A. Raby[3], S.T. Weiss[3], F. Kaplan[1,2,3,4].* 1) McGill Univ-Montreal Child Hosp. Research Inst., Montreal, QC, Canada; 2) Dept. of Human Genetics, McGill University; 3) Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School; 4) Depts of Pediatrics and Biology, McGill University.

Rationale: The prevalence of asthma in children under 4yr has increased dramatically in the last 2 decades. Existing evidence suggests that this increase in prevalence derives from early environmental exposures acting on a pre-existing asthma-susceptible genotype. We are exploring the origins of asthma susceptibility in developing lung in rat strains that model the distinct phenotypes of airway hyperresponsiveness (AHR, Fisher) and atopy (Brown Norway). Methods: We assessed lung function, cell proliferation, epithelial repair, goblet cell number, and BAL cell differentials from postnatal day (PN) 1-14. mRNA (Real-time PCR) and protein (immunohistochemistry [IHC]) were used to assess gene expression in lung tissue and primary lung cell culture. Illumina genome wide profiling was used to compare global gene expression patterns. Results: Initially, we established that BN and Fisher pups modeled early changes in lung structure / function. BN and Fisher pups showed differences in lung resistance at PN14 (the earliest age at which lung function can be reliably assessed) when compared to normoresponsive Lewis pups. At PN7, BN lungs showed increased epithelial proliferation; both BN and Fisher lungs had increased fibroblast proliferation. BN and Fisher lungs also demonstrated altered epithelial repair. Newborn BN rats displayed tracheal goblet cell hyperplasia. Fisher pups had persistent elevation of BAL neutrophils. Having established developmental differences in the respiratory phenotype of these models, we next explored associated changes in gene expression. Principal components analysis confirmed distinct global gene expression differences between strains over time. Linear regression analysis (n=4 per strain per time point) identified developmental variation in expression of 1,376 genes, including several previously implicated in asthma. Biological processes heavily represented were growth and development, ECM and cell adhesion, cell signaling and immune function. Differential mRNA and protein expression was validated for EGFR, PDGFRβ GR, IPO13 and ADAM33. Conclusion: Innate differences in patterns of gene expression in developing lung contribute to individual variation in respiratory phenotype and are likely to contribute to the pathogenesis of asthma.

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## 2420/T/Poster Board #969

**Lgl1 deficiency exacerbates the effects of neonatal hyperoxia-induced lung injury.** *K. Nadeau[1,2], I. Mandeville[1], T. Bao[1,3], L. Ribeiro[1,2], N.B. Sweezey[4], F. Kaplan[1,2,3].* 1) Dept Human Genetics, McGill- Univ Montreal Children's Hospital Res Inst, Montreal, PQ, Canada; 2) Dept of Pediatrics, McGill University, Montreal, PQ, Canada; 3) Dept of Biology, McGill University, Montreal, PQ, Canada; 4) Hospital for Sick Children, Toronto, ON, Canada.

Bronchopulmonary dysplasia (BPD) is a leading cause of neonatal morbidity with sequelae that persist into adulthood. Impaired alveolarization in BPD is associated with disruption of elastin fibres and mucus cell hyperplasia. Exposure of the immature lung to hyperoxia is a primary risk factor for BPD. Yet the molecular mechanisms that link oxygen exposure to disrupted alveolarization and postnatal lung inflammation remain unclear. We cloned Lgl1, encoding a developmentally regulated glycoprotein in the lung. Absence of Lgl1 is lethal prior to lung formation. Lgl1+/- mice display a complex phenotype with features of inflammation in the post-natal period and altered lung mechanics at maturity. The present study was designed to explore whether deficiency of Lgl1 in Lgl1+/- mice exacerbates hyperoxia-induced neonatal lung injury. We report that, compared to their Lgl1+/+ littermates, Lgl1+/- mice exposed to hyperoxia for 4 weeks had more profound neutrophilia, increased macrophage influx, more intense fragmentation and disorganization of the elastin scaffold and reduced lung elastance following methacholine provocation. Oxygen exposure was associated with reduced Lgl1 expression in all mouse pups. Lgl1 expression in hyperoxia-exposed Lgl1+/- mice at 4 weeks was ~25% of that observed in Lgl1+/+ littermates. Exposure to hyperoxia for 2 weeks led to profound goblet cell hyperplasia and elevated MUC5AC levels in a subset of Lgl1+/- mice, suggesting that genetic modifiers influence the inflammatory phenotype. Our results suggest that Lgl1 contributes to the complex phenotype of BPD in infancy and that compromised developmental expression of Lgl1 may increase susceptibility to respiratory pathology in adulthood.

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## 2421/T/Poster Board #970

**Intracellular function of neuron-specific TAF1 and its isoform in Neuro-2a mouse neuroblastoma cells.** *S. Makino[1], G. Tamiya[2], I. Tooyama[1].* 1) Molecular Neuroscience Research Center, Shiga University of Medical Science; 2) Advanced Molecular Epidemiology Research Institute, Yamagata University School of Medicine.

We previously found a neuron-specific isoform of the *TAF1* (TATA box binding protein-associated factor 1), which is the disease causative gene of X-linked recessive dystonia-parkinsonism showing severe neurodegeneration in striatum (XDP/DYT3; MIM314250). The *TAF1* gene encodes the largest component of the TFIID complex involved in RNA polymerase II-mediated expression of many genes related cell division. The neuron-specific isoform of the *TAF1* gene, named *N-TAF1*, may have an essential role in neuronal survival in the striatum through transcriptional regulation of many neuron-specific genes. To investigate the detailed function of the neuron-specific isoform of the *TAF1* gene, we carried out over-expression of *N-TAF1* in the mouse Neuro-2a (N2a) cell line. The cell lines were prepared by stable transfection of N2a-TetR cells with an inducible mouse N-TAF1/TAF1 expression vector. The doxycycline treatment of N-TAF1/TAF1 inducible cells allowed a substantial expression of its products. We subsequently performed MTS assay, which could easily be interpreted as cellular mitochondria activities, to examine if the over-expression of N-TAF1/TAF1 affect cell proliferation in Neuro-2a. The MTS assay showed that the N-TAF1 induction for 7 days significantly inhibited N2a proliferation whereas the TAF1 induction elevated cell proliferation. In addition, we examined a possible difference in the intracellular trafficking between N-TAF1 and TAF1 using FLAG-tagged protein. Through these in vitro experiments, we demonstrated that *N-TAF1* varies from *TAF1* gene in intracellular transportation, probably reflecting the difference in physiological roles between *N-TAF1* and *TAF1*. These experiments would reveal the essential role of these genes and shed light on the molecular pathogenesis of disease caused by transcriptional dysregulation as well as XDP.

## 2422/T/Poster Board #971

**Biochemical Analysis of NALP1 Inflammasome Variants Associated with Autoimmune Vitiligo.** *C. Mailloux[1], C. Dinarello[2], R. Spritz[1].* 1) Human Med Gen, Univ Colorado Denver, Aurora, CO; 2) Div Infectious Diseases, Dept Medicine, Univ Colorado Denver, Aurora, CO.

Generalized vitiligo is a common autoimmune disease that is often associated with other autoimmune disorders such as autoimmune thyroid disease, Addison's disease, pernicious anemia, lupus, rheumatoid arthritis, psoriasis, and adult onset insulin dependent diabetes. We previously showed that common high-risk genetic variants of the *NALP1* (*NLRP1*) gene are associated with susceptibility to vitiligo-associated multiple autoimmune disease. NALP1, the canonical member of the NLR (NACHT-leucine rich repeat) protein family, forms the backbone of the inflammasome, a complex that regulates apoptotsis and activation of the innate immune system in response to extracellular triggers. Of the vitiligo-associated *NALP1* variants, there is only one non-synonymous SNP, rs12150220, that defines disease susceptibility haplotypes. SNP rs12150220 is located between the N-terminal Pyrin and internal NACHT domains, and corresponds to a low-risk leucine or a high-risk histidine at codon 155. To investigate functional differences between high-risk and low-risk *NALP1* alleles defined by SNP rs12150220, we first developed an antibody that recognizes full-length NALP1 protein, and found that NALP1 expression is highest in B cell lineages. We observed no evident difference in the amount of steady-state NALP1 protein in lymphoblastoid cell lines homozygous for the high-risk 155His and low-risk 155Leu alleles of rs12150220, indicating that expression of these two alleles, and stability of the corresponding 155Leu and 155His NALP1 polypeptides, is similar. This suggests that susceptibility to autoimmune disease may result from differential binding of the NALP1 155Leu versus 155His polypeptides to other components of the inflammasome, thereby differentially activating innate immunity and apoptotic pathways in response to environmental triggers. We are currently investigating binding of NALP1 155Leu versus 155His to known components of the NALP1 inflammasome, as well as to other potential interaction partners that may participate in a complex variety of functionally different novel inflammasomes.

## 2423/T/Poster Board #972

**STRUCTURE-FUNCTION ANALYSIS OF HUMAN HEMATOPOIETIC PBX INTERACTING PROTEIN (HPIP): A NOVEL HUMAN STEM CELL REGULATORY PROTEIN.** *P. Kaur[1], C. Stadler[1], W. Hiddemann[1], K.R. Humpheries[2], S. Bohlander[1], C. Buske[1], M. Feuring-Buske[1].* 1) Helmholtz-zentrum, Med III, Klinikum Groβhadern, Muenchen, Germany; 2) The Terry fox laboratories, Vancouver, BC, Cannada.

Human hematopoietic PBX interacting protein (HPIP) is a 731 amino acid protein, discovered as a novel interacting partner of the PBX homeodomain protein. HPIP inhibits the ability of PBX-HOX heterodimers to bind to target sequences and strongly inhibits the transactivation activity of E2A-PBX1 [t(1;19) translocation, which occurs in 25% of pediatric pre-B cell acute lymphocytic leukaemia] (Abramovich C. et al JBC, 2000; Oncogene, 2002). HPIP cDNA was cloned in pMSCV-IRES-YFP cassette. Umbilical cord blood enriched with CD34+ population of stem cells was obtained to perform in vitro and in vivo experiments. Mutants, with deletions of the microtubule binding region (ΔMBR-HPIP), and NRPID; deletion of LXXLL motif/ PBX interacting domain (ΔNRPID-HPIP) were generated and tested in vitro and in vivo. The constitutive expression of HPIP wt and ΔMBR-HPIP in human cord blood cells (CD34+) enhanced erythroid colony formation in CFC assay ($p=0.008$, $n=6$) while the ΔNRPID-HPIP mutant nullified the effect. Both mutants of HPIP augmented significantly, the formation of primitive colonies (GEMM and GM) in methylcellulose assay ($p \leq 0.01$, $n=6$) as compared to YFP control and HPIP wt. In replating CFC assays ΔNRPID-HPIP showed an increased number of myeloid colonies ($p \leq 0.01$, $n=6$) and GM (p=ns) colonies but a decrease in granulocytic colonies ($p \leq 0.05$, $n=6$) compared to YFP control and HPIP wt. Long-term culture initiating cell assay (LTC-IC) demonstrated that HPIP protein enhanced the frequency of LTC-IC ($p \leq 0.1$, $n=3$) while mutants did not show any significant increase as compared to control YFP. HPIP wt and the mutants did not enhance the yield of LTC-IC derived CFC per million cells (p=ns, n=3). Infected cells were transplanted into NOD/SCID mice. HPIP induced, a significant increase in CD34+CD19+, CD10+ and CD117+ ($p \leq 0.05$ and $p \leq 0.1$) cells. Intriguingly, there was a significant increase in scid repopulating cell frequency introduced by HPIP wt as compared to control YFP in NOD/SCID mice. Interestingly, a decrease in SRC frequency was observed in the case of ΔNRPID-HPIP mutant form as compared to HPIP wt. Affymetrix differential gene expression analysis was performed on CB CD34+ cells transduced with HPIP wt and its mutant forms. A deregulation of genes involved in cytokine-cytokine pathway and MAPK pathway was observed.

## 2424/T/Poster Board #973

**LINE-1 endonuclease-independent retrotransposition.** *H. Kopera[1], J. Garcia-Perez[3], J. Moran[1,2].* 1) Dept Human Genetics, Univ Michigan, Ann Arbor, MI 48109 USA; 2) Howard Hughes Medical Institute; 3) Spanish Stem Cell Bank, Center for Biomedical Research, University of Granada, Avda Conocimiento s/n Armilla, Granada 18100, Spain.

The mobile element Long INterspersed Element-1 (LINE-1 or L1) is a non-LTR retrotransposon that comprises ~17% of the human genome. A full length LINE-1 is 6kb in length and encodes two open reading frames, ORF1 and ORF2. ORF1 encodes for a 40kDa RNA binding protein and ORF2 encodes for a 150kDa protein with endonuclease (EN) and reverse transcriptase (RT) domains. Both ORF1p and ORF2p are required for retrotransposition. Despite its abundance in human DNA, only ~80-100 LINE-1s per human genome remain retrotransposition-competent. Recent evidence shows that LINE-1s are still active in humans and that L1-mediated retrotransposition events are responsible for about 1 in 1000 disease causing mutations. LINE-1 integrates into genomic DNA through a process called target site primed reverse transcription. ORF2p cleaves genomic DNA and the reverse transcriptase activity then uses the exposed 3' hydroxyl as a primer for L1 cDNA synthesis. Our laboratory has recently described retrotransposition of an L1-ORF2p EN mutant in a hamster cell line deficient for the non-homologous end-joining (NHEJ) DNA repair pathway. Analyses of these endonuclease-independent retrotransposition events revealed some LINE-1s integrated at dysfunctional telomeres. Interestingly, when the integration junction was sequenced, the LINE-1 poly(A) sequence was flanked by the complement of perfect telomeric repeats (5'-CCCTAA-3'). Using L1 ribonucleoprotein particles (RNPs), and an oligonucleotide that ends in a series of telomeric repeats as a substrate to mimic a deprotected telomere, we have recapitulated this step of L1 retrotransposition *in vitro*. Interestingly, wild type L1 RNPs contain a putative endonuclease activity that processes the telomeric oligonucleotide before it is used as a substrate for reverse transcription. We currently are examining a cohort of L1-ORF2 endonuclease mutants for their ability to both retrotranspose in a NHEJ-deficient hamster cell line and use oligonucleotides ending in telomeric repeats as substrates for reverse transcription *in vitro*. Thus, these data further our understanding of LINE-1 retrotransposition and integration.

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### 2425/T/Poster Board #974

**Impaired responses to sensory stimuli in miR-183 morphant zebrafish.** *P.D. Witmer[1,2], H.A. Burgess[4], S. Xu[3], S. Fisher[1], D. Valle[1].* 1) Human Gen Program, Johns Hopkins Univ, Baltimore, MD; 2) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 3) Department of Ophthalmology/Neurological Sciences, Rush Univ Medical Ctr, Chicago, IL.D; 4) Unit on Behavioral Neurogenetics, Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, MD.

The miR-183 miRNA family (miR-183, -96 and -182) is highly enriched in the mammalian retina and other sensory organs. Our own results (Xu et al., JBC 2007) and other recently reported studies including reports implicating miR-96 as a deafness gene in humans and mice (Mencía et al., Lewis et al., Nat Genet 2009) show that these miRNAs play important roles in sensory neural biology. Sequence conservation extends to teleost fish, where expression is detected in the lateral line, a mechanosensory organ. To investigate the functions of the miR-183 family during development, we utilized morpholino-directed knockdown of miR-183, -96 and -182 expression in zebrafish. We produced morphant larvae with abnormal balance, swimming abnormalities and an attenuated response to vibrational stimuli: a phenotype similar to those reported for zebrafish circler mutants. To examine sensory function further in these triple-morphant fish, we tested auditory responses to acoustic startle and visual responses using dark flash. Our results indicate a significantly reduced response to startle and a significantly increased latency in response to dark flash stimuli. Taken together these results strongly suggest that these fish hear and see less well than their sibling controls. Interestingly, knockdown of a single miRNA in the cluster, miR-183, results in bilateral loss of the anterior otolith, a structure necessary for auditory function. Even so, in miR-183 morphant fish that retain their otoliths, we observe the same abnormal response to dark flash seen in the triple-morphant group. In contrast, no changes in morphology or response to sensory stimuli were observed for miR-96 or miR-182 single morphants. Thus, it appears that loss of miR-183 alone accounts for the sensory impairments observed in morphant fish. We conclude that members of the miR-183 family are important for sensory neuron development and/or function. Additional studies are underway to characterize the nature of the sensory deficits in these fish and relate these observations to human phenotypes.

### 2426/T/Poster Board #975

**Human lysyl oxidase-like protein 2 (LOXL2) functions as an amine oxidase toward collagen and elastin.** *Y. Kim, Y.M. kim.* Department of Biochemistry, Wonkwang University School of Medicine, Iksan, Jeollabuk-Do, Korea.

The lysyl oxidase-like protein 2 (LOXL2) is a member of the emerging family of lysyl oxidase (LOX), several of which have been shown to function as copper-dependent amine oxidases catalyzing lysine-derived cross-links in extracellular matrix proteins, such as collagen and elastin. LOXL2 contains four scavenger receptor cysteine-rich domains in addition to the characteristic domains of the LOX family, including a copper-binding domain, a cytokine receptor-like domain, and residues of the lysyl-tyrosyl quinine cofactor. Recently, LOXL2 was also reported to mediate the induction of epithelial-mesenchymal transition for tumor progression. In an effort to assess its amine oxidase activity, we expressed LOXL2 as recombinant forms attached with hexa-histidine residues at the carboxy terminus. The purified LOXL2 proteins showed an amine oxidase activity toward different types of collagens and elastin. These results indicate that LOXL2, with the four copies of SRCR domains, also function as an active amine oxidase. Availability of the pure and active forms of LOXL2 will be significantly helpful for functional studies related to substrate specificity and crystal structure of this amine oxidase, which should provide significant insights into functional differences within the LOX family members.

### 2427/T/Poster Board #976

**Investigating the Mechanism of APOBEC3-Mediated Inhibition of LINE-1 Retrotransposition.** *S.R. Richardson[1], I. Narvaiza[4], A.E. Hulme[1,5], A.J. Doucet[1], J.L. Garcia-Perez[1,6], M.D. Weitzman[4], J.V. Moran[1,2,3].* 1) Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI, USA; 2) Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, USA; 3) Howard Hughes Medical Institute; 4) The Salk Institute for Biological Studies, La Jolla, CA, USA; 5) Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; 6) Spanish Stem Cell Bank, Center for Biomedical Research, University of Granada, Spain.

Long INterspersed Element 1 (LINE-1 or L1) is an endogenous retrotransposon whose sequences comprise about 17% of human DNA. Although most L1 sequences are nonfunctional molecular “fossils”, an estimated 80-100 L1 elements per human genome are able to mobilize by a mechanism termed retrotransposition. Retrotransposition involves cleavage of genomic DNA by the L1-encoded endonuclease, and the use of the liberated 3’OH as a primer by the L1-encoded reverse transcriptase to generate a cDNA using the L1 mRNA as a template. L1 retrotransposition frequently is associated with alterations to genomic DNA, and about 65 cases of human disease have been attributed to L1-mediated retrotransposition events. L1 therefore poses a substantial threat to genomic integrity, and it follows that host genomes may have evolved mechanisms to curtail L1 mobility. One such defense appears to be the APOBEC3 (A3) family of cytidine deaminases. Specifically, APOBEC3A (A3A) and APOBEC3B (A3B) have been demonstrated to inhibit L1 retrotransposition in a cultured-cell assay; however, the mechanism of inhibition is not known. Here, we describe our efforts to determine how A3A and A3B inhibit L1 retrotransposition. Our results suggest that A3-mediated inhibition may occur independently of the sequence of the LINE element, as both A3A and A3B are able to restrict retrotransposition of LINE-1 elements from mouse and a LINE-2 element from zebrafish, which bear little identity to human L1 sequences. Our data also suggest that A3-mediated inhibition of L1 retrotransposition is not exerted during the first step of integration, as we have found that endonuclease-independent L1 retrotransposition is inhibited by both A3A and A3B. We currently are employing in vitro assays to determine the impact of A3A and A3B on other L1-encoded activities. Our ultimate goal is to achieve a mechanistic understanding of A3-mediated inhibition of L1 retrotransposition.

### 2428/T/Poster Board #977

**Mutation profile of CYP1B1 Gene in North Indian Congenital Glaucoma Population.** *M. Tanwar[1], T. Dada[2], R. Sihota[2], TK. Das[3], R. Dada[1].* 1) Dept Anatomy, AIIMS, New Delhi, India; 2) Dr. R.P.Centre for Ophthalmic Sciences, AIIMS, New Delhi, India; 3) Electron Microscope Facility, AIIMS.

Mutations in CYP1B1 gene are a predominant cause of congenital glaucoma. This study was planned with the aim to identify the mutation profile of CYP1B1 gene in north-Indian PCG patients. After ethical clearance, fifty congenital glaucoma patients and 50 ethnically matched controlled were recruited. Genomic DNA was isolated from blood and trabecular meshwork and CYP1B1 gene was screened for six most prevalent mutations (Termination at 223, Gly61Glu, Pro193Leu, Glu229Lys, Arg368His and Arg390Cys) by PCR-RFLP. DNA sequencing was done to identify other mutations and for confirmation of RFLP positive samples. On PCR-RFLP, 21/50 (42%) cases were found positive for one/more of these mutation.However on sequencing we found that 23/50(46%) harbored CYPIBI mutation. Ter@223 was found in 18%,p.R390H in 16% and p.R368H in 8% cases. Three novel mutations p.L24R, p.F190L, p.G329D were identified by DNA sequencing. Leucine, phenylalanine and glycine are conserved at 24,190 and 329 positions in CYP1B1 protein in different species suggestive of important functions at these loci. Ter@223 was found to be most prevalent mutation in our patients while p.R368H was most prevalent in southern India. Difference in frequency and mutation profile may be due to heterogeneous Indian population. CYP1B1 mutation impairs anterior chamber development and differentiation with block in aqueous outflow and raised intraocular pressure (IOP). In conclusion three novel mutations have been identified. Studies of pathogenic sequence variants in CYP1B1gene in different populations may contribute to a better understanding of disease pathogenesis. This may lead to the development of novel therapeutic approaches in near future.

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## 2429/T/Poster Board #978

**Collection, Classification, and Assessment of Variants of the Wilson Disease Copper Transporter ATP7B.** *D.A. Bugbee, L. Prat Davies, S.M. Kenney, G. Macintyre, D.W. Cox.* Medical Genetics, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Wilson Disease (WND) is an autosomal recessive disease caused by impaired or abolished capacity to remove copper from the body. Patients with WND accumulate copper in multiple organs. This excess copper produces various clinical symptoms (hepatic and/or neurological) presenting anywhere from 3 to 70 years of age. Several biochemical tests aid in diagnosis of WND, however none is abnormal in all patients. Thus, diagnosis proves challenging. In 1993 the gene responsible for causing WND was identified as encoding a P-type ATPase, ATP7B. This protein traffics and transports copper within, and out of, the cell. Numerous variants of *ATP7B* have been identified in both affected and non-affected individuals. To better understand the implications of variation in *ATP7B*, we have curated variant information in a searchable Wilson Disease Database (http://www.wilsondisease.med.ualberta.ca/database.asp) that adheres to HGVS nomenclature guidelines. Over 1600 submissions have been entered into the database from: our data, the literature, and direct submission. In total, these entries include 644 variants, of which 54 are suspected to be non-disease-causing (NDV), 57 are silent changes (Sil), and 510 are possible disease-causing variants (DV). Of this 510 DV category there are: 150 deletion/insertions/duplications, and 360 substitutions. The remaining 23 variants have been submitted as both DV and NDV from various sources. The classification for each variant is supported by several criteria, including functional data. In our laboratory, variants are functionally assessed by a combination of a Chinese Hamster Ovary (CHO) cell viability assay, an ATP7B trafficking assay, and a yeast growth assay. The CHO viability assay examines the ability of each variant to protect cells from toxic amounts of copper. This result indirectly indicates a variant's capacity to perform its *in vivo* roles, both in transport and trafficking. These cells can then be directly observed for trafficking using immunofluorescence microscopy. The yeast assay examines copper transport function, as trafficking is thought to be absent in yeast. Many variants still require functional assessment and classification support as indicated in the Wilson Disease Database.

## 2430/T/Poster Board #979

**FOXD3 in the genetic syndrome of Carney triad, [paragangliomas associated with gastrointestinal stromal tumors (GISTs) and pulmonary chondromas] and in sporadic GISTs.** *A. Horvath[1], F. Faucz[1], G. Assie[2,3], S. Kim[4], M. Lodish[1], M. Raygada[1], E. Bimpaki[1], M. Nesterova[1], K. Tsang[1], E. Remmers[5], M. Almeida[1], J.A. Carney[6], J. Bertherat[3], C. Antonescu[7], L. Helman[4], C. Eng[2], C. Stratakis[1].* 1) Section on Endocrinology & Genetics, PDEGEN, NICHD, NIH, Bethesda, MD 20892, USA; 2) Genomic Medicine Institute and Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195, USA; 3) Department of Endocrinology, CHU Cochin & Institut Cochin, INSERM U567, CNRS UMR8104, IFR 116, Université Paris V-René Descartes, 75014 Paris, France; 4) Pediatric Oncology Branch, National Cancer Institute, NIH, Bethesda, MD 20892; 5) Genetics and Genomics Branch, National Institute of Arthritis, Musculoskeletal and Skin Diseases, NIH, Bethesda, MD 20892; 6) Department of Laboratory Medicine and Pathology (Emeritus member), Mayo Clinic and Foundation, Rochester, MN 55905, USA; 7) Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, USA.

The discovery of constitutive KIT and PDGFRA activation in the majority of gastrointestinal stromal tumors (GIST) led to the identification of a distinct group of GISTs that do not harbor KIT and PDGFRA mutations (non-KIT/PDGFRA-GISTs). These tumors respond poorly to imatinib mesylate or other kinase activity inhibitors and affect mostly pediatric and young adult patients that: (1) do not have other associated lesions or a family history of GIST ("sporadic"); or, (2) exhibit the Carney-Stratakis syndrome [CSS; OMIM#606864], an autosomal dominant syndrome characterized by paraganglioma (PGL) and GIST and caused by mutations in the SDHB, SDHC, and SDHD genes, or the nonfamilial Carney Triad [CT; OMIM#604287], a condition that associates PGLs and GISTs with pulmonary chondromas and other tumors. We analyzed patients with CT and their tumors using a genome-wide approach (Assie G, et al. Am J Hum Genet. 2008;82:903, Matyakhina L, et al. J Clin Endocrinol Metab 2007;92:2938). In 5 tumors from 4 (of 22) patients with CT and non-KIT/PDGFRA-GISTs, we identified somatic deletions on the region 1p31 harboring the FOXD3 gene. FOXD3 encodes the factor "Genesis" that is expressed during early embryogenesis and is a main determinant of embryonic stem cell fate. Western blot analysis and immunohistochemistry confirmed FOXD3 protein downregulation in tumors from CT patients with deletions. FOXD3 sequencing in 22 CT patients showed no additional germline or somatic mutations. However, we found 3 FOXD3 coding variants in 3 unrelated patients with sporadic non-KIT/PDGFRA-GISTs among 24 patients with such tumors: c.161G>A/p.R54H, c.286G>T/p.V96L, and c.255_266del12. The R54H and Del12 were also studied in a total of 930 unrelated control individuals and the V96L in 296 unrelated controls. Analyzed together, R54H and Del12 are more frequent among GIST patients ($p=0.089$). V96L is also more frequent in non-KIT/PDGFRA-GISTs (without reaching statistical significance). R54H and Del12 affect the evolutionarily preserved forkhead box of the FOXD3 protein; R54 is present in all forkhead box-containing proteins in both humans and mice. Electro-mobility shift assays with constructs containing the three FOXD3 mutants suggested impaired interaction between each of the mutants and DNA target sequences. We conclude that FOXD3 defects may be involved in non-KIT/PDGFRA-GIST pathogenesis. Ongoing studies aim at further elucidating the role of FOXD3 in the formation of this tumor.

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## 2431/T/Poster Board #980

**Molecular investigation of an autism risk region on chromosome 12.**
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There is a strong genetic component to autism, but studies to date have demonstrated that the underlying genetic architecture is complex, with numerous genes potentially involved. One strategy for uncovering these genes is the use of large, extended families with multiple, distantly related, affected individuals. Our previous genome-wide linkage study identified a 4 centimorgan region (75-79 cM) on chromosome 12 that demonstrated significant linkage (HLOD = 4.51) across eleven extended multiplex families with only male affected individuals. Our goal is to comprehensively evaluate the chromosome 12 candidate region in order to identify the genetic abnormalities that underlie the strong autism linkage peak. We anticipate that this genetic abnormality will confer a relatively moderate autism risk due to its presence in these large, extended families. We are currently using a three-pronged approach to identify potential causative variants; 1) analysis of copy number variations (CNVs) across the region using comparative genomic hybridization (CGH) arrays, 2) traditional Sanger sequencing to identify variants in known and well annotated genes, and 3) deep re-sequencing of the entire candidate region using the Illumina Genome Analyzer II to identify all potential variants in the region. In order to identify copy number alterations, an Agilent custom CGH array was designed across a broad region containing the linkage peak (51,309,829-66,881,321 bp). Twenty-three affected individuals from 10 distinct extended families were evaluated. Thirteen CNVs were present in at least two affected individuals within a family. However, we were unable to confirm that these CNVs were causative of autism. Sanger sequencing was performed on 12 autistic individuals in 8 distinct families that define the region, as well as 10 control Caucasian individuals for 20 of the annotated genes in the minimal shared region (60,710,030-64,239,801 bp). Preliminary sequencing results failed to show variations of interest in 8 genes; the remaining 12 genes demonstrate one or more alterations that warrant further investigation. Deep re-sequencing using the Illumina GA II next-generation sequencing system of the minimal candidate region to completely catalogue variation in these families is currently underway and studies are ongoing to determine if any identified single nucleotide or copy number variations play a role in the etiology of ASD.

## 2432/T/Poster Board #981

**The alternative glucocerebrosidase promoter is located within a 1000-bp region 2.6 kb upstream of the usual promoter.**
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Gaucher disease is a lysosomal glycolipid storage disorder caused by an inherited deficiency of glucocerebrosidase. The patients carry mutations in the glucocerebrosidase gene (GBA, 1q21). The gene has a promoter (“downstream promoter”) immediately upstream of exon 1. A database search identified ESTs and other partial glucocerebrosidase transcripts that apparently originated at an alternative promoter located 2.6 kb upstream of the downstream promoter. The alternative transcripts from the putative upstream promoter contained one or two extra exons (exon -2 or exons -2, -1, respectively), but the first ATG codon and predicted protein sequence were the same as in transcripts from the downstream promoter. Our main goal was to confirm that the predicted alternative promoter initiates transcription of GBA. A 1000 bp PCR product containing the sequence of the predicted upstream promoter was cloned in both sense and antisense orientations into pGL4 plasmid vector (Promega) into the polylinker site upstream of the firefly luciferase reporter gene. The constructs were transfected to HepG2 (human hepatoblastoma) cells. The pRL-TK vector (Promega) harboring the Renilla luciferase gene was co-transfected as an internal control enabling normalization of the transfection efficiency. A luminescent signal was measured for both upstream and downstream promoters, however, the highest promoter activity (17. 82 ± 1.1 relative units) was observed in the construct containing the sequence of downstream promoter, while the luminescent signal from alternative promoter construct was 3, 01 ± 0.43 relative units. Furthermore, no luminescent signal was measured in constructs with inserts in the opposite orientation used as negative controls. A CPG island overlapping with the putative promoter was completely unmethylated when analyzed by bisulfite sequencing. 5’-RACE (RNA ligase mediated RACE) suggested 3 possible transcription initiation sites. Transcripts from upper promoter were found by RT/PCR in four different human tissues and the ratio between the transcripts from both promoters appeared to be similar in different tissues, the assay, however, was not quantitative. Alternative promoters often play role in tissue-specific proteins with different amino-acid termini - which is not the case in the glucocerebrosidase promoters. Conclusion: We have confirmed that the alternative upper promoter initiates transcription of GBA. Supported by GAUK 121407, VZ MÅM ÄŒR 0021620806.

## 2433/T/Poster Board #982

**Comprehensive targeted re-sequencing of a 150kb contiguous genomic region of chromosome 5p14.1 associated with autism.**
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Recent genome-wide association studies (GWAS) have identified and validated in multiple independent datasets a novel region with significant association to autism on chromosome 5p14.1. The approximately 200kb peak region of association (25.9-26.1Mb) is flanked by the Cadherin 9 (CDH9) and Cadherin 10 (CDH10) genes located approximately 1Mb proximal and 1.5 Mb distal, respectively. CDH10 and CDH are excellent autism candidate genes as they are involved in the regulation of cell-cell junctions in the developing brain. Potential abnormalities in CDH10 expression in autism have been reported. The peak region itself, however, lacks well annotated genes or regulatory sequences though the clustering of significant association signals suggests that a common variant(s) there is responsible for the association. To identify the associated variant(s) and potential rare variants within the candidate genes, we sequenced evolutionarily conserved regions in the peak area, as well as the annotated exons of CDH9 and CDH10 in 100 autism cases and 100 control individuals. We identified 50 known SNPs and 37 novel variants within the conserved regions near the association peak. We also identified 9 known and 26 novel variants in CDH10, including 2 non-synonymous amino acid changes, and 12 known and 9 novel variants in CDH9, including 5 non-synonymous amino acid changes. Analysis of these variations in a larger dataset demonstrated no common or rare variant(s) that by themselves can be shown to contribute to autism. Advances in next-generation sequencing technologies have enhanced our ability to rapidly sequence large targeted genomic regions quickly and in multiple samples. We are in the process of sequencing the entire peak region of association. To facilitate our re-sequencing of this autism association region, we have PCR amplified approximately 150kb of genomic DNA from the peak region, using tiled long-range PCR amplicons ranging from 5 to 11kb, in 48 allele specific autism cases and 48 control individuals. These fragments are being pooled in equimolar amounts to generate libraries of DNA for each individual to be analyzed using multiplexed paired-end sequencing on the Illumina Genome Analyzer II platform. We will identify the complete catalog of genetic variation in this region of association in these individuals in an effort to identify the causative mechanism contributing to autism risk.

## 2434/T/Poster Board #983

**New rare variants in *MAFF* antioxidant gene in Mexican population.**
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Eukaryotic organisms have cellular mechanisms to protection against oxidative and electrophiles damage, which are responsible of several chronic diseases such as cancer and degenerative processes of aging. One of the most important mechanisms is the Nrf2-Keap1 pathway and, for switch on it and to prevent cellular damage is necessary the heterodimerization of Nrf2 with small Maf proteins (MafF, MafG, MafK). Studies in mouse knockout *nrf2* have demonstrated an increased in the sensibility to cancer and degenerative diseases development. Despite of the importance of this mechanism, there are few studies only in Caucasian and Asiatic populations about the SNPs in *NRF2* and small *MAFF* genes. The aim of this study was to determine the structure of *MAFF* gene in 120 Mexican healthy individuals. Genotyping was performed by direct sequencing of 1,000 pb upstream of the transcription start site and the entire coding region. The allelic frequencies of each SNPs were compared with other populations. We identified 20 SNPs: 7 rare novel variants, and 13 polymorphisms previously reported. Fourth of these SNPs were in the promoter, two in introns and seven in the 3’UTR on the exon 3. Furthermore, five SNPs, previously reported in other populations, were not found in Mexicans. The absence of SNPs in the coding regions of small *MAFF* gene suggests that this gene is highly conserved, so rare variants could alter the structure of the MafF protein and therefore, its interaction with Nrf2 protein. By the other way, we observed that allelic frequencies of SNPs located in the 3’UTR region were different to those reported in other studies, pointing out the importance to perform this type of analysis in populations from different ethnic origin. These results could constitute an useful tool for the further analysis of the role of the small-MafF proteins in disease development.

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## 2435/T/Poster Board #984

**Using Drosophila and mammalian model systems to study the effect of MeCP2 on signaling pathways.** *A. Gamliel[1], T.R. Grossman[3], E. Bier[3], M.G. Rosenfeld[1,2].* 1) Deptartment of Medicine, University of California, San Diego, La Jolla, CA; 2) Howard Hughes Medical Institute; 3) Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, CA.

RETT syndrome and some progressive neurological disorders result from mutations as well as increased expression levels of MeCP2. We hypothesize that deregulation of specific target genes by themselves or in particular combinations may affect specific signaling pathways that could play a major role in the progression of the disease. As many of the genetic pathways that guide basic developmental processes in vertebrates and invertebrates have remained largely conserved during evolution, we took advantage of the Drosophila melanogaster wing as an assay system to test for genetic interactions between MeCP2 known signaling pathways. To identify signaling pathways that may be perturbed by altered MeCP2 levels, we generated transgenic Drosophila lines using the UAS-GAL4 system. We found that wing specific over-expression of human MeCP2 led to disorganization that was reminiscent to the changes that occur in specific signaling pathway mutants. We showed by a series of genetic interactions that indeed MeCP2 affected the suspected pathways. We confirmed that the results observed in the fly model were also applicable to the mammalian system by showing that changes in MeCP2 levels affect the same signaling pathway using reporter gene constructs, and quantitative RT-PCR of endogenous proteins in transfected neuroblastoma cells, as well as in mouse brain tissue. In order to identify which genes are direct targets of MeCP2 we performed a genome-wide Solexa ChIP sequencing in neuroblastoma cells that identified many of the previously known MeCP2 target genes, for example, BDNF, DLX5-6, FKBP5, SGK1, ID1-4, as well as many novel target genes that were components of the signaling pathway identified in our fly screen. We are now testing whether the modulation of specific signaling pathways can affect the function of MeCP2. We hypothesize that MeCP2 -dependent neurological defects may be caused by modulation of the specific pathways postnataly, consequently inhibiting neuronal maturation. Taken together our findings may facilitate therapeutic applications targeting the specific pathways, which may enable the reversal of the neurological defects.

## 2436/T/Poster Board #985

**A newly identified Bardet-Biedl Syndrome 3 (ARL6) long isoform has a vision specific function.** *P.R. Pretorius[1,2,3], L.M. Baye[3], R.F. Mullins[4], C.C. Searby[2], D.Y. Nishimura[1], K. Bugge[2], B. Yang[5], E.M. Stone[2,4], D.C. Slusarski[3], V.C. Sheffield[1,2].* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute; 3) Dept. Biology, University of Iowa, Iowa City, IA; 4) Dept. Ophthalmology and Visual Sciences, University of Iowa, Iowa City, IA; 5) Dept. Obstetrics and Gynecology, University of Iowa, Iowa City, IA.

Hundreds of individually rare, but collectively common Mendelian disorders result in visual impairment. One of these disorders is a heterogeneous syndromic form of retinal degeneration, Bardet-Biedl Syndrome (BBS). At least twelve genes are reported to individually cause BBS. Typically, individuals with BBS experience vision loss during childhood leading to blindness by the third decade of life. To gain insight into the mechanism underlying retinal degeneration associated with BBS, we have established mouse and zebrafish models of *BBS3*. A member of the Ras family of small GTP-binding proteins, *BBS3*, is postulated to play a role in vesicular transport. We have identified a second longer transcript of *BBS3* in humans, *BBS3L*, which is enriched in the eye. This transcript is highly conserved in mouse and zebrafish. The eye-specific expression of the BBS3L isoform will facilitate the dissection of BBS function in the retina, independent of alterations to other tissues. To this end, a *Bbs3L* knockout mouse has been generated and histological analysis at 9 months reveals disorganization of the inner segments, indicative of retinal degeneration. To further evaluate the functional effects of *BBS3* deficiency in the eye, an antisense morpholino approach was utilized to knockdown *bbs3* gene expression in zebrafish. Consistent with an eye specific role, knockdown of *bbs3L* results in reduced visual function as assessed by the visual startle response. Moreover, immunohistochemical analysis of *bbs3L* morphant embryos reveals that green opsin expression was not restricted to the outer segments of the photoreceptors but was also detected in the cell bodies of the outer nuclear layer. To dissect the individual functions of *BBS3* and *BBS3L*, rescue experiments using human BBS3 or BBS3L RNA were performed. Co-injection of each RNA with the *bbs3* morpholinos demonstrated that *BBS3L*, but not *BBS3*, is sufficient to rescue the vision defect. Additionally, *BBS3L* RNA restored green opsin localization. These data demonstrate that *BBS3L* is required for proper vision function and retinal organization, and that *BBS3L* has a unique eye-specific role compared to BBS3.

## 2437/T/Poster Board #986

**Functional analysis of EFHC1, a gene involved in Juvenile Myoclonic Epilepsy, in Drosophila.** *M.G. Rossetto[1], E. Zanarella[1], G. Orso[1], A. Daga[1], A. Delgado-Escueta[2].* 1) Scientific Institute E. Medea , Padova, Italy; 2) David Geffen School of Medicine at UCLA and VA GLAHS Epilepsy Center of Excellence, Epilepsy Genetics/Genomics Laboratories, Comprehensive Epilepsy Program.

Juvenile Myoclonic Epilepsy (JME), a common and lifelong epilepsy, is the most common cause of primary grand mal seizures and accounts for 10 to 12% of all epilepsies. Mutations in myoclonin/EFHC1 cause JME in 20% of JME families. We are using Drosophila melanogaster to unravel the cellular role of EFHC1 and generate a model for JME. We identified two Drosophila homologues of myoclonin/EFHC1 which display a similar degree of homology with their human counterpart. We are currently focusing on the study of one of these two fly genes named CG8959. We have generated CG8959 knockout mutants and transgenic Drosophila lines for targeted overexpression of the CG8959 encoded protein. CG8959 knockout mutant flies appear phenotypically normal. However, quantitative analysis reveals increased number of boutons in the neuromuscular junction synapse. CG8959 overexpression reduces the complexity of the NMJ arbor and dendrite arborization in peripheral sensory neurons. Moreover, CG8959 overexpression in the posterior compartment of the fly wing causes loss of posterior margin and wing veins. Modification of this phenotype by mutations in the Notch gene suggests a functional interaction between myoclonin/EFHC1 proteins and the Notch pathway. Modulation of myoclonin/EFHC1 expression in Drosophila affects neuromuscular junction and dendrite arborization. Maintenance of a population of dynamic microtubules is important for axonal extension, synaptic bouton formation and dendritic arborization. Thus changes in microtubule organization could be the common theme underlying the NMJ synapse and dendritic arbor phenotypes produced by loss and overexpression of CG8959.

## 2438/T/Poster Board #987

**The Dynamin-like GTPase atlastin is required for homotypic fusion of endoplasmic reticulum membranes.** *A. Daga[1,4], D. Pendin[1], G. Orso[1], J. Tosetto[1], A. Martinuzzi[2], S. Liu[3], T. Moss[3], J. McNew[3].* 1) DTI at the Scientific Institute E. Medea Padova, Italy; 2) Scientific Institute E. Medea, Conegliano, Italy; 3) Department of Biochemistry and Cell Biology, Rice University, Houston, Texas USA; 4) Department of Neurology, The David Geffen School of Medicine, University of California, Los Angeles, California, USA.

Establishment and maintenance of proper architecture is essential for endoplasmic reticulum (ER) function. Homotypic membrane fusion is required for both the biogenesis and maintenance of the ER and has been shown to depend on GTP hydrolysis. Here we demonstrate that Datlastin, the fly homolog of the GTPase atlastin-1 whose mutation causes Hereditary Spastic Paraplegia (HSP), localizes on ER membranes and that loss of Datlastin causes ER fragmentation. Datlastin embedded in distinct membranes has the ability to form trans-oligomeric complexes and its overexpression induces enlargement of ER profiles, consistent with excessive fusion of ER membranes. In vitro fusion experiments confirm that Datlastin autonomously drives membrane fusion in a GTP dependent fashion. In contrast, GTPase-deficient Datlastin is inactive, unable to form trans-oligomeric complexes due to failure to self-associate, and incapable of promoting fusion in vitro. These results demonstrate that Datlastin mediates membrane tethering and fusion and strongly suggest that it is the GTPase activity required for ER homotypic fusion.

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## 2439/T/Poster Board #988

**Study of the intracellular dynamics of C111Y and C111S mutations identified in Factor IX from Mexican patients with severe hemophilia B.** *J. Mantilla[1,2], N. Enjolrras[3], C. Negrier[3], A. Jaloma-Cruz[1,2].* 1) Dept CIBO, Jalisco, IMSS, Guadalajara, Guadalajara, Mexico; 2) Doctorado en Genética Humana, CUCS, Universidad de Guadalajara, Guadalajara, Jalisco, México; 3) Laboratoire d'Hémobiologie, Faculté de Médecine RTH Laennec, Lyon, France.

ANTECEDENTS. We identified a group of punctual recurrent mutations in non-CpG sites of factor IX gene (FIX) causing severe hemophilia B, relevant in terms of the involved non-random mutagenesis in Latin-American populations and the functional significance of mutations at cysteine (C) which forms disulfide bond in a FIX domain with high structural and functional commitment. We studied two mutations at 17,747 nucleotide in the second-like epidermal growth factor (EGF2) corresponding to the Exon E to identify their effect in the structure-function relationship of the protein by the study of their intracellular trafficking. MATERIALS AND METHODS. C111 wild-type and the mutations C111S and C111Y were inserted by directed-site mutagenesis into an expression vector (pcDNA 3.1®) containing the FIX wild-type (wt) gene. The correct insertion of FIX wt and the two mutations was verified by restriction analysis and sequencing. Transfection on Cos-7 cells by Fugene6® after 48hrs was tested with a control plasmid containing the green fluorescence protein (pGFP) with a good efficiency (64.5%) evaluated by a flux-cytometry. The intracellular FIX amounts and secretion were quantified by ELISA assay. Transfected cells were incubated in presence of inhibitors like Brefeldin A, which blocks protein transport from endoplasmic reticulum (ER) to the Golgi complex; N-Acetyl-Leu-Leu-Norleucinal (ALLN) and Clasto-lactacystin beta-lactone, proteasomal inhibitors, and NH4Cl and Leupeptin, lysosomal inhibitors. RESULTS. Respect to FIX wt, the mutations showed a decreased FIX secretion (28%) and intracellular accumulation of 145% (C111Y) and 157% (C111S). The effects of the inhibitors caused a higher intracellular accumulation of the mutants which led a degradation mainly in lysosomes (NH4Cl) and secondly in proteasomes (ALLN). By the effect of Brefeldin A on C111S we can assume an adequate transport from ER to Golgi complex, opposite to C111Y which seems to be blocked at ER and to have elevated degradation in proteasomes (ALLN effect). The experiment was repeated 5 times (final results). CONCLUSIONS. The disruption of the disulfide bond in the mutants have an important effect on the native folding of FIX protein, evident by the effects on its transport through ER and the degradation mechanisms, with a predominant degradation at proteasomes when the ER transport is blocked and a higher degradation at lysosomes when the transport from ER to Golgi complex is adequate.

## 2440/T/Poster Board #989

**The oligomeric structure of the y+LAT-1/4F2hc amino acid transporter analysed using FRET microscopy.** *M. Tringham[1], M. Toivonen[1], J. Salmi[1], O. Simell[2], J. Mykkänen[2].* 1) Department of Medical Biochemistry and Genetics, University of Turku, Finland; 2) Department of Paediatrics, University of Turku, Finland.

y+LAT-1 and 4F2hc are protein subunits that form a transporter complex for cationic amino acids in the basolateral membrane of epithelial cells, mainly in the small intestine and proximal kidney tubules. Mutations of y+LAT-1, 51 of which are currently known, cause lysinuric protein intolerance (LPI, OMIM #222700), a rare metabolic disorder characterised by diminished intestinal absorption of the cationic amino acids lysine, arginine and ornithine and by severe loss of these amino acids into the urine. The more detailed structure of this transport complex has so far been unclear - it has remained unelucidated whether the complex is formed as a dimer or a tetramer of the subunits. What has been known, however, is that the y+LAT-1 subunits cannot reach the plasma membrane without forming a complex with 4F2hc. We previously established fluorescence resonance energy transfer (FRET) microscopy as a tool in studying the interactions of y+LAT-1 and 4F2hc. We have now applied the acceptor photobleaching application of the FRET technique to the exploration of the heteromerisation status of the y+LAT-1/4F2hc transporter complex. Based on our results, when fused into fluorescent vectors and transfected into the HEK293 cells, the y+LAT-1 proteins interact together in different cellular compartments in the presence as well as absence of 4F2hc. Our findings therefore suggest that the holotransporter is a tetramer of y+LAT-1 and 4F2hc subunits.

## 2441/T/Poster Board #990

**Src kinase modification of human UDP-glucuronosyltransferase-2B7 required for inactivation of genotoxic estrogen metabolites associated with breast cancer.** *I.S. Owens, P.S. Mitra, N.K. Basu, K. Chakraborty.* PDEGEN, NIH, NICHD, Bethesda , MD.

Whereas ER-bound UDP-glucuronosyltransferase (UGT)-2B7 avidly conjugates genotoxic catechol-estrogens (CEs), 2B7 presence in mammary gland suggests it prevents CE-based depurination associated with breast-cancer initiation. As UGTs have been shown to require phosphorylation, 2B7 has PKC and tyrosine kinase (TK) phopshorylation sites, it incorporates immunoprecipitable [33P]orthophosphate and undergoes inhibition by TK inhibitors. 2B7 mutants demonstrate that Y236- and Y438-phosphorylation is required, and Src-specific PP2 inhibition that depleted anti-phospho-Y438-2B7 content supports this claim. Also, PP2 disruption of 2B7 and active-Src co-localization and their cross-linking confirms their in-cellulo interaction. Furthermore, Src incorporated 6.5-fold more [$\gamma$33P]ATP-dependent label into microsomes isolated from 2B7His-transfected SYF-/-cells and solubilized than [$\gamma$33P]ATP alone; parallel Src-modified microsomes using unlabeled ATP generated 6-fold greater activity with 4-OH-estrone. Western blot of the in-vitro modified 2B7His-containing microsomes showed corresponding increases in purified phospho-Y438 kDa-2B7His at 55 kDa that was immuno-confirmed with anti-phospho-Y438-2B7. Moreover, evidence indicates Src-specific scaffold protein, AKAP12, supports Src-dependent ER-signaling associated with 2B7 activity. The results indicate Src and UGT2B7 are closely engaged allowing Src support of regulated 2B7-phosphorylation enabling it to protect against catechol estrogen depurination linked to breast-cancer initiation. This research was supported by the Intramural Research Program of the NIH, NICHD.

## 2442/T/Poster Board #991

**SNP variation in IVS 10 of CFTR gene cause aberrant mRNA splicing in CF patients.** *D.A. Coviello[1], L. Costantino[1], C. Colombo[2], L. Claut[2], V. Paracchini[1], L. Porcaro[1], M. Zanardelli[2], G. Pizzamiglio[2], P. Capasso[1], D. Degiorgio[1], M. Seia[1].* 1) Laboratory of Medical Genetics Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milan, Italy; 2) Regional CF Unit; Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milan, Italy.

Genomic variations in intronic regions affecting mRNA splicing are increasingly reported. Incorrect splicing can generate aberrant transcripts and also modulate the level of normal transcript. CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) gene mutations are usually screened in newborns and in patients, and although diagnostic protocols have a good sensitivity and specificity, a significant percentage (6%) of CF alleles remain unidentified even after extensive studied of the gene by DNA analysis. In the present study we investigate the molecular defect, by CFTR mRNA analysis, of 11 cystic fibrosis (CF) patients, in which only one or no allele were identified by DNA analysis of the whole CFTR gene. CFTR gene is tightly regulated and differentially expressed transcript in many mucosal epithelial cell types, we decided to collect biological material from nasal epithelial cells using cyto-brush, from 11 CF patients and 5 non-CF controls. RNA was extracted with TRIzol reagent, first strand cDNA was synthesized using hexanucleotide primers and high capacity cDNA Archive kit. The cDNA was amplified in six overlapping fragments spanning the entire gene and then visualized on agarose gel for identifying large deletion/insertion and the product of possible alternatives splicing; each fragment was next sequenced and cloned into plasmid vectors. The mRNA analysis has allowed us to detect an extra band of higher molecular weight than the normal cDNA fragment. Sequencing the specific mutated allele a cryptic exon in two patients (18%) was identified: the new exon is a 101 bp insertion located between exons 10 and 11 . Further sequencing of the genomic region of IVS 10 allow us to identify a new SNP. We believe that this sequence variation cause a new donor splice site. The molecular characterization of nine remaining patients is still ongoing. Our results confirm the usefulness of RNA analysis to look for DNA mutations that can affect the normal splicing processes.

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## 2443/T/Poster Board #992

**Fine-scale variation and genetic determinants of alternative splicing across individuals.** *J. Majewski[1, 2], K.L.C. Lam[2], C. Dias[2], J. Coulombe-Huntington[1,2].* 1) Dept Human Genetics, McGill Univ, Montreal, PQ, Canada; 2) McGill University and Genome Quebec Innovation Centre, Montreal, PQ, Canada.

It is only recently, thanks to the increasing throughput of new technologies, that we have been able to explore the full extent of alternative pre-mRNA splicing (AS) in the human transcriptome. This is unveiling a vast layer of complexity in isoform-level expression differences between individuals. In this work, we use splicing sensitive microarray data from lymphoblastoid cell lines to conduct an in-depth analysis on splicing efficiency of known and predicted AS exons. By combining publicly available AS annotation with a novel algorithm specifically designed to search for AS events, we demonstrate that the extent of differential splicing among individuals is far greater than has previously been reported. Specifically, many genes show subtle but significant genetically controlled differences in exon inclusion. PCR validation shows that 43 out of 59 (73 %) of a sample from our new AS candidate gene regions undergo detectable AS. Through sequencing, we were able to identify the most likely causative SNP in most of the validated cases. In 17 of the cases we were able to show, using a splice-site strength prediction tool, that the expected effect of a SNP invariably agreed in directionality with the micro-array results, perfectly explaining the differences in isoform levels between individuals. In 14 of the remaining cases, we show that the splicing changes could be explained by a SNP disrupting predicted splicing enhancer elements. We verified the effects of six candidate SNPs using in vivo transient transfection minigene assays. This study shows that splicing differences between individuals, including quantitative differences in isoform ratios, are frequent in human populations and that causative SNPs can be identified using in silico predictions. Several of these differences were found in disease-relevant genes such as IL6 and MMAB. It is highly likely that such differences are involved in phenotypic diversity and susceptibility to complex diseases.

## 2444/T/Poster Board #993

**Lamin A and telomerase collaborate to trigger cellular senescence.** *K. Cao, C. Blair, D. Faddah, M. Olive, M. Erdos, E. Nabel, F. Collins.* Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Hutchinson-Gilford progeria syndrome (HGPS), a devastating premature aging disease, is caused by a point mutation in the lamin A gene (LMNA, Accession number: NM_170707). This mutation constitutively activates a cryptic splice donor site, resulting in a mutant lamin A protein termed "progerin". Recent studies have demonstrated that progerin is also produced in normal human cells and tissues at low levels. To probe the causative role that progerin might play in senescence of normal cells, we studied early and late passage fibroblasts from the same individual, and found that progerin transcription was activated in senescent cells. Screening various primary and transformed cell lines revealed an interesting inverse correlation between telomere length and progerin transcription. Moreover, shRNA knockdown of telomerase in cancer cell lines resulted in increased progerin production. Exon array analysis further demonstrated that extensive changes in alternative splicing of multiple genes, including LMNA, occur as telomeres shorten and cells approach senescence. Our findings suggest a novel synergism between telomere erosion and progerin production in induction of cellular aging, and provide the first genome-wide analysis of the changes in alternative splicing during cell senescence.

## 2445/T/Poster Board #994

**Intronic c.592-4_C.592-3delTT in *DGUOK* Gene Abolishes mRNA Splicing.** *Q. Ji[1], D. Dimmock[2], L. Tang[1], M. Descartes[3], R. Gomez[3], L. Rutledge[3], E. Schmitt[1], L. Wong[1].* 1) Baylor College of Medicine, Houston, TX; 2) Medical College of Wisconsin, Milwaukee, WI; 3) University of Alabama at Birmingham, Birmingham, AL.

Deoxyguanosine kinase (DGUOK) catalyzes the first step of the mitochondrial deoxypurine salvage pathway, the phosphorylation of purine deoxyribonucleosides. Mutations in the *DGUOK* gene have been linked to inherited mtDNA depletion syndromes, neonatal liver failure, nystagmus, and hypotonia. Previously, we reported the first case of heterozygous intronic c.592-4_C.592-3delTT in *DGUOK* in one patient with DGUOK deficiency (Hum Mutat, 2008. 29(2): pp. 330-1). This alteration was predicted to cause aberrant splicing based upon two computer algorithms. We now report a homozygous c.592-4_c.592-3delTT alteration found in two affected siblings of asymptomatic consanguineous parents. The proband presented with symptoms of idiopathic hepatitis, liver dysfunction, nystagmus, and retinal blindness. This individual died at 6 months of age due to liver failure. This individual's affected sibling presented similarly and has remarkable elevations of tyrosine, methionine, and alanine. Many organic acids were elevated in urine including lactic acid, Krebs cycle intermediates, and para-hydroxy compounds; ketone bodies were also present. RNA studies support aberrant splicing. Total RNA was isolated from whole blood. Sequencing of cDNA clones detected exon 5 skipping in the two affected siblings, but not in the normal control. These results indicate that the homozygous c.592-4_C.592-3delTT is deleterious and responsible for the DGUOK deficiency. The parents were subsequently confirmed to be carriers of this mutation. In summary, we have demonstrated that c.592-4_C.592-3delTT is a pathogenic splice receptor site mutation leading to DGUOK deficiency.

## 2446/T/Poster Board #995

**Prediction and functional validation of expressed SNPs altering mRNA splicing.** *E.J. Mucaki, P.K. Rogan.* Biochemistry, University of Western Ontario, London, ON, Canada.

mRNA splicing changes induced by common, polymorphic SNPs were predicted with information theory-based models of constitutive splice sites and confirmed by expression studies. SNP-related, allele-specific splicing effects, were predicted from differences in the strengths of allelic natural and/or cryptic splice sites. We qualified selected SNPs for quantitative RT-PCR studies using published Affymetrix exon expression microarray data from Hapmap individuals. This study reports splicing effects for SNPs with low coefficients of variation in expression (CVE) among individuals with the same genotype. Predicted SNPs within *XRCC4*, *IL19*, *C21orf2*, *UBASH3A*, *TTC3*, *PRAME*, *ARFGAP3*, *Fλ8*, *BCR*, *BACE2*, *CLDN14*, *TMPRSS3* and *DERL3* were tested for allele-specific splicing effects. For each, multiple, distinct splice forms were quantified using HapMap cell lines heterozygous and homozygous for the relevant SNPs, including allele-specific changes in exon skipping, cryptic splicing, and partial intron retention. Results for minor allele SNPs in each individual were compared with distinct, constitutively spliced, non-polymorphic exons from the same genes and were normalized against external reference genes expressed at similar levels. Nine of 15 SNPs showed significant changes in the use of the splice site affected by the SNP. Homozygotes (n=4) for the allele producing a stronger constitutive splice site (1.3-2.2 bit; 2.5-4.6 fold increase) exhibited higher levels of transcription of the associated gene (45-300% increased expression). Exon skipping (and intron inclusion) was increased 3-9 fold in homozygotes for a SNP adjacent to the skipped exon (due to a 4-fold reduction acceptor strength). Exon skipping was decreased in an allele that improved exon definition due to a SNP that abolished one of a pair of tandem, overlapping acceptors. Increased exon skipping was detected for as little as a 40% reduction (0.5 bits) in splice site strength, however, large CVEs were seen in individuals for 4 SNPs that exhibited small changes in splice site strength. Five SNPs were predicted to activate cryptic splice sites (for 4 acceptors and 1 donor), 4 of which were confirmed in heterozygotes and minor allele homozygotes. Some SNPs appear to contribute to disease by affecting constitutive mRNA structure and abundance. A mutant SNP in *XRCC4* is linked to renal cell carcinoma and bladder cancer, and SNPs in type-1 diabetes and bipolar disorder are in LD with *TTC3* and *BCR*, respectively.

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## 2447/T/Poster Board #996

**Mutation-negative FAP patients with mRNA defects of APC.** *M.W. Condie[1], T.M. Tuohy[1], P. Shires[2], R.W. Burt[1], D.W. Neklason[1].* 1) Huntsman Cancer Inst, Univ Utah, Salt Lake City, UT; 2) Saint Luke's Hospital, Kansas City, MO.

Background: Familial adenomatous polyposis (FAP) is a colon cancer syndrome with a prevalence of 1:10:000, hallmarked by 100s to 1000s of precancerous colonic polyps and nearly 100% risk of developing colon cancer at an average age of 39 years in the absence of colon surveillance and surgery. Mutations in the APC gene lead to FAP and an attenuated form called AFAP with reduced polyp numbers and cancer risk. Mutation detection fails using DNA-based technology in 20% of FAP and 50% of AFAP patients due to testing limitations, inability to determine significance of DNA change, or other responsible genes. A subset of disease causing APC mutations may be due to non-coding or even coding variants that result in RNA splice defects. Methods: A RNA-based assay has been developed to screen APC mRNA for splice defects in mutation-negative FAP and AFAP patients. Primers and PCR conditions were developed for five overlapping amplicons that cover exons 1 - 14 and the beginning of exon 15 in the APC mRNA. PCR products from the cDNA of patients are run on agarose gels and examined for atypical products. These products are then sequenced and results verified by genomic analysis. Results: To date, cDNA from 14 mutation-negative families has been tested, and two mutations resulting in splice defects have been identified when other standard techniques failed to demonstrate loss-of-function mutations. One is a single nucleotide change, deep in intron 4 that generates a splice acceptor site, an additional exon (exon 4A), and a stop codon. The second is a 1.5-kb deletion in intron 14 which causes deletion of exon 14 in the mRNA transcript; exon 13 is spliced to exon 15 resulting in an out-of-frame stop codon. Both families present as AFAP. This is a useful assay to compliment DNA-based testing in APC mutation-negative patients or patients with a variant of uncertain significance. Additionally, to address cases where undetected mutations affect the stability of the resultant transcript, allelic imbalance can be examined in amplicons containing one of the common APC single nucleotide polymorphisms (SNPs) using real time PCR.

## 2448/T/Poster Board #997

**A Comparative Study of The Biology of Two Dominant Retinal Degenerative Disorders on Chromosome 17.** *R. Ramesar[1], A-B. Pandor[1], L. Roberts[1], S. Prince[2], C. Seoighe[3], J. Greenberg[1].* 1) MRC Human Genetics Research Unit, Division of Human Genetics, Institute for Infectious Diseases and Molecular Medicine,; 2) Department of Human Biology, University of Cape Town, Cape Town, Western Cape, South Africa; 3) School of Mathematics, Statistics and Applied Mathematics National University of Ireland, Galway, Ireland.

Retinitis pigmentosa is the major cause of inherited forms of retinal blindness. In the past 20 years, with the use of genetic technologies, the biology of this large group of heterogeneous conditions has begun to unravel, leading to logical therapeutic interventions. Here we report on the biology at two distinct loci, originally identified by ourselves, on chromosome 17, underlying dominant RP. We also report on our current research aimed at therapeutics. Objective: To identify the biological basis of disease in two clinically indistinguishable autosomal dominant forms of retinitis pigmentosa (adRP). Methods and Materials: The RP17 form of adRP is caused by a R14W mutation in the Carbonic Anhydrase IV (CA4) gene on chromosome 17q. Constructs of mutant (R14W) and wildtype versions of the CAIV gene were made and various tagged forms of these constructs were investigated in COS7 and HEK293 cells. The RP13 form of adRP, on the other hand, is caused by a mutation in the universal spliceosomal component, PRP8. We have used several techniques to investigate the possible retinal target genes which may be secondarily compromised as a result of the primary PRP8 mutation, including a genome-wide expression survey of splicing. Results: The CA4 gene (RP17) is expressed in the endothelial cells of the choriocapilaris; whereas the PRP8 protein is universally expressed. Our cell biological work shows mis-folding, mis-trafficking of mutant CA4, and raised markers of misfolding and apoptosis in mutant cell lines. The genome-wide expression data on the RP13 gene has shown that a large number of genes are misspliced as a result of a defective PRP8 gene; it would seem that there is a global downstream effect resulting in biological compromise. Discussion: Although clinically indistinguishable, these two forms of adRP, are the result of totally disparate pathological mechanisms. The mutant CA4, results in apoptosis of endothelial cells, and deterioration of the choriocapillary vascular supply, resulting in ischemia-related photoreceptor death. Preliminary work with synthetic chaperones has shown that it is possible to relieve protein misfolding in cell culture, leading to rescue, of mutant CA4-carrying cells, from apoptosis. RP13 reflects only a retinal phenotype. The most significantly affected genes (from the expression data) are currently being investigated for their possible contribution to the retinal phenotype.

## 2449/T/Poster Board #998

**Validity and Repeatability of Two PCR Methods for Determining CGG Size and X Inactivation Skewing Percent of the FMR1 Gene.** *A. Hadd[1], J. Kline[2], K. Oppenheimer[3], S. Brown[3], B. Levin[2], A. Kinney[2], S. Sah[1], S. Filipovic-Sadic[1], T. Stenzel[1], G. Latham[1].* 1) Asuragen, Inc, Austin, TX; 2) Columbia University, Mailman School of Public Health, New York, NY; 3) University of Vermont College of Medicine, Burlington, VT.

INTRODUCTION. Fragile X Syndrome is caused predominantly by the expansion of CGG sequences in the FMR1 gene. The specific number of CGG repeats is associated with many disorders, including Fragile X Primary Ovarian Insufficiency, the leading genetic cause of premature menopause. As a precursor to a study to test whether repeat lengths of intermediate size are related to ovarian aging, two PCR methods were evaluated for determining CGG repeat length and X-chromosome inactivation (XCI) skewing percent. METHODS. The validation and repeatability studies compared prototype reagents from Asuragen and a laboratory developed test (LDT) based on published methods (Tassone et al, JMD, 2008). PCR amplicons were sized using capillary electrophoresis. Validity was determined for 25 female samples comparing predicted CGG repeats with DNA sequencing. The reliability of measuring the XCI skewing percent was determined in another sample of 25 women by splitting gDNA into two reactions; one digested with HpaII and amplified with a HEX-labeled primer, and the other undigested and amplified with a FAM-labeled primer. Ratios of HEX to FAM peak heights were used to calculate the XCI skewing percent. RESULTS. The validation set comprised 49 alleles of size 20-80 repeats (one allele >200 repeats was omitted because it was outside the quantifiable range of DNA sequencing). Sample pass rates were 47 and 49 for the two Asuragen assays, and 49 and 46 for the two LDT assays. Rounded (i.e., integer) predicted CGG repeat values were within ±1 CGG from the true value for all alleles and equal to the true value in 90% to 98% of alleles, depending on the assay and run. Comparing unrounded predicted values with true values, the Asuragen method showed a slightly higher negative bias and a slightly lower standard deviation than the LDT method. The reliability set comprised 50 alleles sized between 20-43 CGG repeats; the XCI skewing percents ranged from 50.9%-89.6%. The Asuragen method yielded XCI skewing percents that were, on average, about 2% lower and less dispersed than percents yielded by the LDT assay. CONCLUSIONS. Both methods showed excellent validity for sizing and excellent repeatability for the XCI skewing percent. The Asuragen method is preferred because it has slightly better validity for sizing and is less time intensive. These results, which demonstrate a simple two-color methylation workflow, form the basis for using the Asuragen assay in epidemiologic studies.

## 2450/T/Poster Board #999

**New Paradigms in Fragile X Testing by PCR.** *F. Tassone[1,2], W. Zhang[1], S. Filipovic-Sadic[3], L. Chen[3], S. Sah[3], J. Kempannien[3], A. Hadd[3], T. Stenzel[3], G. Latham[3], P. Hagerman[1,2].* 1) Dept Biochemistry & Molec Med, Univ California, Davis, Davis, CA; 2) M.I.N.D. Institute, University of California Davis Medical Center, Sacramento, CA; 3) Asuragen, Inc. Austin, TX.

INTRODUCTION: Expansion of CGG repeats in the fragile X mental retardation (FMR1) gene is associated with a constellation of clinical involvements. There is a growing and acute need for larger-scale screening efforts for expanded FMR1 alleles, and a major push for screening of all newborns (~500,000/yr in California alone). PCR methods are generally limited to fewer than ~100-200 CGG repeats, and all samples detected with premutation or full mutation alleles are still analyzed by Southern blot (SB). Sensitive and accurate detection of full mutation alleles, with the ability to reconcile zygosity and methylation statuswithout the need for SBhas the potential to ultimately sidestep current technological hurdles for broader testing. METHODS: De-identified clinical samples from the the M.I.N.D. Institute at UC Davis were tested by three novel Asuragen PCR methods: gene-specific PCR, CGG Repeat Primed PCR and methylation PCR. Amplicons were resolved by agarose gel and/or capillary electrophoresis. CGG repeats were calculated by comparing size to sequenced FMR1 standards or by counting distinct CGG peaks. Methylation status was assessed by comparing a sample aliquot treated with HpaII and amplified with a HEX-labeled primer to an undigested aliquot amplified with a FAM-labeled primer. Specificity was confirmed by CGG probe blotting. PCR results from blinded samples were submitted prior to comparison with SB results. Blood spot card punches were washed, dried and suspended directly in PCR reagents prior to thermal cycling. RESULTS: Over 200 clinical samples comprising the full-spectrum of clinical genotypes were correctly sized by PCR. Results were concordant with SB for all samples; 2 premutation samples were detected with full mutations not detected by SB results. All female homozygous samples were correctly identified. Alleles were accurately sized for 27/27 blood spot samples with 7/7 full mutation alleles directly determined by PCR. Methylation status of full- and premutation alleles was quantified and concordant with Southern blot. CONCLUSIONS: The ability of these novel PCR research reagents to detect large full mutations, reliably interpret female homozygous samples and assign methylation status was demonstrated. PCR-based sizing was also demonstrated using direct analysis of blood spot card DNA. Together, these results suggest a PCR-only workflow that may shift the current fragile X diagnostic paradigm.

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## 2451/T/Poster Board #1000

**Androgen Receptor VNTRs and SNP as Clinical Biomarkers in Humans.** *A. Freeman, H. Qian, L. O'Brien, P. Haslett, K. Landschulz.* Eli Lilly and Company, Indianapolis, IN. 46285.

The Androgen Receptor gene (AR, Chr X) encodes a transcription factor that is activated by androgens, such as testosterone, to regulate downstream gene and protein expression. The activating domain in AR exon 1, contains two variable number of tandem repeats (VNTRs), CAG and GGC, and a synonymous, E211 single nucleotide polymorphism (SNP). While the VNTRs, resulting in glutamine (CAG) or glycine (GGC) stretches in the receptor, are known to be inversely associated with androgen activity, sensitivity and disease, the E211 SNP, a possible surrogate marker for the VNTRs, has not been extensively studied. We sought to better understand these genetic variants; their ethnic distribution, heritable relationship and potential as clinical biomarkers for disease and drug response. We used fragment analysis and allelic discrimination to genotype the two AR VNTRs and E211 SNP in the HapMap DNAs from 90 African, 88 European (CEPH) and 90 Asian subjects. A chi-square test was performed to determine the difference and significance of variant allele frequencies and Linkage Disequilibrium (LD), which was estimated using the method described by Weir and Cockerham (1979). On average, ethnic distribution of the AR variant alleles was observed. For the CAG VNTR, Africans had 17-18 repeats, Asians had 22-25 repeats and the CEPH population had a bimodal distribution ranging from 20-21 and 23-25 repeats. Conversely, the GGC VNTR in the African population showed a bimodal distribution around 14-16 and 18-19 repeats, while the CEPH and Asian populations had 17-18 repeats. In the African, CEPH and Asian populations, the "G" allele of the E211 SNP had 24.4%, 83.3% and 100% frequency, respectively. We confirmed that both VNTRs were in LD in all three ethnic populations ($p<0.05$), although the LD between the E211 SNP and each VNTR was population dependent. In the CEPH population, the E211 SNP was not in LD with either VNTR. However, in the African population, we found the E211 SNP was in LD with the GGC VNTR only ($p<0.01$), despite reports of partial LD with both VNTRs in African-American men (Ross et al, 1998). Our data show an inherent ethnic variability and linkage between the AR VNTRs that is not fully represented by the SNP in the human population. Future in vitro and in vivo studies will be required to elucidate how these genetic markers are associated with androgen sensitivity and to assess their potential as clinical predictors of androgenic drug response.

## 2452/T/Poster Board #1001

**Identification of CUGBP1 mRNA targets by CUGBP1/RNA immunocapture.** *L.L. Bachinski[1], C. Huichalaf[2], K.A. Baggerly[1], B. Schoser[3], B. Udd[4], L.T. Timchenko[2], R. Krahe[1].* 1) Univ Texas MD Anderson CA Ctr, Houston, TX; 2) Baylor College of Medicine, Houston, TX; 3) Friedrich-Baur Institute, Ludwig-Maximillians-University, Munich, Germany; 4) Tampere University Hospital and Medical School, Tampere, Finland.

In myotonic dystrophy (DM) CUGBP1 is hyper-phosphorylated and steady-state protein levels are up. In both phosphorylated and unphosphorylated isoforms, CUGBP1 is a key protein in regulation of alternative splicing and protein translation. To identify CUGBP1 mRNA targets and to determine whether these targets are dysregulated in DM, we extracted RNA from CUGBP1 immunoprecipitates of ribonuclear protein fractions from normal and DM cells and hybridized these to Affymetrix exon arrays. To determine the normal targets of CUGBP1, we compared immunoprecipitated results to those from whole transcriptome. Captured probes were differentially enriched for targets from 5' ends, 3' ends, and alternative exons (overall p value =0.024 for top 50 probes). Of the 26 known CUGBP1 targets, we identified 14, most of which had enriched probes in the expected regions of the target genes. Known targets identified included *CLCN1, MAPT, MEF2A, MELK, MTMR1, SRF, WEE1, AURKA, AURKB, CDC2, CEBPB, CSKN2B, MOS*, and *RBM9*. Focusing on genes known to be important in muscle differentiation and maintenance, we identified novel candidate CUGBP1 mRNA targets from all three predicted target classes. Interesting novel candidates included *CRYBB3* (lens crystalline), *PPEF1* (Ca+2 binding), *KCNN2* (Ca+2-activated K+ channel) and *RBM6* (RNA binding motif protein). Identification of CUGBP1 target mRNAs is an important step in the elucidation of pathways which are affected in DM by elevation of CUGBP1.

## 2453/T/Poster Board #1002

**The digenic hypothesis unraveled: Epigenetic effects of GJB6 deletion del(GJB6-D13S1330) cause allele-specific loss of GJB2 expression in cis.** *J.M. Rodriguez-Paris[1], I. Schrijver[2].* 1) Department of Pathology, Stanford University School of Medicine, Palo Alto, CA; 2) Department of Pathology and Pediatrics, Stanford University School of Medicine, Stanford, CA.

Connexin 26 and connexin 30 are the major connexins expressed in the cochlea, where they are co-localized and form heteromeric gap junctions. Mutations in the GJB2 gene, which encodes connexin 26, are the most common cause of prelingual non-syndromic sensorineural hearing loss. The large ~309 kb deletion del(GJB6-D13S1830) which involves GJB6 (connexin 30), causes hearing loss in homozygous individuals, or when compound heterozygous with a GJB2 mutation. We demonstrate that mutations in these two genes do not cause hearing loss through a digenic mechanism of inheritance as was postulated previously, but that, instead, GJB2 expression is abolished through an epigenetic effect in cis. Allele-specific expression of GJB2 was investigated in three unrelated individuals compound heterozygous for a GJB2 mutation and del(GJB6-D13S1830). The mutated GJB2 allele in trans with del(GJB6-D13S1830) was expressed in all three individuals whereas the GJB2 allele located in cis with the deletion was not expressed at all. Thus, the GJB6 deletion results in allele-specific loss of GJB2 expression. Our data support the hypothesis that del(GJB6-D13S1830) disrupts a putative cis-regulatory element probably located within the deleted region, which leads to complete loss of expression of the GJB2 allele in cis with the GJB6 deletion.

## 2454/T/Poster Board #1003

**MicroRNA expression profile in fragile X syndrome.** *L.P. Capelli[1,2], Z. Kutalik[2], J.J. Médard[2], P. Descombes[3], S. Jacquemont[4], G. Tanackovic[2].* 1) Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo, SP, Brazil; 2) Department of Medical Genetics, University of Lausanne, Lausanne, Switzerland; 3) Genomics Platform - NCCR, Frontiers in Genetics, University of Geneva, Geneva, Switzerland; 4) Service of Medical Genetics, CHUV-UNIL, Lausanne, Switzerland.

Fragile X syndrome (FXS) is the most common form of inherited intellectual disability (1:2500). It is caused by an expansion of CGG repeats in the 5' untranslated region of the *FMR1* gene. Affected individuals carry a full mutation (>200 CGG) that leads to hypermethylation of the expanded sequence and upstream CpG islands, causing transcriptional silencing of the gene. Normal individuals (6 to 54 CGG repeats) and premutated carriers (55 to 200 CGGs) produce the protein. The precise function of the *FMR1* product, FMRP, is unknown. It is involved in transport of mRNAs along synapses and regulation of their translation, as well as in neuronal maturation. More recently, a role of FMRP in the microRNA (miRNA) pathway was proposed. MiRNAs are small non-coding RNAs which, as part of the miRISC (RNA-interference induced silencing complex), regulate gene expression at the level of mRNA translation or stability, by virtue of imperfect base-pairing with mRNAs. At present, there is no clear understanding of the role that FMRP plays in the miRNA pathway, how it regulates protein synthesis at synapses and weather the two functions are somehow linked. FMRP is part of the protein complex responsible for the miRNA processing, as well as of the miRISC. Therefore, the absence of FMRP could affect miRNA processing or function, and consequently this could cause a global miRNA/mRNA imbalance and contribute to FXS. To address this question, we performed a comparative analysis of miRNA profiles in six *Fmr1* KO mice and six controls using mice miRNA microarrays. In a total of 380 miRNAs, we observed 26 miRNAs that were over or under expressed (20% fold change with $p<0.05$) in the KO animals. Further, using quantitative PCR, we quantified the amounts of 17 transcripts known to be translationally regulated by FMRP and involved in neuronal maturation, neuronal functioning and synaptic plasticity in both the KO and control animals. Thirteen out of 17 transcripts studied were differentially expressed in KO animals and, interestingly, six of them were predicted as target of a subset of miRNAs that we showed to be misregulated in the KO animals. Our results point to a role of FMRP in miRNA processing/stability and indicate that this might be a link between the miRNA pathway and deregulation of mRNA translation in FXS. (First author sponsored by CAPES).

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## 2455/T/Poster Board #1004

**Evidence for protein-coding transcript connectivity networks composed of chimeric RNAs in human cells.** *S.E. Antonarakis[1], R. Guigo[2], S. Djebali[2], P. Kapranov[3], A. Reymond[4], K. Salehi-Ashtiani[5], J. Lagarde[2], V. Lacroix[2], S. Foissac[2], P. Ribera[2], D. Martin[2], C. Ucla[1], J. Drenkow[6], T. Gingeras[6].* 1) Gen Med & Dev, CMU9180, Univ Geneva, Geneva, Switzerland; 2) CRG, Barcelona, Spain; 3) Helicos, Boston, MA, USA; 4) Univ Lausanne, Switzerland; 5) Harvard Univ, Boston, MA, USA; 6) CSHL, New York, USA.

The organization of gene structures have followed the Jacob and Monod bacterial gene model proposed more than 50 years ago. Since then, the unraveling of the complexity of the transcriptomes found in cells from yeast to human has blurred the boundaries of genes. Using rapid amplification of cDNA ends (RACE), tiling arrays, RT-PCR, cloning and sequencing we have sought to characterize the extent upon which individual gene boundaries are defined on two human chromosomes (21 and 22). Analyses of the locations of the 5'and 3' transcriptional termini of 492 protein coding genes revealed that for 85% of these genes the boundaries of these genes extend well beyond the annotated termini. In these cases, the average distance from the distal 5' end to the index site in a gene is about 1 Mbp. The transcripts encoding these extended genes are chimeras composed of exonic and intronic sequences found comprising other intervening genic regions. The biological importance of these chimeric transcripts is underscored by the non-random interconnections of genes involved and the increase likelihood of the genes involved in many chimeric interactions being evolutionarily older. The characterization of these RNA networks made up of chimeric transcripts further highlights the non-linearity of information stored in the genome and expressed in the transcriptomes as well as the possible way cells have to monitor the complex and distal transcriptional activities ongoing in cells.

## 2456/T/Poster Board #1005

**Characterization and functional analysis of *OTC* gene regulatory regions.** *O. Luksan[1], J. Eberova[1], M. Bouckova[2], M. Jirsa[1], M. Hrebicek[2], L. Dvorakova[2].* 1) Laboratory of Experimental Hepatology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; 2) Institite of Inherited Metabolic Disorders, 1st Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Czech Republic.

Ornithine carbamoyltransferase (OTC, EC 2.1.3.3) is an enzyme of the mitochondrial matrix catalyzing the synthesis of citrulline from ornithine and carbamoyl phosphate. The *OTC* gene is located on Xp21.1. OTC deficiency is the most common inherited urea cycle disorder manifesting in a spectrum of severity ranging from neonatal hyperammonemic coma followed by death to later onset of milder symptoms or asymptomatic course. The published data bring evidence that mutation analysis of the protein coding regions fails in 20-25% of cases. The disease may be caused by gross gene rerrangements or defects in regulatory regions of the gene in such cases. In our study we identified and functionally characterized regulatory regions of *OTC*. We determined 3 alternative transcription start sites of the *OTC* gene at positions c.-95, c.-119 and c.-169 upstream of the translation origin. Based on analogy with model organisms we predicted and examined a promoter (linked directly to the 5'UTR) and enhancer (located 9 kb upstream the transcription origin) region of *OTC*. Using the dual luciferase reporter assay we found a significant increase in transcriptional activity of the promoter when compared with the control plasmid. Moreover, three times higher expression of the reporter gene was observed when enhancer was subcloned into the plasmid containing the promoter. In one male patient with mild phenotype of OTC deficiency, we evaluated the influence of single nucleotide substitution c.-366A>G in the promoter. No statistically significant difference in the transcriptional activity was observed when comparing normal and mutated promoter, however, a 50% difference in the reporter gene expression was found when promoter interacted with the subcloned enhancer. This finding indicates pathogenicity of the c.-366A>G variation. Bisulfite sequencing revealed that the methylation pattern of the *OTC* promoter is tissue specific and the degree of methylation inversely correlated with *OTC* expression. These tissue differences were more prominent in male samples in which X-inactivation did not interfere with methylation. In conclusion our results show that expression of *OTC* is controlled by a weak promoter and a strong enhancer. Single nucleotide substitutions in the regulatory regions such as the c.-366A>G variation may cause OTC deficiency. Tissue specific expression of *OTC* may be partially affected by differential methylation. Support: IGA MZ CR NR/9364 and IKEM MZO 00023001.

## 2457/T/Poster Board #1006

**miR-204 is required for vertebrate eye development.** *S. Banfi[1], S. Carrella[1], R. Avellino[1], M. Karali[1], R. Marco-Ferreres[2], P. Bovolenta[2], I. Conte[1,3].* 1) TIGEM, Fondazione Telethon, Naples, Italy; 2) Departamento de Neurobiología Molecular Celular y del Desarrollo, Instituto Cajal, CSIC, and CIBER de Enfermedades Raras (CIBERER), Madrid, Spain; 3) Institute of Genetics and Biophysics Adriano Buzzati Traverso CNR, Naples, Italy.

The functional role of specific microRNAs in controlling the morphogenetic and cell differentiation events involved in normal eye development in vertebrates is still largely unknown. Here we show that a single microRNA, miR-204, is capable to regulate multiple aspects of eye development in medaka fish. Targeted ablation of miR-204 function by morpholino injections in medaka determined a severe eye phenotype characterized by microphthalmia, aberrant lens formation, incorrect retinal cell differentiation and coloboma. Through a variety of in vitro and in vivo approaches, we found that Meis2 is a key target of miR-204 and plays a pivotal role in the generation of this phenotype via the regulation of the Pax6 pathway. These data demonstrate for the first time that a specific microRNA is involved in the regulation of basic processes underlying eye development and open new avenues on a better comprehension of the pathogenetic mechanisms underlying eye developmental disorders.

## 2458/T/Poster Board #1007

***Dube3a* regulates monoamine synthesis by increasing Punch (GTPCH1) activity in the Drosophila brain.** *K. Summers[1], F. Ferdousy[3], J. O'Donnell[3], N. Elsisi[2], G. Hilliard[2], L.T. Reiter[1].* 1) Department of Neurology, University of Tennessee Health Science Center, Memphis, TN; 2) Department of Molecular Sciences, University of Tennessee Health Science Center, Memphis, TN; 3) Department of Biology, University of Alabama, Tuscaloosa, AL.

The subtle regulation of proteins and transcripts at the synapse may explain the changes in synaptic plasticity and function that lead to autism spectrum disorders (ASD). The human *UBE3A* gene has clearly been implicated in ASD pathogenesis when duplicated and, in some cases in conjunction with Angelman syndrome. Our lab has been using the genetic model organism *Drosophila melanogaster* to screen for proteins regulated by UBE3A and we recently identified Punch (GTP cyclohydrolase I), a key regulator of dopamine/serotonin synthesis as a potential UBE3A target. Individuals with ASD often respond well to serotonin reuptake inhibitors, which increase free pools of serotonin at the synapse and can ameliorate some ASD symptoms. Here we show that *Dube3a* is able to regulate Punch levels in the nervous system. We detected elevated levels of both tetrahydrobiopterin (BH4), the rate-limiting co-factor in dopamine/serotonin synthesis, and dopamine in fly heads over-expressing *Dube3a*. As expected, expression of *Dube3a*-RNAi decreased both BH4 and dopamine levels in flies. These changes in neurochemistry were also associated with pronounced activity changes dependent on dopamine levels.

*Punch* mutants display profound neuronal pathfinding defects as embryos and reduced survival rates to adulthood. We expressed *Punch*-RNAi in a subset of PNS neurons and found abnormal dendritic branching phenotypes that were similar to the phenotype in *Dube3a* loss of function animals. These data suggest that Punch functions in synaptic stability and growth during neuronal development. These results provide a connection between dopamine/serotonin and *Dube3a* expression that may be directly applicable to the treatment of individuals with 15q duplication autism and may also explain why some idiopathic ASD patients respond to selective serotonin reuptake inhibitors better than others.

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## 2459/T/Poster Board #1008

**Auditory electrophysiology of the *mdxCv3* mouse shows abnormal latencies which may provide evidence for the role of dystrophin and the dystrophin-associated protein complex in proper auditory function.**

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Dystrophin is a cytostructural protein in muscle that links the actin cytoskeleton to extracellular laminin via the dystrophin-associated protein complex (DAPC). The Dystrophin gene encodes several different sized isoforms, and their defective production in Duchenne muscular dystrophy (DMD) underlies the many muscle, eye, brain, and heart pathologies of that disease. There is evidence to implicate the DAPC in auditory function. The laminin-deficient *dy* mouse has an abnormal ABR audiometry threshold response (Pillers, 2002). We have also shown that dystrophin mutations in the *mdx$^{Cv3}$* mouse result in altered ABR latency responses (Pillers et al., ASHG 2004). To better characterize the role of dystrophin and the DAPC in hearing, ABR was used to evaluate auditory function in the *mdx*, *mdx$^{Cv3}$*, and in age-matched C57BL/6J controls. Cochlear dystrophin gene and protein expression was evaluated to identify which isoforms are normally present and thus potentially defective in muscular dystrophy mouse models. Both the wild-type C57BL/6J and mdx mice(which lack full-length dystrophin, Dp427m) demonstrated normal ABR threshold and latency responses. However, the *mdx$^{Cv3}$* mouse that lacks all forms of dystrophin displayed shorter ABR latency responses. Western blot analysis of C57 cochlea showed expression of Dp427m and the isoforms Dp116, and Dp71. None of these dystrophin proteins were detected in the cochlea of the *mdx$^{Cv3}$*. Immunohistochemistry showed multiple areas of dystrophin expression in C57 and *mdx* inner ear tissues, including the organ of Corti, and the stria vascularis. No dystrophin labeling was seen in the cochlea in the *mdx$^{Cv3}$*. RT-PCR analysis using primers sets and probes for full-length dystrophin Dp427m, four commonly detected isoforms, and two alternate forms of Dp427m (Dp427c (cortex) and Dp427p (Purkinje)), showed that all isoforms were expressed in the cochlea of C57 mice with the exception of Dp260. The sum of the expression of Dp427m, Dp427c, Dp427p, Dp140, Dp116, and Dp71 appeared to account for all cochlear isoforms. Proper integration between the intracellular cytoskeleton and the extracellular matrix (ECM) appears to play a role in normal hearing. Disrupting the ECM (laminin) has been shown to alter threshold audiometry responses. Here we show that disrupting the intracellular component by removing all dystrophin isoforms alters latency thresholds, providing more evidence that the DAPC plays a role in normal auditory function.

## 2460/T/Poster Board #1009

**Charactereization of the transcription complex regulating Surival Motor Neuron (SMN) gene expression.**

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Spinal muscular atrophy (SMA), a lower motor neuron disorder, is a leading cause of early infant death. Mutations in the *SMN1* gene are responsible for SMA. Because there are two nearly identical *SMN* genes in humans, *SMN2* is an attractive therapeutic target even though it predominantly produces transcripts lacking exon 7. Two HDAC inhibitors are currently being tested in SMA clinical trials; however, these drugs can target up to 40% of genes. We hypothesized that understanding the mechanisms regulating *SMN* gene expression, especially with regards to observed post-natal down-regulation, could help identify more specific therapeutic targets. Our published studies indicated that the core transcription complex was different in undifferentiated and neuronal-like P19 cells and involved numerous transcription factors. In this study, linker-scanning mutagenesis of Smn promoter-luciferase gene constructs and competitor-electrophoresis mobility shift assay (EMSA) strategies allowed us to exclude C/EBPβ, NF1 and Sp1 from the two major protein-binding domains of the core Smn promoter. Competitor-EMSA allowed us to demonstrate Ets binding; but, excluded the Pea3, Ets1, Ets2, and Elk1 family members. Consequently, we used a proteomics approach to identify the specific Ets transcription factor regulating Smn promoter activity in P19 cells. Step-wise purification was monitored by EMSA and the final step relied upon DNA-affinity chromatography. Fractions containing our protein of interest were pooled, subjected to in-gel trypsinization and LC-MS/MS separation. Spectra were interpreted using Spectrum Mill software. This allowed us to identify GABPα as the Ets family member binding to the Smn promoter. GABPα is a unique Ets transcription factor as it binds as a heterotetramer comprised of two GABPα and two GABPβ molecules. Site-directed mutagenesis of the Smn promoter confirmed the need for 2 functional Ets cis-elements and GABPα binding was confirmed by supershift-EMSA and chromatin immunoprecipitation. Taken together, we now have a working model of the Smn transcription complex and have initiated chromatin immunoprecipitation studies to investigate Ets and CREB (a previously reported transcription factor binding to the SMN promoter) for their involvement in Smn gene expression in neuronal an non-neuronal tissues during the critical period of Smn down-regualtion post-natally. *Funded by CIHR, ALS Society, Muscular Dystrophy Canada, and Families of SMA..*

## 2461/T/Poster Board #1010

**Small RNA associated with transcriptional start sites and repetitive elements in postnatally derived mouse neural stem cells.**

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Increasingly complex networks of small RNAs act through RNA interference (RNAi) pathways to regulate gene expression, which could shape diverse cellular pathways. Neurogenesis is regulated at many levels by both extrinsic and intrinsic factors. Recent studies have revealed the important roles of small regulatory RNAs in neurogenesis. To further explore the role of small regulatory RNAs in neurogenesis, we examined expression of small RNA 14-30 nucleotides in length in a homogenous population a postnatally derived neural stem cells. ~43 million small RNA derived cDNA molecules were sequenced. After identification of sequences overlapping currently annotated miRNA, snoRNA, tRNA, rRNA, we identified >100,000 previously unannotated small RNA. These RNA were dominated by a particular class of low abundance 17-19nt RNA that associate with divergent transcription at protein coding transcriptional start sites (TSSa-RNA). We also observed an additional ≈30,000 sequences that overlapped repetitive elements, including many LINE, SINE, and LTR associated small RNA. By comparing the genomic locations from which small RNA were derived we found that relative to RefSeq TSSs there was an overabundance of small RNA within a ≈500bp region in both the sense and antisense orientation directly surrounding TSSs, while there was not significant enrichment of small RNA sequences beyond 750bp of TSSs. These data provide a unique assessment of transcriptional output in postnatally derived neural stem cells and will be generally useful for comparison with genetically defined human neurodevelopmental disease models in mice.

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## 2462/T/Poster Board #1011

**Identification of Deregulated Direct Target Genes of the Hepatitis B Viral (HBV) protein, HBx using chromatin immunoprecipitation and expression microarray profiling.** 
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The hepatitis B X (HBx) protein is strongly implicated in hepatocarcinogenesis (HCC). Although HBx does not bind DNA directly, it interacts with transcriptional regulators resulting in aberrant gene expression and deregulated cellular pathways. We integrated chip-based chromatin-immunoprecipitation(ChIP-chip) and expression microarray profiling and identified 184 gene targets directly deregulated by HBx. We also computationally infer 144 transcription factors that interact with HBx. Interaction of HBx and some of the predicted transcription factors (pTF) as well as the promoters of the deregulated target genes of these pTFs were experimentally validated. Significantly, we also demonstrated that the pTFs interact with the promoters of the deregulated HBx target genes and that deregulation by HBx of these HBx target-genes carrying the pTF consensus sequences can be reversed using pTF siRNAs. The roles of these deregulated direct HBx target genes and their relevance in cancer was inferred via querying against biogroup/cancer-related microarray databases using the web-based NextBioTM software. Integrating ChIP-chip and expression microarray data, 6 pathways, including the Jak-Stat pathway, were predicted to be significantly deregulated when HBx binds indirectly to direct target gene promoters. In summary, this is the first demonstration of the utilization of ChIP-chip to identify deregulated direct gene targets from indirect protein-DNA binding and of the feasibility of identifying TFs that interact with HBx to deregulate target gene expression. Increased knowledge of the gene/transcriptional factor targets of HBx will enhance our understanding of the role HBx in hepatocarcinogenesis and facilitate design of better strategies in combating Hepatitis-B virus associated hepatocellular-carcinoma.

## 2463/T/Poster Board #1012

**Activation of human delta globin gene in transgenic mice.** 
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The δ globin gene, located between the γ and β genes on human chromosome 11, produces a small amount of δ globin in children and adults, participating to the HbA2 synthesis. Our previous work showed in vitro that creation of the CACCC box consensus sequence on the δ globin gene promoter is sufficient to enhance its expression to a considerable extent. The δ globin chain could be a valid substitute of the β globin chain in thalassemia disorder and an antisickling agent in sickle cell anemia. Here we show that: 1) the δ globin gene promoter can be activated in vivo in a transgenic mouse model; 2) the activated δ globin gene promoter is able to drive the expression of δ globin gene to a high level. We have produced transgenic mice lines with a DNA construct in which the wild type (wt) β globin gene promoter and the proximal CACCC box containing δ globin gene promoter are linked in cis to a single erythroid specific enhancer. The order of the different elements in our construct mimics the spatial organization of the β globin cluster were the δ globin gene is situated 5' to the β gene and relatively closer to the LCR. We have also produced transgenic control lines, bearing the wt δ globin gene promoter on place of the CACCC containing δ. The δ and β globin gene promoters are respectively linked to two different (firefly and renilla) luciferase reporter genes. We have analyzed 3 independent transgenic lines bearing the CACCC containing δ globin gene promoter construct, and 3 independent lines bearing the control construct (wt δ globin gene promoter). While the expression level of the wt δ globin gene promoter remains around 20% compared to the β globin gene promoter in each time point observed (12.5, 14.5 and 16.5 days post coitum), the expression level of CACCC containing δ globin gene promoter reached high expression levels in fetal liver at 12.5 p.c. (82% ± 17), 14.5 pc (75% ± 23) and 16.5 pc (97% ± 29). We also have produced two transgenic lines carrying the mini LCR and the δ gene driven by the CACCC containing δ promoter (CACCCδ-LCR). Our preliminary results on a single copy transgenic line show an expression level of the δ gene of 30% compared to the endogenous β major. This level of expression could be considered curative both for β thalassemia and sickle cell disease. We plan to obtain the final validation of the δ globin gene as a therapeutic gene by the rescue of a thalassemic mouse model (th3/th3).

## 2464/T/Poster Board #1013

**Vitamin D receptor associated with late-onset Alzheimer disease promotes amyloid precursor transcription.** 
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Late-onset Alzheimer disease (LOAD) has a strong genetic component. The vitamin D receptor (VDR) is one potential genetic risk factor for LOAD. Genetic association of VDR with LOAD has been described in a Turkish population and we have previously identified the strongest association with LOAD at rs11568820 (p=7.76x10-6, Odds ratio =1.69), which is known to be a promoter SNP that has functional effects on VDR transcription. It has been shown that vitamin D deficiency is associated with cognitive impairment and LOAD. However, a molecular role of VDR in AD pathogenesis remains to be elucidated. VDR is a known transcription factor that interacts with SMAD3, which affects amyloid precursor protein (APP) transcription by promoting TGF beta signaling. Previously, we tested the hypothesis that VDR affects APP promoter activity and demonstrated, using the luciferase reporter assay, that APP promoter activity was significantly up-regulated by VDR over-expression independently from vitamin D. Vitamin D, in fact, inhibited APP over-transcription in neuro2A cells. Additionally, the relative expression levels of endogenous APP mRNA is increased significantly by transient VDR over-expression in both human astrocytes and neurons, confirming the VDR effect on APP transcription and expression in the nervous system. Here we extend these results to human brain by showing that VDR mRNA is significantly over-expressed in 10 LOAD brains compared to 10 age-matched controls. Immunohistochemical assay also revealed stronger staining for VDR in neurons in the LOAD frontal cortices compared to those of controls. These results suggest that VDR over-expression may be involved in LOAD pathogenesis, APP may be a target gene for VDR signaling and that Vitamin D, in at least some cases, may inhibit as well as activate the effects of VDR.

## 2465/T/Poster Board #1014

**mRNA-Seq Whole Transcriptome Analysis of a Single Cell.** 
*K. Lao[1], F. Tang[2], C. Barbacioru[1], Y. Wang[1], E. Nordman[1], C. Lee[1], N. Xu[1], X. Wang[1], J. Bodeau[1], A. Surani[2].* 1) Molec Cell Biol, Applied Biosystems, Foster City, CA; 2) Wellcome Trust/Cancer Research UK Gurdon Institute of Cancer and Developmental Biology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QN, UK.

We developed a digital gene expression profiling assay at single cell resolution by combining a modified single cell whole transcriptome amplification method with the next generation sequencing technique, SOLiD System. Using only a single mouse blastomere, our mRNA-Seq assay can detect the expression of 74% (5,270) more genes than microarray techniques. Moreover, 8 - 19 % of the genes with multiple known transcript isoforms We developed a digital gene expression profiling assay at single cell resolution by combining a modified single cell whole transcriptome amplification method with the next generation sequencing technique, SOLiD System. Using only a single mouse blastomere, our mRNA-Seq assay can detect the expression of 74% (5,270) more genes than microarray techniques. Moreover, 8 - 19 % of the genes with multiple known transcript isoforms express at least two isoforms in the same blastomere or mature oocyte, which unambiguously demonstrated the complexity of the transcript variants at whole genome scale. With conventional transcriptome assays it was not possible to ascertain if the multiple transcript isoforms found in a tissue or organ coexist in the same cell, or if they are just expressed in different cell types or at different cell cycle stages of the same cell type. This knowledge is crucial to provide a real understanding of the transcriptome complexity within individual cells. Finally, for Dicer and Ago2 knockout oocytes, we also showed that in Dicer knockout and Ago2 knockout mature oocytes, 1,924 and 1,687 genes respectively were abnormally upregulated, and 1,343 and 987 transcripts respectively were downregulated compared to wildtype controls, which proves the global importance of small RNAs (including microRNAs and endogenous siRNAs) for oogenesis. The main technical novelty of this work is the combination of an improved unbiased amplification of cDNAs from single cells with well over a 100 million reads, or a few gigabases of cDNAs on SOLIDTM. This not only allowed us to discover many novel transcripts that have been overlooked but also to get a quantitative estimate of their abundance in the cell by the frequency with which the sequence occurs in the mRNA-Seq reads. This single cell mRNA-Seq assay will greatly enhance our ability to analyze transcriptome complexity during mammalian development, especially for early embryonic development and for stem cells, which are usually rare cell population in vivo.

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## 2466/T/Poster Board #1015

**Arsenic induces differential expression of Nrf2-dependent enzymes HO-1 and NQO1.** *F. Centeno[1], E. Córdova[1], J.L. Cruz-Colín[1], M.E. Morales-Marín[2], L. Orozco[1,2].* 1) Investigacion, Inst Natl de Medicina Genomica, Mexico D.F., Mexico; 2) Posgrado en Ciencias Genómicas, Universidad Autónoma de la Cd. de México.

Ground water arsenic is the main source of human exposure worldwide and it represents an important health issue. Arsenic exposure has been associated with different kinds of cancer, vascular diseases and skin lesions. It has been reported that arsenic induces oxidative stress in liver, brain and erythrocytes, being the oxidative damage the major mechanism of arsenic-mediated cellular toxicity. Nrf2 transcription factor (Nuclear Factor Erythroid 2-related Factor 2 ) activation has been proposed as an important mechanism against arsenic exposure in different cell types, by means of the regulation of a battery of genes encoding antioxidant and phase II detoxification enzymes. The aim of this study was to evaluate the Nrf2 response to arsenic exposition in two different cell lines. In this context, we analyzed the expression of two Nrf2-responsive enzymes: HO-1 (Heme-Oxygenase 1) and NQO1 (NAD(P)H dehydrogenase quinone 1), in a liver-derived cell line (HepG2) and in a lymphoblastoid cell line treated with 2.5, 5 and 10μM sodium arsenite. Transcriptional activation of Nrf2, HO-1 and NQO1 genes was evaluated by Real-Time RT-PCR and protein levels were determined by Western blot. A high transcription induction of HO-1 by arsenic treatment was observed in both HepG2 and lymphoblastoid cells. Interestingly, a higher activation of HO-1 was observed in lymphoblastoid cells after 10μM arsenite treatment, this cell line reached above 200 fold HO-1 transcription induction compared with those with out treatment, while the HO-1 induction in HepG2 was around 20 fold. An increase in the HO-1 protein levels was documented in both cell lines after arsenic exposure. In contrast to the high NQO1 induction previously reported by other well-characterized Nrf2-inducers, like sulphoraphan, NQO1 showed a slight induction by arsenic. Nrf2 mRNA levels remained constant in HepG2 but it showed and slight increase in lymphoblastoid cell line. Our results suggest that there is a difference in the induction mediated by arsenic of Nrf2-dependent enzymes HO-1 and NQO1 and that HO-1 expression is the main Nrf2-induced mechanism against arsenic-mediated cell toxicity, mainly in lymphoblastoid cells.

## 2467/T/Poster Board #1016

**Identification of decreased expression of transcripts with alternative 3'UTRs in the brains of *Gtf2ird1*$^{-/-}$ mice.** *J. O'Leary, E.J. Young, L.R. Osborne.* Departments of Molecular Genetics and Medicine, University of Toronto, Toronto, Ontario.

Williams-Beuren Syndrome (WBS) is an autosomal dominant neurodevelopmental disorder caused by the hemizygous deletion of a 1.5 Mb region from chromosome 7q11.23. The clinical symptoms of WBS are numerous and include behavioural and cognitive components. WBS patients usually have overly friendly personalities and suffer from generalized anxiety, simple phobias, ADHD, and mild mental retardation with relative preservation of language skills. Two of the genes deleted in WBS, *GTF2I* and *GTF2IRD1*, are transcription factors which show widespread but distinct expression patterns during development. Genotype-phenotype studies in patients with atypical deletions of the WBS region have implicated these genes in the neurological features of WBS and *Gtf2ird1*-targeted mice have features consistent with the WBS phenotype, namely impairments in fear response, increased sociability and decreased aggression. In order to identify neural targets of GTF2IRD1, microarray analysis was performed comparing gene expression in the brain of *Gtf2ird1*$^{-/-}$ and WT mice, both at embryonic day 15.5 and at birth. Overall, the changes in gene expression in the mutant mice were not striking with most changes falling in the range of 0.3 to 2 fold. Real-Time PCR was used to verify the expression levels of candidate genes identified in the arrays using primer pairs within the microarray probe region in the 3'UTR, as well as within the coding region of each gene. Interestingly, the majority of genes whose transcript level alteration was verified by PCR showed decreased expression only with assays targeting the 3' UTR, but not with assays targeting the coding region. These include *Stx3*, *Actl6b*, *Taf6* and *Zfp68*. Analysis of these genes using 3' RACE identified transcripts using alternative polyadenylation sites that could result in probe-specific alterations in transcript level. Although the significance of these alternate transcripts is not yet known, the 3' UTR plays a pivotal role in transcript localization, stability and translation. Micro-RNA binding sites have been associated with longer 3'UTRs and in the brain, alternate 3'UTR sequences have been shown to influence spine morphology and synaptic plasticity. Given the emerging roles of alternative 3'UTRs it is feasible that the changes in gene expression we have identified could contribute to the behavioural phenotype of the *Gtf2ird1*$^{-/-}$ mice.

## 2468/T/Poster Board #1017

**Comparative Analysis of Transcriptional Activity of PERV LTR Subtypes in Human, Monkey, and Pig Cell Lines.** *H. Kim[1], Y. Jung[1], J. Huh[2], D. Kim[3], B. Cho[4], H. Ha[1], K. Ahn[1].* 1) Department of Biological Science, Pusan National University, Busan, Korea; 2) National Primate Research Center, KRIBB, Ochang, Chungbuk, Korea; 3) Korea Bioinformation Center, KRIBB, Daejeon, Korea; 4) Department of Animal Science, College of Life Sciences, Pusan National University, Miryang, Korea.

Porcine endogenous retroviruses (PERV) in the pig genome have the potential to act as harmful factors in xenotransplantation (pig-to-human). Long terminal repeats (LTRs) of the PERV elements have showed promoter activity that could affect neighboring functional genes. Transcription activities of 11 long terminal repeat subtypes were analyzed using kidney cell line of various species (HEK293, Cos7, PK15). They showed different transcription regulation activity in different cell lines. The E4 type (H2-2) derived from pig kidney showed distinct promoter activity in the human kidney HEK293 cell line. Deletion mutants based on the boundary of U3, R and U5 region of the H2-2 clone were constructed and examined transient transfection assay, indicating that U3 region containing binding sites for transcription factors (MAZR, HIF-1, STAT5A, and GATA-4) was crucial for transcription regulation.

## 2469/T/Poster Board #1018

**Complex regulatory network of transcription factors and interacting proteins involved in Axenfeld-Reiger syndrome.** *M. Acharya, L. Huang, M. Walter.* Department of Medical Genetics, University of Alberta, Edmonton, AB, Canada.

Axenfeld-Rieger (AR) malformation is an autosomal dominant disorder of the anterior segment of the eye associated with glaucoma. Mutations in the PITX2 "homeobox" transcription factor and FOXC1 "forkhead box" transcription factor have been reported to cause AR malformation linked to chromosome 4q25 and 6p25 respectively. Interaction between these two transcription factors has been established previously from our lab, where PITX2 has been shown to have a repressor effect on FOXC1 transcription activation. To understand the complex regulatory network of PITX2, we performed yeast two-hybrid (Y2H) screening of the human trabecular meshwork (HTM) cDNA library using PITX2 as "bait". The trabecular meshwork is a region reportedly associated with anterior segment dysgenesis of the eye and glaucoma. We identified PRKC apoptosis WT1 regulator (PAWR) as a novel PITX2-interacting protein that downregulates PITX2 transactivation in HTM cells. PAWR has already been established as an inducer of apoptosis in cancer and transcription repressor to WT1 thereby blocking expression of the anti-apoptotic protein Bcl2. We further identified FOXC2 as an interacting partner of PAWR from our Y2H screening. Mutations in FOXC2 cause Lymphedema-distichiasis, which includes developmental defect in anterior segment of the eye. FOXC2 has also been found to have structural and functional similarity with FOXC1. We, therefore, confirmed the binary interaction between PAWR and FOXC2 in vitro by Ni-pulldown assay and in vivo by co-immunoprecipitation assays. We also observed FOXC1-PAWR and FOXC1-FOXC2 interactions when tested in vitro by Ni-pulldown assays. We further confirmed that the same C-terminal leucine-zipper (LZ) domain in PAWR interacts with PITX2, FOXC1 and FOXC2. Interestingly, we found an upregulation effect on transcription activity of FOXC2 in presence of PAWR. Based on these results, we think there is a complex regulatory network operating in the eye involving PITX2, PAWR, FOXC1, and FOXC2. Therefore, we are looking forward to do more in-depth study of the assembly and functional significance of the protein-complexes involving PITX2, PAWR, FOXC1, FOXC2 for better understanding of the pathogenesis underlying the AR malformation, glaucoma and associated anterior segment diseases in the eye.

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## 2470/T/Poster Board #1019

**Analysis of the 3'UTR insertion/deletion polymorphism of ARMS2 gene in age-related macular degeneration.** *G. Wang[1], KL. Spencer[2], WK. Scott[1], P. Whitehead[1], BL. Court[1], J. Ayala-Haedo[1], SG. Schwartz[3], JL. Kovach[3], P. Gallins[1], M. Polk[1], A. Agarwal[4], EA. Postel[5], JL. Haines[2], MA. Pericak-Vance[1].* 1) Miami Institute for Human Genomics, Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami, Miami, FL 33136; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN 37232; 3) Bascom Palmer Eye Institute, University of Miami, Miami, FL 33136; 4) Department of Ophthalmology, Vanderbilt University, Nashville, TN 37232; 5) Duke Eye Center, Duke University Medical Center, Durham, NC 27710.

Controversy remains concerning which gene at the chromosome 10q26 locus confers risk for AMD and resolution of statistical genetic analysis is limited by strong linkage disequilibrium across the region. Functional analysis of the related genetic variations and biological studies on the genes ARMS2 and HTRA1 can be helpful to solve this puzzle. Recently Fritsche et al reported that AMD is associated with unstable ARMS2 transcripts caused mainly by a complex insertion/deletion (indel; consisting of a 443bp deletion and an adjacent 54bp insertion) in its 3'UTR. To validate this indel, we analyzed our samples by sequencing and quantitative PCR. We found that the indel is more complex than originally reported (443 bp deletion and 54 bp insertion) and composed of two side-by-side indels separated by 17 bp: (1) 9 bp deletion with 10bp insertion; (2) 417 bp deletion with 27 bp insertion. ARMS2 is ubiquitously expressed in human tissues. Comparatively, ARMS2 is preferentially expressed in the occipital cortex while HTRA1 is expressed in a much lower level in both retina and occipital cortex suggesting a possibly more important role for ARMS2 in the physiology of vision than HTRA1. No association between the genotype of the indel and ARMS2 mRNA level was found in human blood samples, suggesting due to the reported strong linkage disequilibrium between the A69S variant and the indel, that it could be A69S variant, not the indel, that confers risk of AMD.

## 2471/T/Poster Board #1020

**Study on fgf9 and fgfr3 gene expression profiling in mice.** *M.M. GU[1], X.Y. CHEN[1], X.L. WU[1], S.Y. LU[2], Z.G. WANG[1,2].* 1) Department of Medical Genetics, Shanghai Jiao Tong University School of Medicine, Shanghai, China; 2) Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China.

The fibroblast growth factor 9 (FGF9) belongs to the FGF superfamily, which consists of 22 members involved in various developmental processes including development of various tissues such as limb, nervous and skeletal systems in vertebrates. FGF9 is widely distributed in brain, kidney, motor neurons and bone tissue at embryonic period. By binding with its receptor (e.g. FGFR3), FGF9 can promote the proliferation of glial cells, dental mesenchymal cells, prostate epithelial cells and stem cell. FGF9 also can promote development of lung and the reproductive system during the embryonic period. However, it was not identified that the expression profiling of fgf9 and fgfr3 in adult mice. We analyzed cDNA from twelve tissues (heart, liver, spleen, cerebellum, kidney, lung, brain, thymus, muscle, stomach, joint and marrow) using RT-PCR. The results showed that fgf9 was strongly expressed in heart, cerebellum, kidney and muscle, with somewhat lower expression in brain and joint. We could not be detect in any other tissues. Whereas the fgfr3 was widely expressed in all twelve tissues, especially higher expression in lung and brain. Using immunohistochemistry method, we also evaluated the expression of fgf9 and fgfr3 proteins. The fgf9 was widely expressed in brain. Whereas the fgfr3 protein was widely expressed in liver and brain. In different cells of other tissues, fgf9 and fgfr3 were both expressed in muscle fiber of heart and muscle, in tubular cells of kidney and in proliferating and hypertrophic chondrocytes of joint. In the joints of different age mice, the fgf9 and fgfr3 were both detected strongly in the growth plate of newborn and 1 week old mice, with somewhat lower expression in the joints of 4 week mice. But both of them were not expressed in the growth plate of 8 week old mice. These results led us to study how FGF9 played important roles in human bone and joint development and diseases such as multiple synostoses syndrome.

## 2472/T/Poster Board #1021

**A functional approach to understand the role of the Kinesin Binding Protein (KBP) in Goldberg-Shprintzen syndrome.** *M.M.M. Alves[1,5], G. Burzynski[2], A. Brooks[3], C.C. Hoogenraad[4], I. Shepherd[2], B.J.L. Eggen[5], R.M.W. Hofstra[1].* 1) Department of Genetics, University Medical Center Groningen, Groningen, Netherlands; 2) Department of Biology, Emory University, Atlanta, USA; 3) Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, Netherlands; 4) Department of Neuroscience, Erasmus Medical Center, Rotterdam, Netherlands; 5) Department of Developmental Genetics, Faculty of Mathematics and Natural Sciences, University of Groningen, Haren, Netherlands.

Goldberg-Shprintzen syndrome is a rare autosomal recessive disorder characterized by polymicrogyria, mental retardation and Hirschsprung disease. Recently, we showed that patients suffering from this disease have inactivating mutations in the *KBP/KIAA1279* gene. However, the function of its encoded protein is largely unknown. Considering that mutations in KBP are associated with both enteric and central nervous system defects, clarifying its function will lead to new insights about the protein network associated with neuronal development. Based on its primary sequence, KBP is predicted to have two tetratricopeptide repeats (TPRs). Since the basic function of TPR motifs is to mediate protein-protein interactions, a yeast two-hybrid screen was performed to identify possible KBP interacting proteins. We obtained various possible interactors, of which the two major categories were kinesin like proteins involved in the transport of vesicles and organelles towards the plus end of the microtubules, and microtubule destabilizing proteins implicated in axonal growth. These interactions were further confirmed by co-localization studies, co-immunoprecipitation assays and expression studies in mice primary cortical neurons. Moreover, an epistatitc interaction between KBP and SCG10 was also found in our zebrafish model supporting these findings. As several kinesins were identified, a probable interaction with microtubules was explored, both by co-localization and in vitro binding assays. Moreover, an implication in cell differentiation and neurite development was analysed by KBP knock down in PC12 cells.

Based on the results obtained, we postulate that KBP plays an important role in neuronal development and it is likely involved in microtubule organization/stability by direct interaction with SCG10 but it is not directly associated to microtubules.

In conclusion, our results bring new insights about Hirschsprung disease and polymicrogyria development pointing towards a microtubule associated problem and present KBP as a new/major player involved in neuronal development.

## 2473/T/Poster Board #1022

**A dual role for hMSH5 in DNA damage response and DSB repair.** *C. Her.* Sch Molec Bioscience, Washington State Univ, Pullman, WA.

Increasing evidence has suggested that the human MutS homolog hMSH5 may possess diverse functions in several biological processes which include meiotic recombination, class switch recombination, and mitotic DNA double-strand break (DSB) repair. In order to establish a foundation for delineating the precise functions of hMSH5 in these processes, we have investigated the roles of hMSH5-c-Abl interaction in both DNA damage response and DSB repair. The results of these recent studies have demonstrated that the hMSH5 protein can be subjected to induction by DNA damage, in which the elevated levels of hMSH5 promote ionizing radiation (IR)-induced caspase-3-dependent apoptotic response. This role of hMSH5 is entirely dependent on the interaction with c-Abl, resulting in enhanced c-Abl activation and the subsequent p73 activation. The significance of this observation can be mirrored by the effects of the peculiar interaction between hMSH5P29S and c-Abl on sensitizing cells to IR, indicating that mutations impairing this process could significantly affect normal cellular responses to anti-cancer treatments. On the other hand, our most recent data have also demonstrated a functional requirement for c-Abl-mediated hMSH5 phosphorylation in DSB repair. Using a chromosomally integrated recombination reporter, we have demonstrated that DSB-triggered tyrosine phosphorylation of hMSH5 is an essential early event for non-crossover HR repair of DSBs. In fact, cells expressing a phosphorylation deficient hMSH5 mutant are more sensitive to DSB-inducing agent cisplatin. Collectively, our studies tend to suggest that a significant induction of hMSH5 protein in cells could promote DNA damage-triggered apoptotic response, conversely a moderate increase in the level of hMSH5 might facilitate DSB repair.

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## 2474/T/Poster Board #1023

**The tumour suppressor CYLD and the ubiquitin ligase MID1 associate at the microtubules and have counteracting effects on microtubule stability.** *J. So[1, 2], A. Wilde[3], M. Huber[4], S. Krauβ[2], A. Köhler[5], R. Schneider[5], S. Schweiger[2, 6].* 1) Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, Canada; 2) Max Planck Institute for Molecular Genetics, Berlin, Germany; 3) Department of Molecular Genetics, University of Toronto, Toronto, Canada; 4) Department of Dermatology, University Hospital Center and University of Lausanne, Lausanne, Switzerland; 5) Institute for Biochemistry, Center for Molecular Biosciences, University Innsbruck, Innsbruck, Austria; 6) Division of Medical Sciences, University of Dundee, Dundee, UK.

Mutations in the CYLD tumour suppressor have been identified in patients with familial cylindromatosis and familial trichoepithelioma, which are both autosomal dominant genetic predispositions to multiple tumours of the skin appendages. CYLD has been shown to deubiquitinate TRAF proteins and Bcl-3, both leading to inhibition of NF-κB activation. Recent studies have demonstrated the localization of CYLD at the microtubules, and its putative role in the cell cycle. We have now found that CYLD is a microtubule-associated protein that accelerates microtubule destruction in cells treated with the microtubule depolymerizing agent nocodazole. CYLD protein carrying a point mutation that truncates the protein at a.a. 485 and thereby deletes the C-terminus, including the majority of the third predicted CAP-GLY domain and the ubiquitin hydrolase domain, still associates to microtubules, but has no influence on microtubule stability. Accordingly, specific knockdown of CYLD results in an increase of microtubule stability and faster recovery after nocodazole withdrawal. In addition, we demonstrate that the association of CYLD to microtubules may be effected by its interaction with a known microtubule stabilizer, the ubiquitin ligase MID1. MID1 mutations result in X-linked Opitz G/BBB syndrome, a ventral midline malformation syndrome characterized by hypertelorism, hypospadias in males, heart defects, laryngotracheoesophageal anomalies, structural brain anomalies, and developmental delay. We show by immunofluorescence, yeast two-hybrid assay and co-immunoprecipitation that MID1 and CYLD interact and co-localize to the microtubules. We further demonstrate that they have counteracting effects on microtubule de- and re-polymerization in nocodazole challenge and withdrawal experiments in in vivo settings. Our data strongly suggest that, in addition to upregulation of NF-κB signaling, CYLD plays an important role in microtubule dynamics by acting as a destabilizer and as an antagonist to the microtubule-stabilizing ubiquitin ligase MID1. This interaction has implications for the pathogenetic mechanisms involved in tumour formation and development of ventral midline malformations.

## 2475/T/Poster Board #1024

***C15orf2* is imprinted in brain and encodes a nuclear protein that is present in cortex and hypothalamus.** *M. Wawrzik[1], R. Herrmann[2], J. van de Nes[3], K. Buiting[1], B. Horsthemke[1].* 1) Institut für Humangenetik, Universitätsklinikum Essen, Essen, Germany; 2) Department of Pediatrics and Pediatric Neurology, University Hospital Essen, Essen, Germany; 3) Department of Pathology and Neuropathology, University Hospital Essen, Essen, Germany.

The Prader-Willi syndrome (PWS) is a neurogenetic disorder which results from the loss of paternal contribution for a 1.5 Mb imprinted region on the proximal long arm of chromosome 15. In 2000, we identified an intronless gene in this region (*C15orf2*), which maps between *NDN* and *SNURF-SNRPN* and encodes an 1156-amino-acid protein of unknown function. By Northern-Blot analysis we detected expression only in adult testis. Based on these findings we had suggested that *C15orf2* might play a role in spermatogenesis. Nielsen at al. (2005) have suggested that *C15orf2* is under strong positive selection in primates. Interestingly, there is no orthologuous gene in the mouse. Using RT-PCR, we have now detected expression of *C15orf2* in a number of different tissues including fetal brain. By analysing expressed fragment-length polymorphisms in *C15orf2*, we found biallelic expression in adult testis but paternal only expression in fetal brain. The latter finding is compatible with a role of this gene in PWS. By Western-Blot analysis of protein extracts from human brain with an anti-C15orf2 antibody we could detect C15orf2 in cortex and hypothalamus. Analysis of the amino acid sequence by PSORTII, a software for the prediction of eukaryotic protein subcellular localization, revealed a possible localization of C15orf2 in the nucleus. We could confirm this prediction by transient overexpression of *C15orf2* in Hela and HEK293 cells. The nuclear localization of C15orf2 suggests that the protein may have a direct or indirect function in the regulation of gene expression.

## 2476/T/Poster Board #1025

**A simple denaturing HPLC method to determine phase in amplicons with double heterozygosity.** *B.Y.L. Wong[1], D.E.C. Cole[1,2,3,4].* 1) Clinical Pathology, Sunnybrook Health Sciences Centre, Toronto, ON, Canada; 2) Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada; 3) Pediatrics (Genetics), University of Toronto, Toronto, ON, Canada; 4) Medicine, University of Toronto, Toronto, ON, Canada.

INTRODUCTION: The calcium-sensing receptor (CASR) helps regulate extracellular calcium homeostasis. In the human *CASR* gene, three common SNPs (A986S, R990G and Q1011E) have been studied for their impact on protein function, but results are not conclusive. Considering their opposing individual effects and variable allele frequency in different populations, a tri-locus haplotype could potentially provide unique information on overall function. Here, we describe a procedure to determine the *cis/trans* configuration in *CASR* double heterozygotes by denaturing HPLC. MATERIALS AND METHODS: The sequence containing codons 960 to 1030 was amplified by standard PCR. Doubly heterozygous control samples in *trans* configuration were obtained by mixing the PCR amplicons from samples homozygous at one of the polymorphic sites. Controls in *cis* configuration were constructed by amplifying DNA homozygous at one site with a long mutagenic primer containing the mutant base at the other site. To exclude any artifact from amplification with mutagenic long primer, the same long primers were also used to amplify normal samples to generate amplicons singly homozygous which were then mixed to produce the *trans* double heterozygous controls. Chromatograms of amplicons from both short and long primers were compared. All PCR products were sequenced to confirm their expected genotypes. DHPLC analysis was carried out in the Transgenomic WAVE® system equipped with a DNASep column. Seventy-seven double heterozygotes (39 A986S+R990G, 23 A986S+Q1011E, 15 R990G+Q1011E) previously identified among 2107 samples were analyzed by this method. RESULTS AND DISCUSSION: The sequencing results showed that doubly homozygous amplicons constructed with the long mutagenic primers produced only the expected haplotypes. The DHPLC chromatograms of the *trans* double heterozygotes produced by both short and long primers were identical. The chromatograms of mixtures with *cis* configuration were readily distinguishable from their trans counterparts. All 77 double heterozygotes were found to be in *trans* configuration. In some cases, evidently, the partial denaturation of doubly heterozygous amplicons at the optimum melting temperature depends on the configuration of the mismatches and their flanking sequences. Our results with the three *CASR* variants suggest that DHPLC may be a simple and easy method to determine phase when double heterozygosity involves closely clustered SNPs.

## 2477/T/Poster Board #1026

**PANArray cancer related miRNAs profiling with direct labeling on chip.** *H. Kim, JJ. Choi, M. Cho, H. Park.* PANAGENE Inc., Institute of Bioscience, Yuseong-gu, Daejeon, Korea.

MicroRNAs(miRNAs) are short non-coding RNAs that play a critical role in many important biological processes. MiRNA expression profiling is important to understand the biological mechanisms, develop drugs, and diagnose. However, analyzed method of expression profiling is limited for short base of miRNA. Microarray which is hybridization based technique is the best for detection tool on miRNA characteristic. So, we have developed a PNA-based microarray that is highly sensitive, specific and reproducible to analyze miRNA expression profiles with direct miRNA labeling on chip after hybridization. PNA probes are well known to have stronger binding affinity, specificity to its complementary DNA or RNA strand and stability to biological enzymes. Unlabeled total RNA is hybridized to the PNA-based microarray and then only hybridized miRNA labeling is performed by using enzymatic ligation with pCp-Cy3. Labeling method of only hybridized miRNAs is an important technique for reproducibility and reliable results of microarray. Our data showed very low cross hybridization for miRNAs differing by single nucleotide such as human let7(a-i) family. Also, this PNA-based microarray platform can detect small amounts of individual miRNAs from <400ng of total RNA with above 99% correlation between independent replicates in 6hrs. Therefore, PANArray™ miRNA can be a powerful tool to analyze miRNA expression profiling in cancer diagnosis and prognosis for high throughput screening with high accuracy.

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## 2478/T/Poster Board #1027

**Microarray gene expression profiling of limiting biological samples.** *M. Watt[1], R. Alexandridis[1], C. Skalitzky[1], N. Burns[1], J.D. Heath[2], B. Kwong[2], R. James[1], R. Selzer[1].* 1) Roche NimbleGen, Inc., Madison, WI; 2) NuGEN Technologies Inc., San Carlos, CA.

In microarray-based gene expression profiling experiments, the expression levels of thousands of genes in individual samples are simultaneously monitored to study the effects of certain treatments, diseases, or developmental stages on gene expression. Standard microarray hybridization and detection protocols require microgram amounts of sample RNA. Also, the highest quality of purified RNA is essential for a successful and reproducible gene expression profiling experiment. Often, samples (for example, clinical needle biopsies and micro-dissected tissues) are extremely small and typically result in nanogram amounts of RNA as starting materials for analysis. For expression profiling analysis of such limited RNA samples, a global RNA amplification scheme is required. Optimally, the RNA amplification procedure will generate microgram amounts of RNA (or cDNA) and maintain the relative transcript abundance present in the initial mRNA sample. Here, we present our findings on the microarray hybridization performance of a range of amplified eukaryotic RNA samples (2 to 20 ng total RNA input). Reproducibility and reliability of linear amplification methods (mRNA-based and whole transcriptome), compared to standard (non-amplified) sample preparation, were assessed using both qRT-PCR and high-density oligonucleotide microarray platforms. The correlation between the results of microarray experiments derived from non-amplified and amplified samples is presented. This study indicates that high quality microarray data can be generated from small amounts of RNA; RNA amplification allows the detection of a large number of genes expressed in the starting RNA population without significantly altering their relative signals. The continued validation of RNA amplification technologies is recommended, particularly as sample limits are further expanded. Robust RNA amplification methods, partnered with expression profiling platforms, have fostered advancements in the understanding of important pathophysiological scenarios and their transition into clinical settings.

## 2479/T/Poster Board #1028

**Overexpression of modified cyclophilin A ragulated endoplasmic reticulum stress.** *H. Kim, W. Choe.* Kyung Hee Univ Department of Biochemistry and Molecular Biology, Medical Science and Engineering Research Center for Bioreaction to Reactive Oxygen Species, Biomedical Science Institute, School of Medicine, Kyunghee University, Seoul 130-701, Korea.,.

The protein is guided to the ER by a signal sequence. signal sequence of preprolactin, after translocation into microsomes and cleavage by signal peptidase, is converted to an intermediate form. The KDEL sequence(lys-asp-glu-leu) is a signal for permanent retention of proteins in the endoplasmicreticulum(ER). Cyclophilin A(CypA) is a relatively abundant small immunophilin exist in the cytoplasm of all mammalian cells. These immunophilins link to cytoplasmic dynein indirectly through the association of the immunophilin peptidylprolyl isomerase (PPIase) domain with dynamitin, a component of the dynein-associated dynactin complex. our study showed that the localization of cyclophilin A(cypA) is regulated by prolactin signal sequence and the KDEL sequence. The ER stress can be induced not only by alterations in physiological conditions such asproteins undergo post-translational modifications in the ER, which requires high levels of luminal Ca2+ and oxidative components. Exposure of cells to glucose starvation, inhibition of protein glycosylation, disturbance of Ca2+ homeostasis, or oxygen deprivation devastates ER environments and causes unfolded proteins to accumulate in the ER (ER stress). we identified ER existence of a modified cyclophilin A expressed in the Endoplasmic reticulum directed by the prolactin signal sequence and the KDEL sequence that resisted ER-stress in chang cell.

## 2480/T/Poster Board #1029

**Characterization and multimerization of recombinant cyclophilin A mutant.** *I. Oh, W. Choe.* Department of Biochemistry and Molecular Biology, Medical Science and Engineering Research Center for Bioreaction to Reactive Oxygen Species, Biomedical Science Institute, School of Medicine, Kyunghee University, Seoul 130-701, Korea.

Cyclophilins belong to a group of proteins that have peptidyl-prolyl cis-trans isomerase activity. The first member of the cyclophilins to be identified in mammals, cyclophilin A (CypA), is the major cellular target for, and thus mediates the actions of, the immunosuppressive drug cyclosporin A. To determine the multimerization of CypA and mutant, Substitution of CypA serine 16 (Ser16) into proline (Pro) and alanine (Ala55) into arginine (Arg) were conducted by PCR in two steps, we investigated the induction of CypA-GST fusion protein. Fusion protein could be purified from crude lysate by affinity chromatography on immobilized glutathione. Bound fusion proteins can be eluted with reduced glutathione under non-denaturing conditions. Eluted fusion protein cleavaged using thrombin agarose and remove GST from CypA. Purified CypA was analyzed by Native-PAGE and immunoblot analysis. Native-PAGE revealed that wild type CypA formed monomer but P16S mutant formed dimmers. Peptidyl-prolyl cis-trans isomerase activity was assayed for the mutants using peptide substrate N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide, which was lower than wild-type CypA. The antioxidant activity of the CypA and mutants have been examined by total antioxidant capacity assay using cu++ reagent. These assay reveal that CypA mutant lost antioxidant activity.

## 2481/T/Poster Board #1030

**Discovery And Profiling Of Human Short RNAs Using Single Molecule Sequencing (tSMS).** *P. Kapranov[1], F. Ozsolak[1], S. Foissac[2], E. Giladi[1], D. Lipson[1], J. Reifenberger[1], C. Hart[1], J. Thompson[1], P. Milos[1].* 1) Helicos BioSciences Corporation, 1 Kendall Sq Ste B7301 Cambridge, MA 02139-1671; 2) Integromics, S.L., Grisolía, 2 - 28760 Tres Cantos Madrid, Spain.

The repertoires of RNA molecules produced by eukaryotic cells have proven to be exceptionally complex. A large fraction of these molecules are produced from the regions of the genome not considered to be protein coding. Furthermore, as shown by the ENCODE Consortium, structures of RNA molecules can be complex, bringing together distal regions of the genome and portions of different genes, thus challenging our very definition of what we have described as a gene.

The population of short RNAs (sRNAs, <200nt) has also seen a dramatic increase in complexity with the discovery of whole new classes of RNAs such as promoter-associated and termini-associated short RNAs (PASRs & TASRs) and others along with the realization that the sRNA population of a human cell line can comprise 100's of thousands of different un-annotated species. Profiling of sRNAs using next-generation sequencing confirmed the complexity of the un-annotated sRNAs as well as presence of at least one new functional class of novel sRNAs, the class of PASRs.

True Single Molecule Sequencing (tSMS) which requires minimal sample manipulation and no amplification or ligation, could provide better estimate of the complexity of an RNA population and abundances of individual RNA species. We show the feasibility of analysis of human sRNA population via tSMS approach using both no-amplification and amplification-bases methods. As expected, the ligation/amplification-based approach while correlative with the no-amplification method, resulted in skewing abundancies of known sRNAs by up to 1-2 orders of magnitude. Overall, profiling of human sRNAs using tSMS has detected large numbers of un-annotated RNA species, including the class PASRs. In addition, we report a novel class of sRNAs that contain a stretch of non-genomically encoded polyU residues at their 5' ends and are sense to the 5' ends and antisense to the very 3' ends of genes thus strongly suggesting that they are produced by copying polyadenylated RNA species using RNA-dependent RNA-polymerase like activity.

This data suggests that a portion of the vast complexity of sRNA populations can only be partially revealed by any single technology. In depth analysis of these results will be presented.

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## 2482/T/Poster Board #1031

**Length of ORF, position of the first AUG and Kozak motif are important factors for possible dual-coding transcripts.** *X. Kong, H. Xu, P. Wang, Y. Fu, Y. Zheng, L. Hu.* Molec Gen, Inst Hlth Sci, Shanghai, China.

A single mammalian transcript normally encodes one protein. But the transcript of GNAS (G-protein alpha subunit)contains two reading frames and produces two structurally unrelated proteins, XLαs and ALEX. However, no other confirmed GNAS-like dual-coding transcript was reported up to now, even though several candidate genes were predicted by bioinformatics analysis. In this study, we constructed a series of vectors to test how two protein products could be translated with a single transcript in vitro. The length of ORF (open reading frame), position of first AUG and Kozak motif were found to be important factors. These factors as well as 55-bp NMD (nonsense-mediated mRNA decay) rule were used in our bioinformatics search for candidate dual-coding transcripts. 1307, 750 and 474 two-ORF containing transcripts were found in human, mouse and rat, respectively, of which 170, 89 and 70 were potential dual-coding according to our study. Most transcripts showed low conservation between those species. Additionally, the dual-coding transcripts were found to be significantly rich in transcripts of zinc finger protein family which are usually DNA-binding proteins involved in transcription process and regulation.

## 2483/T/Poster Board #1032

**Identification of human sphingomyelin synthase 1 (*SMS1*) gene splice variants.** *S.A. Limborska[1], A.V. Rozhkova[1], V.G. Dmitrieva[1], O.N. Zhapparova[2], E.S. Nadezhdina[2], L.V. Dergunova[1].* 1) Institute of Molecular Genetics RAS, Moscow, Russian Federation; 2) Institute of Protein Research RAS, Pushchino, Moscow Region, Russian Federation.

Recently sphingomyelin synthase 1 (SMS1) gene has been identified. The enzyme SMS1 occupies a central position in sphingolipid metabolism: it catalyses the conversion of ceramide and phosphatidylcholine to sphingomyelin and diacylglycerol (DAG). Since SMS1 was assumed to be involved in both cell death and survival by regulating pro-apoptic mediator ceramide and pro-survival mediator DAG it is of a great importance to investigate the regulation of SMS1 in the cell. To date we have found 12 new alternative transcripts of SMS1 gene using in silico and in vitro methods. Most of them differ from SMS1 mRNA in 5'-untranslated regions (5'-UTR). New-revealed 5'-UTRs are located in front of exon I and also between exons I and II, II and III, VI and VII. These exons were identified in SMS1 gene earlier. We also found alternative transcripts of this gene that include not-known before exons, situated between exons VII and VIII. Analysis with GENOMATIX software revealed several putative promoter regions adjacent to four new exons. Variations in levels of different transcripts between tissues were obtained by semi-quantitative RT-PCR. We presume that regulation of SMS1 gene functional activity is provided by using alternative promoters and synthesis of alternative mRNAs. It was shown that some of predicted open reading frames (ORFs) of new transcripts encode full-length SMS1 protein while others encode C- or N-terminal truncated forms of the protein. In particular circumstances the presence of different alternative transcripts of SMS1 gene may give rise to at least three different proteins. The cDNA containing ORF for C-truncated SMS1 isoform was cloned into the eukaryotic expression vector pEGFP-C2 and the resulting plasmid was used to transfect HeLa and Vero cells. It was demonstrated that C-truncated isoform localized to the Golgi complex like the full-length SMS1 isoform. We suggest that regulation of SMS1 gene function is provided at the level of transcription as well as at the level of translation.

## 2484/T/Poster Board #1033

**Recovery of Alu SINE *de novo* inserts in tissue culture.** *B. Wagstaff, A. Engel.* Tulane Cancer Ctr and Department of Epidemiology, Tulane Univ, New Orleans, LA.

A tissue culture assay for tagged Alu SINE retrotransposition has been in use for several years. However, recovery of *de novo* Alu integrants from this assay is extremely time consuming and data is very limited. A method for easy recover of L1 insertions has previously been developed. This approach relies on the introduction of a plasmid origin of replication that later is used in conjunction with a selectable marker to rescue circularized L1-containing genomic DNA in bacteria. However, the L1-construct could not be directly applied for the recovery of tagged Alu inserts. We developed an alternate construct that utilizes a similar approach to rescue inserts from a tagged Alu vector. This new Alu Rescue vector can be recovered from transfected HeLa cells following retrotransposition. We currently have recovered and analyzed multiple *de novo* integrants and report our findings here. Sequencing data revealed that recovered inserts faithfully mimic many aspects of Alu sequences present in the primate genomes, such as the presence of direct repeats and of 3' polyA tails. Alu insertions occurred randomly dispersed throughout the chromosomes. However several Alu inserts were localized in the A-tail of an existing Alu or L1 element. Overall this is a powerful technique with useful applications to analyze Alu insertion in cells with different genetic backgrounds.

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## 2485/F/Poster Board #1

**Further delination of a 46,XX Disorder of Sexual Development in a syndromic presentation.** *P. Hurtado[1], H. Pachajoa[2].* 1) Genetist, Universidad del Valle, Cali, Colombia; 2) PhD student, Universidad del Valle, Cali, Colombia.

Case report We described a patient with multiple malformations, born of non consanguineous parents. No inconvenient is register in her pregnancy. Born by C section at 38 weeks of gestation. Length: 45 cms Weight: 2130 grs. At physical examination at birth it was noticed: unilateral cleft lip and palate and bilateral microphthalmia. He stayed at the NICU for 5 days because of this. In further examination it was found a congenital heart disease, bilateral inguinal hernia and bilateral cryptorchidism. He has had a global retardation and has not achieved the goals for his age. At the actual physical examination we found: short stature, low weight microcephaly, anteverted narins, bilateral hernia, "clenched-like" hand, left clef lip and palate, bilateral microphthalmia. Hypoplasic scrotum with no testicles in it. Bilateral inguinal hernia. He is hypertonic in arms and legs and has a speech delay. Paraclinics karyotype report 46, XX. Total testosterone: less than 10.00 ng/dl (241-827 normal range for men). Dehidroepiandrostenidione: less than 0.044 ug/ml. 17-Hydroxiprogesterone: 7.01 ng/ml. Echocardiogram: permeable oval foramen. At the pelvic magnetic resonance: there is no uterus, ovaries neither vagina. None prostate or seminal glands. There are penis, corpora cavernosa, and small scrotum. In the inguinal canal there are images suggestive of testicles. SRY pending. Discussion 46,XX testicular disorder of sex development (46,XX testicular DSD) is characterized by the presence of a 46,XX karyotype; male external genitalia ranging from normal to ambiguous; two testicles; azoospermia; and absence of Müllerian structures. Approximately 20% of individuals with 46,XX testicular DSD present at birth with ambiguous genitalia. We reported a patient with a syndromic 46,XX DSD due by the microphtalmia, but also with the cleft lip and palate and congenital heart disease. We didn't found a similar report in the literature. We consider this case of special interest.

## 2486/F/Poster Board #2

**CLINICAL AND CYTOGENETIC EVALUATION OF TWO CASES WITH WILLIAMS SYNDROME.** *L. Bobadilla-Morales[1,2], R. Silva-Cruz[1], C.E. Monterrubio-Ledezma[1], H.J. Pimentel-Gutierrez[1], A. Corona-Rivera[1,2].* 1) Lab. Citogenet G y B, Inst de GH DrECR, Dpto. Biol Molecular Genomica, Univ Guadalajara, CUCS, Guadalajara, Guadalajara, Jalisco, Mexico; 2) Unidad de Citogenética, Servicio de Hematología Oncología Pediátrica, División de Pediatría, Nuevo Hospital Civil "Dr. Juan I. Menchaca", Guadalajara, Jalisco, México.

Williams syndrome (WS) is caused by a hemizygous submicroscopic deletion in 7q11.23 including the elastin gene. WS shows dysmorphic facies, cardiovascular disease, mental retardation, a characteristic cognitive profile and idiopathic hypercalcemia. Case 1. Male, 7 years old. Both parents were healthy. Healthy pregnancy. Weigh birth 3500g. He shows developmental and speech delay. At examitation, height 112cm, cefalic perimeter 50cm, mouth 5.5cm. Triangular facies, stellate iris pattern, strabismus, anteverted nares, flat nasal bridge, oligodontia, hoarse voice, hypoplastic nails, attention deficit disorder and extroverted personality. Normal serum calcium (9.8mg/dl), IgE 149 UI/ml, normal hematic and thyroid function. He presents stenosis in pulmonary valve. Clinical evaluation from the American Academy of Pediatrics (AAP) score was 11 points. Karyotype, 46,XY,9qh-,21s+. FISH study was positive to WS microdeletion (LSI WSR Dc 7q11.2 and 7q31). Case 2. Female, 2 years old from healthy parents. The mother presented gestational diabetes. Caesarean delivery, APGAR 7/8, weight 4150gr, 50cm height. Physical examination at 5 months old: weight 4680 (-3.9 DE), height 58cm (-2.8 DE), cephalic perimeter 38.5cm (-2.46 DE), triangular facies, stellate iris pattern, depressed nasal bridge and wide, café au lait spots. Laboratory: serum creatinine 0.6mg/dl. serum calcium 10mg/dl, T3 1.75ng/ml, T4 2.6mcg/dl, TSH 3.3mU/ml. Subaortic interventricular communication with moderate pulmonary hypertension and persistent ductus arteriosus was observed by echocardiography at 2 months old. Kidney showed loss of differentiation corticomedular, multicystic renal dysplasia, bilateral hypotrophy renal. Cardiovascular surgery was performed at one year old. AAP score was 7. Karyotype, 46,XX,9qh+. FISH study was negative to WS deletion. WS represent a diagnostic challenge. Evolution, detailed clinical signs such as cardiological findings and AAP score helps to the WS diagnosis. Clearly positive score of 11 obtained from patient 1 is compatible with FISH positive WS deletion result. However in patient 2, who showed a positive score of 7, the FISH result was negative. In this patient cardiological alterations do not represent the most frequent cardiopathy but renal abnormalities and most of the clinical findings support WS diagnosis and the heterogeneity postulated to this patients.

## 2487/F/Poster Board #3

**Confirmation of a Distinct Phenotype Associated with Interstitial Long Arm Deletion of Chromosome 16q22.2→q24.1.** *M. Golabi[1], B. Hall[2], P. Cotter[3], S. Chan[1].* 1) Pediatrics Genetics, California Pacific Medical Center, San Francisco, CA; 2) Division of Clinical/Biochemical Genetics and Dysmorphology, Department of Pediatrics, University of Kentucky, Lexington, KY; 3) Children's Hospital and Research Center at Oakland, Oakland, CA.

Deletions of 16q are rare with few cases reported. We report a new case of interstitial deletion of chromosome 16; involving 16q22.2→q24.1, describe the clinical findings of the proband, review literature with similar deletion, and document a distinct and recognizable phenotype associated with this deletion. The proband was a full term female infant with birth weight 1950 grams, length 44 cm, and head circumference 30 cm. She had intrauterine growth retardation. She had high, broad forehead with recessed hairline, wide split metopic and sagittal sutures with large fontanelles, flat occiput, hypertelorism with epicanthal and lower canthal folds, upslanted palpebral fissures, significant lipoatropy of infraorbital regions, broad nasal bridge, anteverted nostrils, simple long philtrum, small mouth, one natal tooth, asymmetric cry, and micrognathia. Ears were dysplastic, triangular shaped, low set, and posteriorly rotated. She had a short neck with redundant tissue, small chest, wide set hypoplastic nipples, and atrial septal defect. There was significant cutis aplasia around the umbilical ring and diastasis recti. The infant had long, tapered digits with small nails, adducted thumbs, and fingers passively flexed with no camptodactyly. Her toes were long and held in flexion. There was an abnormal hair pattern with frontal and parietal hypotrichosis and body hypertrichosis. Skin was thin and hands were wrinkled. Neurological exam was noted for jitteriness, truncal hypotonia and appendicular hypertonia. The brain MRI and ophthalmological evaluations were normal. High resolution karyotype revealed a de novo interstitial deletion of 16q with breakpoints at 16q22.2 and 16q24.1. The oligonucleotide array CGH analysis showed a single copy number loss of 13.4 Mb of the region 16q22.2→q24.1. Follow-up at one month revealed poor feeding and poor growth. Review of literature revealed 2 cases with overlapping deletions involving 16q22.1→q24.1 and 16q22.3→q24.2. These 2 cases had identical clinical features to the proband including high forehead, large anterior fontanelle, diastasis of cranial sutures, broad and flat nasal bridge, hypertelorism, short and up slanted palpebral fissures, high arched eyebrows, dysmorphic and low set ears, natal teeth, micrognathia, flexed fingers, and abnormal position of toes. We confirm that there is a distinct phenotype associated with monosomy of 16q22.2→q24.1. Lipoatrophy and cutis aplasia should be sought for in future cases.

## 2488/F/Poster Board #4

**Interstitial microdeletion of 4p16.3: Contribution of *WHSC1* haploinsufficiency to the pathogenesis of developmental delay in Wolf-Hirschhorn syndrome.** *K. Kosaki[1], K. Izumi[1,2], H. Okuno[1,3], K. Maeyama[3], S. Sato[3], T. Yamamoto[4], C. Torii[1], R. Kosaki[1,5], T. Takahashi[1].* 1) Department of Pediatrics, Division of Medical Genetics, Keio University, Tokyo, Japan; 2) Center for Human Genetics, University Hospitals Case Medical Center, Cleveland, OH; 3) Department of Pediatrics, Saitama City Hospital, Saitama, Japan; 4) International Research and Educational Institute for Integrated Medical Sciences (IREIIMS), Tokyo Women's Medical University, Tokyo, Japan; 5) Department of Clinical Genetics and Molecular Medicine, National Center for Child Health and Development, Tokyo, Japan.

The critical interval for Wolf-Hirshhorn syndrome pheotype has been assigned to a region called WHSCR2 at chromosome 4p16.3. The pathological significance of the haploinsufficiency of the *WHSC1* gene, which is at the centromeric end of WHSCR2, remains unclear. Recently, a patient with the classical facial features of WHS who exhibited only a mild developmental delay and deletion involving WHSCR2 but with an intact *WHSC1* gene was reported in the journal. We here report a patient with WHS who had a comparable but distinctive deletion that disrupted the *WHSC1* gene and a severer degree of developmental delay. The documentation of a much severer developmental delay in the present patient, compared with the patient with intact *WHSC1* alleles, does not prove but does support the notion that *WHSC1* contribute to the severe developmental delays typically observed in patients with WHS. The recently elucidated biological role of a transcript of the *WHSC1* gene (*RE-IIBP*) as a histone H3 lysine 27 methyltransferase is compatible with this notion in that the histone modification of H3-K27 is an essential process in neuronal cell lineage specification. Fine-mapping of the extents of the deletions in more Wolf-Hirschhorn syndrome cases would be helpful to determine whether the disruption/retention of the *WHSC1* locus is predictive of the developmental outcome in patients with Wolf-Hirschhorn syndrome.

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## 2489/F/Poster Board #5

**Postnatal diagnosis of trisomy 8 mosaicism after normal amniocentesis.** *S. Ramanathan[1], K. Kessler[2], P. Koduru[3], L. Mehta[4].* 1) Peds Genetics, Loma Linda Univ. Health Care, Loma Linda, CA; 2) Medical Genetics, Schneider Children's Hospital, Manhasset, NY; 3) Dept. Pathology, Schneider Children's Hospital, Manhasset, NY; 4) Dept. of Genetics and Genomic Sci, Mt Sinai SOM, New York, NY.

Constitutional trisomy 8 mosaicism (CT8M) is rare but known to be associated with a widely variable phenotype ranging from dysmorphic features and mental retardation to normal intelligence. Renal and cardiac defects are well documented. We report on two patients with normal amniocentesis karyotypes, diagnosed postnatally with CT8M. Patient 1 was evaluated at age 5 for global delays, hypotonia and prematurely gray hair. He had bushy eyebrows, high forehead and long, mildly upslanted palpebral fissures. Digits were unusual and tapered with prominent pads at the base, index finger clinodactyly and single transverse crease on the left hand. There was increased first-second toe gap on the left foot; right foot showed overlapping toes. Prior amniotic fluid chromosome analysis, done for maternal age, was normal. Blood chromosome analysis showed trisomy 8 in 4/30 cells. Patient 2 had abnormal prenatal ultrasound findings of bilateral hydronephrosis, ventricular septal defect (VSD), enlarged cisterna magna and questionable hemivertebrae. The pregnancy was complicated by maternal diabetes which was well controlled. Amniotic fluid chromosome analysis was normal. At birth, evaluation showed short upturned nose, full mouth, overfolded right ear and deep, single to bridged, palmar creases. Bilateral hydronephrosis and VSD were confirmed. X-rays of the spine were normal. Blood chromosome analysis showed trisomy 8 in 12/20 cells. Chromosome analysis on circumcised foreskin showed trisomy 8 in 9/20 cells. Both patients had deep plantar creases. Trisomy 8 mosaicism is caused by mitotic non-disjunction. Prenatal diagnosis is complicated by the higher distribution of abnormal cells in extra-embryonic mesoderm rather than in cytotrophoblasts and amniocytes. Therefore, fetal blood karyotyping may be necessary to evaluate for CT8M (van Haelst, 2001). Alternately array CGH may detect low-level mosaicism on amniocytes that may be missed by conventional cytogenetics (Wood, 2008). Our patients further illustrate the pitfalls of prenatal diagnosis. With regard to postnatal diagnosis, both our patients had minor dysmorphisms, but CT8 mosaicism was suspected by the deep plantar creases, which are highly suggestive of this diagnosis and prompted further evaluation even after a normal prenatal karyotype.

## 2490/F/Poster Board #6

**Survival of Down syndrome patients in Lithuania.** *A. Sinkus, L. Jurkeniene, I. Andriuskeviciute, L. Salomskiene, G. Sinkute.* Inst Biology, Lab Cytogenetics, Kaunas University of Medicine, Kaunas, Lithuania.

Every year the medical boards in Lithuania (with the population 3.4 million people)ascribe about 800 sixteen-year-olds to disablement groups, which makes 1.6% of the population. Half of these individuals, about 400 people, are disabled because of severe mental retardation. Each year in Lithuania 70 babies suffering from Down syndrome (DS) are born but at the age of 16 DS is diagnosed only for 12-16 individuals (1.8% of disabled patients, or 3.5% of mentally retarded patients). The specified reasons of death are usually non-informative in medical histories ("Down syndrome", "chromosomal disease"). In informative 100 certificates the following reasons of death were enumerated: bronchopneumony in 60 patients, acute respirative infection - in 4, staphylococcous infection -1, tuberculosis -2, congenital vitium cordis -20, other congenital malformations -4, leukemy -4, accidents -5. In our country we have registered 641 alive DS patients: 374 children under 16 years of age and 267 adults (two oldest patients were 48 years of age). We have made karyotype analysis in lymphocyte culture for 393 of them (61.3%). The chromosomal investigation has confirmed the diagnosis of DS in 372 (94,6%) patients. Normal karyotype was found in 19 (4.8%) patients.In two patients chromosome anomalies other than trisomy-21 were found. The karyotype in one 3-year-old boy was 49,XXXXY.The deeply mentally retarded boy had many malformations typical of DS patients. The other chromosome patient without trisomy-21 was 29-year-old mild mentally retarded woman in whose karyotype ring chromosome 9 was found. For both latter patients the physicians suspected DS. In two patients nearby trisomy-21 an additionally reciprocal translocations of other chromosomes were present. Therefore, in Lithuanian population false-positive diagnosis of Down syndrome was found in 5.3% of patients, i.e. markedly rarely as compared with analogical investigations in other countries. One of the reasons of such situation is the racial homogeneity of Lithuanian population presented almost exclusively by Caucasians. But in newborn Lithuanian babies the freguency of false-positive diagnoses arises up to 32.1%: among 134 patients with clinical diagnosis of DS the normal karyotype was found in 43. The main clinical feature - mental retardation - cannot be recognized in infants, and the neonatologists send the patients for karyotype analysys due to isolated microanomalies.

## 2491/F/Poster Board #7

**Proximal Deletion 1p36 syndrome identified by CGH array: A small (~2 MB) deletion extends the phenotype to include pupillary colobomas and preauricular pits.** *C. Bay, A. Rutherford, T. Bonilla.* Clinical Pediatric Genetics J415, Univ Kentucky, 740 S. Limestone Dr. Lexington, KY 40536.

Among our 4 patients with 1p36 deletions is a patient recently identified to have an apparently de novo 2 MB interstitial deletion of proximal 1p36, identified by CGH array (oligoV7.4 Baylor) with loss of 1p36.12p36.11. The clinical phenotype was not consistent with the classic 1p36 monosomy syndrome (Battaglia, 2008). This patient has features more consistent with the phenotype recently outlined by Kang et al (2007) and Rudnik-Schoneborn et al (2008), which is now called Proximal monosomy 1p36 syndrome. This patient, however, has a smaller, more proximal deletion than the few previously reported patients. Clinical features: 2 week old white male, born at term to a 20 year old G2P1 white female. Paternal age was 27 years. Pregnancy remarkable only for decreased fetal movements. No known teratogenic exposures. Birth length and weight both ~25-50 centile. Bilateral pupillary colobomas were appreciated immediately and were the indication for referral. PE at 2 weeks included: length 50th centile; weight 10th centile; OFC 50-75 centile. Features: hypertelorism, downslanting palpebral fissures, normally arched eyebrows, prominent nasal bone, unilateral preauricular pit, posteriorly angulated ears with overfolded superior helices, mild micrognathia with mildly pointed chin, increased skin of posterior neck, widely spaced nipples and mild 5th finger clinodactyly. Pertinent negatives: normal sized anterior fontanelle, normal arch to eyebrows, eyes not deep set; no brachy/camptodactyly. Echo: small VSD; Renal US: normal; CXR: normal; Parental FISH studies: normal. This patient extends the phenotype of Proximal interstitial 1p36 deletion syndrome to include pupillary colobomas and preauricular pits. He has a deletion which appears to be proximal to all previously reported cases known to us, and provides support for CGH array testing in neonates with colobomata and preauricular pits.

## 2492/F/Poster Board #8

**Cytogenetic and molecular study in patients with 22q11.2 deletion syndrome phenotype.** *S.I.N. Belangero[1], A.N.X. Pacanaro[1], D.M. Christofolini[1], F.T.S. Bellucco[1], L.D. Kulikowski[1], M.C. Cernach[2], C.M. Lourenço[3], C.A. Kim[4], A.M. Hacker[5], B.S. Emanuel[5,6], M.I. Melaragno[1].* 1) Genetics, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil; 2) Development Biology, Universidade Federal de São Paulo, São Paulo, Brazil; 3) Neurogenetics, Universidade de São Paulo, Ribeirão Preto, Brazil; 4) Children's Institute, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; 5) Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, USA; 6) Department of Pediatrics, The University of Pennsylvania School of Medicine, Philadelphia, USA.

The 22q11.2 deletion syndrome (22qDS) is characterized by facial dysmorphism, cleft palate and heart defect. Its main etiology is a hemizygous deletion in chromosome 22 which occurs in about 1:4000 livebirths, making it the most common microdeletion syndrome. Most deletions (80-90%) are de novo alterations and the vast majority of patients (~90%) have the same 3Mb deletion. Although smaller deletions occur, they do not necessarily result in milder symptoms, making genotype-phenotype correlations difficult. We present a molecular and cytogenetic study of 74 patients with 22qDS phenotype, using classical cytogenetics, FISH-BAC (fluorescent in situ hybridization with bacterial artificial chromosome probes) using a TBX1 region probe, and MLPA (multiplex ligation-dependent probe amplification). The karyotypes appeared to be normal, while FISH analysis showed 17 deletions. The MLPA study, performed in 51 patients, revealed 16 deletions, of which 14 had the 3Mb deletion and one a 1.5Mb deletion. One patient presented two non-continuous deletions, a proximal one of 1.5Mb, and a distal one of 1.7Mb, separated by about 1.5Mb. Other regions associated with the 22qDS phenotype, such as 10p, 4q, 8p, and 17p, were also studied by MLPA and showed normal results for all patients, reinforcing that the 22q11.2 region is the main locus causing this phenotype. Forty-nine patients were studied by both approaches, MLPA and FISH, and all results were concordant. The overall deletion frequency (26%) was lower than reported in the literature, probably due to less strict clinical diagnosis criteria in our sample. One of the 17 deletions was inherited from the mother, revealing a 94% de novo deletion rate, which is similar to previous reports. We determined the size in 16 deletions, of which 87.5% were typical 3Mb deletions and 1.3% atypical deletions, in line with the literature that indicates 87% and 2%, respectively, although the 1.5Mb deletion frequency (1.3%) was lower than in the literature (7%). This kind of genetic investigation is important for an appropriate genetic counseling to the patients and their families. Further 22qDS clinical studies, using different approaches, can help in the diagnosis and in the establishment of genotype-phenotype correlations. Moreover, a better understanding of the disease pathogenesis can improve the prognosis and treatment of the symptoms, for a better quality of life and a longer life span of patients.(Financial support:FAPESP;CNPq).

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## 2493/F/Poster Board #9

**Partial deletion of the BRAF gene is associated with a unique phenotype.** *W. Graf, s. Yu.* children's mercy hospital, kansas city, MO.

Background: The human BRAF gene contains 18 exons encoding a Braf protein with 766 amino acids. BRAF is a serine/threonine kinase involved in the RAS/RAF/MEK/ERK/MAPK pathway and the transduction of mitogenic signals from the cell membrane to the nucleus. BRAF is expressed in most tissues with high expression in neuronal tissue. Clinically, BRAF gene mutations cause a typical cardiofaciocutaneous (CFC) syndrome characterized by congenital heart defects, recognizable facial features, ectodermal abnormalities, and intellectual disability. Phenotype variants of CFC syndrome overlap with Noonan and Costello syndromes, which collectively include a heterogeneous spectrum of KRAS, BRAF, MAP2K1(MEK1) and MAP2K2(-MEK2)gene mutations. Methods: We screened the genome of a male infant with severe postnatal recurrent emesis, growth failure, mild non-specific facial dysmorphism, scoliosis, and severe diffuse developmental encephalopathy by microarray-based comparative genomic hybridization (aCGH). Results: We identified a novel 93 Kb genomic deletion of chromosome 7q34 (chr 7:140080282-140271033) involving the BRAF gene. The deleted region in our patient spans 3' part of first intron, exons 2-7, and 5' part of the seventh intron of BRAF gene. Clinically, the child shared some of the major features of CFC, Noonan, and Costello syndromes. However, the child in this report had sparse straight hair, normal cardiac function, and normal neuroimaging with postnatally acquired microcephaly. Conclusion: This is the first reported BRAF gene deletion. The partial BRAF deletion in this patient could represent a alternative genetic mechanism to point mutation leading to a unique phenotype with features overlapping CFC, Noonan, and Costello syndromes. This predicted truncated protein would not form heterodimers with its partner, RAF1, and would not bind to either upstream or downstream molecules as it does not contain any of the functional CR domains which are encoded by the missing exons. This suggests that the partially deleted BRAF gene may lead to the uunique clinical phenotype via loss-of-function of the BRAF gene. Other individuals with CFC syndrome without identified gene mutation by sequencing may have deletions of BRAF gene detectable by aCGH techniques.

## 2494/F/Poster Board #10

**The 22q13 Deletion Syndrome: An Under-Diagnosed Cause Of Severe Speech And Language Delay in Singaporean Children.** *A. Lai[1], E. Lim[2], M.H. Yong[3], L. Knight[3], E.C. Tan[2].* 1) Genetics Service, Dept of Ped Med, KK Women's and Children's Hospital; 2) KK Research Centre, KK Women's and Children's Hospital; 3) Cytogenetics Laboratory, KK Women's and Children's Hospital.

The 22q13 deletion syndrome is characterised by neonatal hypotonia, global developmental delay, delayed or absent speech, normal to accelerated growth and minor dysmorphism. Due to lack of clinical awareness, it is under-diagnosed and its prevalence unknown. The deletion can result from a simple chromosome deletion, an unbalanced translocation, or a ring chromosome. The critical region has been identified as the region containing the SHANK3 gene. Although the deletion can occasionally be detected by high resolution chromosome analysis, most cases require fluorescent in-situ hybridisation (FISH) analysis, multiplex ligation-dependent probe amplification (MLPA) or array comparative genomic hybridisation (aCGH) for diagnosis. We present two patients with this condition. Patient 1 is a 6-year-old girl who presented with the VACTERL association of congenital anomalies at birth, including cleft lip and palate, congenital heart defect, unilateral arm deformity and multicystic dysplastic kidneys. The 22q13 deletion was detected on FISH analysis that showed deletion of the ARSA (control) probe when performing a FISH analysis for the velocardiofacial syndrome (deletion 22q11.2). Patient 2 is a 5-year-old girl who presented at 2 years old with hypotonia and global developmental delay. The 22q13 deletion was detected by MLPA and confirmed by FISH analysis and aCGH. Both patients have mild dysmorphism, normal growth and marked speech delay. With increased awareness, we now consider this condition in the differential diagnosis of children with hypotonia, developmental delay, speech and language disability, autistic-like features, minor dysmorphism, and normal or accelerated growth. FISH analysis for confirmation of the diagnosis is available locally.

## 2495/F/Poster Board #11

**Silver-Russell Syndrome caused by a deletion encompassing *H19*.** *K. Gronskov[1], S.E. Boonen[1], J. Ek[1], B. Dolmer[2], K. Brondum-Nielsen[1].* 1) Kennedy Center, Glostrup, Denmark; 2) Pediatric dept., Naestved Hospital, Naestved, Denmark.

Silver-Russell syndrome (SRS) is characterized by pre- and postnatal growth retardation, facial dysmorphism and growth asymmetry. Recently, it has been discovered that approximately 35% of SRS patients show abnormalities in 11p15. Abnormalities at 11p15 can also cause the overgrowth syndrome Beckwith-Wiedemann syndrome (BWS) in 50-60% of cases. SRS and BWS thus seem to be opposite syndromes. 11p15 harbours two imprinting control regions, ICR1 and ICR2, which regulate the expression of 14 imprinted genes predominantly involved in growth. The H19DMR region in ICR1 contains 7 CTCF binding sites. This region is methylated on the paternal chromosome, which abolishes CTCF binding. This enables expression of the growth factor IGF2 and prevents expression of H19. From the maternal allele H19 is expressed whereas IGF2 is repressed. Several mechanisms can lead to BWS and SRS; BWS can thus be due to maternal microdeletion, paternal duplication or paternal uniparental disomy of 11p15, or isolated loss of methylation of KvDMR1 or isolated hypermethylation of H19. These defects lead to either loss of methylation of KvDMR1 or hypermethylation of H19 or both. Molecular mechanisms leading to SRS revealed so far are isolated loss of methylation of H19 and a single case of maternal duplication of 11p15 leading to loss of methylation of H19 and hypermethylation of LIT1. We present here a patient referred to us for SRS. Methylation sensitive MLPA analysis showed loss of methylation of H19DMR. Conventional MLPA analysis showed a deletion of H19DMR leaving CTCF binding sites 1-5 intact whereas the analysis showed deletion of CTCF binding site 7. It was not possible to determine whether CTCF binding site 6 was deleted. MLPA analysis using subtelomeric probes showed a normal result. Microsatellite analysis indicated that the deletion was paternally inherited. This is the first case to our knowledge of a deletion in 11p15 causing SRS.

## 2496/F/Poster Board #12

**New case of the recurrent 15q24 microdeletion presenting with hypotonia, tethered cord, and seizures.** *K. Herman[1], S.L. Davis[2], A.M. Hata[1].* 1) Genetics, UC Davis, Sacramento, CA; 2) Pediatric Neurology, Children's Hospital Oakland, Oakland, CA.

We present a 7-year-old Hispanic male with a history of hypotonia, developmental delay, seizures, and tethered cord. The patient is reported to have had hypotonia, muscle weakness, and poor balance since infancy. At age 5, he was noted to have increased urinary frequency and a decrease in balance and was found to have a tethered cord. In addition, this patient had onset of seizures at the age of 4 and is currently well controlled on medication. He is also noted to have sleep apnea and developmental delays. Based on these features, testing for a mitochondrial disorder was initiated at an outside institution and deletion studies were negative. Array CGH was also ordered and revealed a 15q24.1-q24.3 deletion. Parental studies have not yet been completed. Several reports in the literature have described this deletion and defined this region of chromosome 15q24 as a site of a recurrent microdeletion syndrome. Features reported include unusual facial features, developmental delay, microcephaly, digital abnormalities, genital abnormalities, and joint laxity. Our patient presented with tethered cord which has not previously been reported in individuals with this microdeletion. In addition, seizures have only been reported in one previous patient and hypotonia in our patient appears more significant that suggested in other cases. Finally, unusual facial features (high anterior hair line, broad medial eyebrows, hypertelorism, downslanted palpebral fissures, broad nasal base, long smooth philtrum and full lower lip) have been reported. This patient, however, presented with such mild facial dysmorphisms that a clinical diagnosis was not apparent. We suggest that this case further characterizes the phenotype of the recurrent 15q24 microdeletion syndrome.

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## 2497/F/Poster Board #13

**Detection of uniparental disomy using linkage mapping set markers.** *A. Felton, J. Lee, A. Chhibber, B. Johnson, C. Davidson, D. Rodriguez, A. Pradhan, R. Padilla, R. Fish, S. Berosik, S. Hung, R. Petraroli, L. Joe.* Life Technologies, Foster City, CA.

According to Mendel's Law, a child inherits one set of alleles from each parent. However, there are some exceptions, as a few imprinted genes are expressed from alleles inherited from only one parent, either maternal or paternal. Uniparental disomy (UPD) occurs when an individual inherits both copies of a chromosome pair from only one parent and no copies from the other parent. Children with UPD 15, deficiency in the expression of imprinted genes from both parents on Chromosome 15, are subject to two genetic disorders, Prader-Willi syndrome (PWS) and Angelman syndrome (AS). DNA from potential candidates for PWS or AS can be screened using PCR and capillary electrophoresis (CE). DNA from the both parents and the child are evaluated using microsatellites, or STRs (Short Tandem Repeats) with fluorescently labeled primers. We demonstrate new CE and software methods for the comparison of the set of three DNA samples. Such methods could help in providing the researcher with automated results, consistent across a wide range of laboratory environments.

## 2498/F/Poster Board #14

**Phenotypic Spectrum in Waardenburg Syndrome Types I and II: Associations of Clinical Manifestations.** *D. Lin, S. Lin, H. Lin, H. Lee, H. Huang, M. Shu, C. Hsu, C. Chuang.* Dept Pediatrics, Mackay Memorial Hosp, Taipei, Taipei, Taiwan.

Waardenburg syndrome (WS), an inherited disorder, is characterized by a pigmentation anomaly, hearing impairment and various defects of neural crest derived tissues. Based upon the diagnostic criteria, eighty-six individuals from forty four families affected with WS are classified into WS type-1 (WS-1), and type-2 (WS-2) in about equal incidence. There are no evidences of statistically difference between the two groups with respect to hearing loss, iris pigmentary disorder, white forelock, skin hypopigmentation and other associated anomalies. However, WS-2 individuals are significantly more likely to having pigmentation disorders compared to WS-1 individuals in taking account of iris, hair and skin pigmentary disturbances as a whole. Among the clinical traits, iris pigmentary disorder is the most predominant manifestation with nearly a half composed of hypoplastic blue irides. Hearing loss, affecting a half of the WS individuals, is significantly associated with iris pigmentary disorder. Furthermore, the severity of hearing loss is significantly correlated with the extent of forms of iris pigmentary disorder. These results may suggest same mechanism in controlling both severity of deafness and extent of iris pigmentary disorder. The close relationship of hearing loss with iris pigmentary disorder may warrant further studies on molecular basis, as well as embryogenesis regarding mechanism in controlling melanoblasts formation and differentiation.

## 2499/F/Poster Board #15

**Moyamoya Syndrome with Concomitant Renovascular Disease in a Young Girl with Neurofibromatosis Type 1 (NF1). Should Patients with NF1 and Renal Artery Stenosis be Screened for Moyamoya?** *A. McGregor, D. Jones, S. Einhaus, E. Pivnick.* Pediatrics, University of Tennesse Health Science Center, Memphis, TN.

A young girl presented to the emergency room with a tick bite and was found to have severe hypertension. She was started on amlodipine and referred to Nephrology, where she complained of dizziness that began after starting amlodipine. She was noted to have cafe au lait spots. Renovascular hypertension and Neurofibromatosis Type 1 (NF1) were suspected. Her renal ultrasound appeared normal, but her plasma renin level was elevated. She underwent a renal angiogram, which revealed moderate to severe right renal artery stenosis. An angioplasty was successfully performed; subsequently, no anti-hypertensive medications have been required. She was referred to Genetics. Prenatal, birth, and family history was unremarkable. The physical examination revealed many cafe au lait spots and axillary freckling, but no other physical stigmata of NF1. Due to complaints of headaches, a MRI of the brain was performed. This showed increased vascular flow-voids in the perimesencephalic and medial sylvian regions concerning for early changes of distal carotid occlusive disease. Due to concerns about moyamoya syndrome, she was referred to Neurology. During the visit, she reported episodes of hand numbness and tingling with dropping of objects. This was concerning for possible transient ischemic attacks. Neurology ordered a magnetic resonance angiogram (MRA), which was consistent with possible moyamoya. She was referred to a neurosurgeon who recommended a cerebral angiogram. It showed severe stenoses of portions of the right anterior cerebral and middle cerebral arteries, occlusion of portion of the left anterior cerebral artery, and a moyamoya pattern of dilated collateral perforating vessels. Surgical intervention is planned. Moyamoya and renal artery stenosis are well-described complications of NF1 but the prevalence of co-occurrence is not known. We suspect that the patient became dizzy on amlodipine due to decreased cerebral perfusion. This case raises the question of whether screening for moyamoya is justified in patients with NF1 and renal artery stenosis, particularly if they have been having headaches and/or sensory symptoms. If moyamoya is present, a sudden decrease in blood pressure may result in stroke. We propose that the prevalence of the co-existence of renal artery stenosis and moyamoya in NF1 warrants further investigation as it impacts treatment of hypertension.

## 2500/F/Poster Board #16

**POLYMORPHISM ILE587VAL IN THE EIF2B5 GENE AS SUSCEPTIBILITY FACTOR IN MULTIPLE SCLEROSIS.** *C. Ungaro[1], F.L. Conforti[1], T. Sprovieri[1], M. Muglia[1], L. Citrigno[1], A.L. Gabriele[1], A. Gambardella[1,2], R. Mazzei[1].* 1) ISN-CNR, Mangone (CS), Cosenza, Italy; 2) Institute of Neurology, University "Magna Graecia", Catanzaro, Italy.

Multiple Sclerosis (MS) is a demyelinating disease of the Central Nervous System (CNS) due to genetic and environmental factors. It is a chronic progressive pathology characterized by a gradual destruction of myelin and neuron axons in the brain and spinal cord. The risk among the general population to develop MS is about 1/800. MS primarily affects adults, with an age of onset typically between 20 and 40 years, and is more common in women than in men (3:2, respectively). Mutations in EIF2B1-5 genes, encoding for the five subunits of eIF2B, cause the Vanishing White Matter Disease (VWMD) also known as Childhood Ataxia with Central Nervous System Hypomyelination (CACH) syndrome, an autosomal recessive leukoencephalopaty. The onset is usually between ages 2-6 with a rapid progression towards death during the early childhood; however, an adult onset form was been reported with slow progression and mild symptoms, mostly linked to mutations in EIF2B5 gene. Genetic and biochemical data of MS patients, just described, and MRI data showing VWM images similar to MS lesions, have encouraged the present study in which we performed the EIF2B5 gene mutational screening in 130 Italian MS patients and in 215 healthy subjects. The polymorphism Ile587Val in exon 13 resulted very common in the MS patients ($\chi 2$=14.439; p=0.0007). Therefore, the current data suggest that polymorphism Ile587Val in exon 13 of the EIF2B5 gene should be considered as susceptibility factor in the development of MS.

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## 2501/F/Poster Board #17

**Polysplenia and cardiovascular defect heterotaxy in a tunisian girl born after a five years history of infertility related to non-syndromic asthenozoospermia.** *N. Abdelmoula, I. Trabelsi, R. Louati, S. Kammoun, T. Rebai.* Lab Histology, Univ Medicine, Sfax, Tunisia.

Heterotaxy is a clinically and genetically heterogeneous disorder characterized by disturbed body symmetry and malposition of internal organs. It is a developmental condition caused by the disturbance of the left/right axis, in the early embryonic period. Such development of left-right asymmetry requires ciliary motion at the embryonic node. Heterotaxy comprises a broad spectrum of abnormalities and typical manifestations include abnormal symmetry and malposition of the thoraco-abdominal organs and vessels, complex congenital heart disease and extracardiac defects involving midline-associated structures. Here, we describe a Tunisian female newborn with typical findings of heterotaxy syndrome. Cardiovascular defects include dextrocardia and abnormal pulmonary and systemic venous return like abnormal pulmonary venous connection into the coronary sinus, and interruption of the inferior vena cava, with azygos vein continua. Extracardiac defects involve polysplenia and a midline liver. There is no history of consanguineous marriage but record of male infertility for the newborn's father, related to non-syndromic asthenozoospermia. Ciliary structural abnormalities have been recently linked to heterotaxy with identification of mutations in 2 ciliary genes, DNAI1 and DNAH5. Screening for these two genes mutations will be made for our patient and results will be presented and discussed.

## 2502/F/Poster Board #18

**Hemochromatosis: a multigenic and multifactorial disease.** *G. Le Gac, C. Férec.* Inserm U613, EFS-Bretagne, Centre Hospitalier Universitaire; Brest, France.

With the recognition of five causative genes (HFE, HJV, HAMP, TFR2 and SLC40A1) and the presentation of the historic HFE p.C282Y/p.C282Y genotype as a necessary but not sufficient condition to explain an iron overload, position of hemochromatosis (HC) in the complex category of multigenic and multifactorial disorders is now indisputable. Different grades of severity can be attributed to the genetic heterogeneity of the disease, in particular when considering the respective roles of HC proteins in iron homeostasis. These grades range from the HJV and HAMP-related juvenile conditions to the typical adult-onset HFE-related condition, which is usually diagnosed in the fourth decade for men and the fifth for women. The iron overload course in TFR2-related patients is distinct from that of the juvenile conditions, but it can be more rapid and impressive than usually observed in HFE-related patients. Position of the SLC40A1 gene is more ambiguous. Indeed, depending of their impact on the ferroportin iron exporter (loss-of-function vs gain-of-function), mutations of this gene are responsible of different clinical syndromes (ferroportin disease vs HC type 4). Although definition of the p.C282Y/p.C282Y genotype penetrance is still a matter of debates, the expressivity of this predominant genotype clearly depends on a balance between accentuating and reducing factors. The influence of gender, age and lifestyle components, such as alcohol abuse, has been well documented. Involvement of modifier genes or epigenetic mechanisms is less obvious. It is clearly supported by several studies in Hfe knockout mice, but few data are currently available in humans. Overall, pointing that the ferroportin disease refers to a different iron overload syndrome, the large phenotypic heterogeneity observed in hemochromatosis can be viewed as a continuum. Based on certain of our works, and of a review of the literature, we aim to underline the elementary elements that must be considered in a diagnosis process for hemochromatosis. We also aim to justify the use of in vitro and in vivo assays for the characterization of rare mutants and the establishment of new structure/function relationships. Finally, we propose to discuss about the search for the p.C282Y mutation, supporting the idea that, in future, this test could be viewed as not sufficient for a genetic counseling and the organization of a familial screening.

## 2503/F/Poster Board #19

**Genetic and clinical profile of patients registered in the Brazilian Database on Orofacial Clefts.** *I.L. Monlleo[1], T.M. Felix[2], A. Fett-Conte[3], G.F. Leal[4], E.M. Ribeiro[5], J. Souza[6], F.M.P. Vicente[7], P.A. Mossey[8], V.L. Gil-da-Silva-Lopes[7].* 1) Department of Paediatrics, State University of Alagoas, Maceio, AL, Brazil; 2) Servico de Genetica Medica, HCPA, Porto Alegre, RS, BRAZIL; 3) Servico de Genetica da Faculdade de Medicina de Sao Jose do Rio Preto, SP, BRAZIL; 4) Centro de Atencao aos Defeitos da Face, Recife, PE, BRAZIL; 5) Servico de Genetica do HGCC, Fortaleza, CE, BRAZIL; 6) Centro de Atendimento Integral ao Fissurado labiopalatal, Curitiba, PR, BRAZIL; 7) Servico de Genetica Medica do Hospital de Clinicas, Unicamp, Campinas, SP, BRAZIL; 8) University of Dundee, Dental Hospital & School, Dundee, SCOTLAND, UK.

Introduction: Databases are useful tools to gather information not only for prevalence monitoring, but also for investigation of causes, prevention, evaluation of outcomes, and provision of expertise, education and training. On this basis, our team designed the Brazilian database on orofacial clefts (BDOC). The strategy was to start as a nation-wide, prospective, hospital-based, and multi-disciplinary database. Aim: To profile clinical and genetic aspects of persons with orofacial clefts recorded in the first phase of the BDOC. Methods: Seven clinical geneticists collected information using a structured case record form. Eligibility criteria were (a) patients with isolated clefts (CL, CLP and CP), (b) Pierre Robin sequence, (c) X-linked cleft, (d) multiple malformed subjects including orofacial clefts and (e) syndromes with orofacial clefts. Results: Data from 126 patients were recorded from November 2008 to May 2009. The ratio of male to female was 1.93:1. Age ranged from 30 days to 17 years. Clinical presentation was: 71 CLP (47 unilateral and 24 bilateral), 31 CP, and 23 CL (18 unilateral and 5 bilateral). Additional CFA was described in 34 patients whereas other birth defects in 21. Recurrence of clefts in relatives was verified in 33 cases. Conclusion: Clinical and familial characteristics seem to be similar to other populations described. Participation of clinical geneticists possibly improved accuracy of clinical and familial data recorded. The BDOC has the potential to become a clinical network suitable for studies on phenotype-genotype correlation, prevention and quality of healthcare. Support: CAPES, CNPq, Fapeal, Fapesp.

## 2504/F/Poster Board #20

**Clinical variability associated with the chromosome 1q21.1 microdeletion: an additional case with previously unreported skeletal abnormalities.** *N. Dolzhanskaya[1], S. Reddy[2], N. Zellers[1], M. Velinov[1].* 1) Institute for Basic Research in Dev. Disabilities, Staten Island, NY; 2) Richmond University Medical Center, Staten Island, NY.

A 3 months old male infant was evaluated because of large anterior fontanelle, poor feeding and bilateral clavicular pseudoarthrosis. He was born at 36 weeks gestation. The birth weight was 6 lbs 8 oz. The pregnancy was complicated with polyhydramnios. Radiology exam showed bilateral clavicular pseudoarthrosis and 11 ribs. His mother had polycystic ovary syndrome. The child had relative microcephaly with very wide front fontanelle. He had dysmorphic facial appearance with hypertelorism, scarce eyebrows, micrognatia and high arched palate. His nipples were hypoplastic. Array CGH analysis was done using a commercial BAC based array and revealed a microdeletion at the chromosomal band 1q21.1. 244K oligonucleotide array analysis was done and the deleted region was further characterized to span about 2.7 Mb in the region chr1: 144,122,609-146,800,272 (NCBI build 36) and to include at least 40 coding genes. 25 patients with similar sub-microscopic deletion were recently reported. Our patient's deletion includes the 1.35 Mb minimal deleted region noted in the previously reported patients (chr1: 145,000,000-146,350,000 ,NCBI build 36). 1q21.1 microdeletion in the previously reported patients was variably associated with mental retardation, heart defects, joint laxity or cataracts. There was a significant proportion of asymptomatic patients. No skeletal abnormalities as in our patient were reported. However, no radiology evaluations were mentioned in the previously reported patients. Since the skeletal abnormalities in our patient may be asymptomatic later in life, they could be missed on clinical evaluations. Our report further underscores the great clinical variability of the phenotype associated with 1q21.1 microdeletion and may provide skeletal markers for prenatal diagnosis or suspicion for this recently described and apparently common chromosomal abnormality.

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## 2505/F/Poster Board #21

**Impact of environmental factors during pregnancy on minor physical variants in nonsyndromic cleft lip with or without cleft palate (CL/P) families and controls.** *C. Brandon[1], K. Neiswanger[1], R.S. DeSensi[1], M.E. Cooper[1], A.E Czeizel[2], J.T. Hecht[3], M.L. Marazita[1].* 1) Dept Craniofacial & Dental Gen, Univ Pittsburgh, Pittsburgh, PA; 2) F Comm Ctl Hered Dis, Budapest, Hungary; 3) Univ Texas-Houston Med School, Houston, TX.

Exposure to environmental factors during the first trimester of pregnancy impacts the development of the fetus and is associated with birth defects, such as cleft lip and palate. Minor physical variants (MPVs) are congenital anomalies which may have a genetic basis, and also may result from environmental exposures during pregnancy. MPVs are morphological features of the body that are generally clinically insignificant. However, an increased number of MPVs may indicate a more serious condition. The goal of this study was to examine whether environmental exposures during the first trimester of pregnancy result in an increased number of MPVs in offspring. Additionally, we explored whether families with a history of a genetic condition (CL/P) and exposure to environmental insults during pregnancy have an increased number of MPVs compared to controls. Fifty-nine MPVs were assessed in 110 children from 64 mothers. Mothers were asked about their habits of smoking, alcohol consumption, and prenatal vitamin use during the first trimester of each pregnancy. Women who consumed alcohol during the first trimester had a significant increase of MPVs in their children ($p=0.02$). Smoking, as well as alcohol and smoking combined, were not significantly associated with increased number of MPVs in offspring. Vitamin intake was not related to a significant decrease in number of MPVs. The sample was further divided in to 3 groups: CL/P cases (n=56), non-cleft family members (n=40) and controls (n=14). The mean counts of MPVs in the 3 groups were not significantly different. Exposure to alcohol, smoking and vitamin use did not show significant impact on the number of MPVs between CL/P cases, non-cleft family members and controls. However, a non-significant trend was observed showing that non-cleft family members with an exposure to alcohol or smoking had the greatest number of MPVs; whereas controls with no exposures had the lowest number of MPVs. Our results support that exposure to alcohol during pregnancy may increase the number of MPVs in offspring. Our results suggest that increased MPVs are seen in non-cleft family members when also exposed to alcohol and smoking, even with a small sample size. Supported by NIH Grants R01-DE016148, P50-DE016215.

## 2506/F/Poster Board #22

**Developing a clinical genetics diagnostic approach to cleft lip and/or palate.** *E. Goh[1], R. Klatt[1], C. Forrest[2], D. Fisher[2], S. Bowdin[1].* 1) Division of Clinical & Metabolic Genetics, Hospital for Sick Children, Toronto, ON, Canada; 2) Division of Plastic Surgery, Hospital for Sick Children, Toronto, ON, Canada.

Cleft lip, with and without cleft palate, has an incidence of approximately 1 in 700 births worldwide, with a higher incidence depending on the ethnic population. Cleft palate alone has an incidence of approximately 1 in 2000 births. Multiple publications have been produced looking at the optimal way to address the various medical, social, emotional and counseling issues surrounding clefting. Many papers stress the importance of a multidisciplinary approach including the interaction of surgeons, speech pathologists, otolaryngologists, audiologists, geneticists, genetic counselors, and allied health professionals. From the perspective of clinical genetics, the main goal is to assess for other congenital abnormalities, as well as to determine an etiology if possible, in order to counsel the families about recurrence risk. However, with over 500 genetic syndromes associated with clefting of the lip, palate or uvula, not to mention the environmental exposures and chromosomal abnormalities that have been reported, the search for an etiology may be challenging. To this end, we have developed an algorithmic approach to cleft lip and/or palate with a focus on both syndromic and non-syndromic genetic causes. We have also developed clinic material to standardize the questions and examination in order to increase the efficiency and accuracy of genetic diagnosis in the clinic environment. We present a pilot study of 50 index patients seen over an intensive one month period at the Hospital for Sick Children using this algorithm to streamline the clinical experience. Our results support the literature indicating the majority of cases are non-syndromic and isolated, however the range of syndromic diagnoses made emphasizes the utility of genetic evaluation and genetic counseling in seemingly isolated cases. The use of a systemic algorithm as an approach to cleft lip and/or palate has beneficial effects in the clinical environment as well as potentially as an educational aid for patients, trainees and educators alike.

## 2507/F/Poster Board #23

***Fgfrl1* null mice have reduced expression of *Tpm3* and other sarcomere genes in the diaphragm.** *N. LopezJimenez[1], S. Gerber[2], B. Trueb[2], A. Slavotinek[1].* 1) Pediatrics, Univ California, San Francisco, San Francisco, CA; 2) Dept. of Clinical Research, University of Bern, Switzerland.

*Fgfrl1* (also known as *Fgfr5*) homozygous null (-/-) mice have thin, amuscular diaphragms and die at birth because of an inability to inflate their lungs. The myofibers of the diaphragm are normal, suggesting that reduced muscle cell migration or proliferation in the diaphragm causes the diaphragm hypoplasia. Fgfrl1 forms constitutive homodimers and is thought to promote cell adhesion mediated by heparan sulphate. Fgfrl1 also binds Fgf2, a potent stimulator of muscle cell proliferation. We hypothesized that patients with diaphragm defects (DD) would have sequence variants in *FGFRL1* that reduced gene expression and altered diaphragm thickness. We sequenced the coding exons and exon-intron boundaries of *FGFRL1* in 100 patients with DD, confirming six known, coding SNPs: c.209G>A (p.P20P), c.977G>A (p.P276P), c.1040C>T (p.D297D), c.1120C>A (p.P362Q), c.1306G>T (p.R424L), and c.1426C>T (p.P464L). We found no novel sequence alterations. The allele frequency for the 6 coding SNPs did not differ significantly between patients with DD and patients with craniosynostosis or normal controls ($p>0.05$). However, one patient with a DD and a 4p16.3 deletion including *FGFRL1* was hemizygous for the minor allele for 3 of the 6 SNPs on the remaining 4p16.3 allele, raising the possibility that the DD was related to the 4p16.3 deletion, with the minor alleles of the SNPs further reducing *FGFRL1* expression. We then hypothesized that genes involved in muscle formation would be differentially expressed between *Fgfrl1* wildtype mice and -/- mice. We used RNA from the diaphragms of *Fgfrl1* wildtype and -/- mice and Affymetrix Genechip Mouse Gene 1.0 ST arrays, and found 8 genes with significantly reduced expression levels ($p<0.05$) in -/- mice compared to wildtype mice - *Tpm3*, *Fgfrl1* (as expected; $p = 0.004$), *Myl2*, *Lrtm1*, *Myh4*, *Myl3*, *Myh7* and *Hephl1*. Interestingly, *Tpm3* is mutated in patients with nemaline myopathy, in which diaphragm hypoplasia is recognized. *Myl2*, *Myh7* and *Myl3* encode sarcomeric proteins and are mutated in hypertrophic and dilated cardiomyopathy. We consider that the diaphragm hypoplasia is likely to be caused by reduced Fgfrl1 levels, either associated with decreased heparan sulphate and Fgf2 binding with reduced muscle cell proliferation, or associated with reduced myoblast adhesion and diaphragm thinning. Alternatively, the decreased levels of the muscle-related genes seen in our expression arrays could reflect the diaphragm hypoplasia seen in the -/- mice.

## 2508/F/Poster Board #24

**Minor physical variants in nonsyndromic cleft lip with or without cleft palate (CL/P) families and controls.** *K. Neiswanger[1], C.A. Brandon[1], R.S. DeSensi[1], K. T.Cuenco[1], A.E. Czeizel[2], J.T. Hecht[3], M.L. Marazita[1].* 1) Dept Oral Biol, Univ Pittsburgh Sch Dent Med, Pittsburgh, PA; 2) F Comm Ctl Hered Dis, Budapest, Hungary; 3) Univ Texas-Houston Med School, Houston,TX.

Minor physical variants (MPVs) are physical traits such as strabismus or single palmar creases that occur as part of the normal variation in a population. However, they may also be increased in individuals with clinically significant birth defects or in disorders like schizophrenia. Furthermore, individuals with multiple MPVs are at increased risk for a major anomaly. The Pittsburgh Oral Facial Cleft study assesses MPVs in multiplex CL/P families and controls, to determine if the overall rate of MPVs is increased in cleft families, compared to controls. We analyzed two sets of MPVs: 1) MPVs that occurred with a frequency of less than 20% of our sample (N= 59 MPVs), and 2) MPVs occurring in less than 5% of our sample (N=32 MPVs). The sample included 279 individuals (126 male, 153 female) from Pittsburgh, PA, Budapest, Hungary, and Houston, TX. More than 98% of the sample were Caucasians; <2% were African-Americans or biracial. There were 82 individuals with nonsyndromic CL/P, 150 non-cleft family members, and 47 controls with no family history of clefting. When MPVs that occurred at a frequency of <20% were considered, the entire sample had an average of 3.48 MPVs (CL/P cases: 3.37; non-cleft relatives: 3.65; controls: 3.15; NS). For the less common set of MPVs that occurred at a frequency under 5%, the average number of MPVs in the sample was 0.85 (CL/P cases: 0.80; non-cleft relatives: 0.95; controls: 0.62, NS). The number of subjects without any MPVs did not differ significantly between CL/P families and controls [for MPVs < 20%: CL/P cases 10/82 (12%), non-cleft relatives 22/150 (15%), controls: 6/47 (13%); for MPVs < 5%: CL/P cases 43/82 (52%), non-cleft relatives 79/150 (53%), controls: 29/47 (62%)]. When sub-groups of MPVs were examined using two-tailed t tests, there were no significant differences in the mean number of hand/arm, foot/leg, or limb MPVs between CL/P subjects, family controls, and pure controls for either the common or the rarer set of MPVs. There was a trend toward more head MPVs in family controls than in unrelated controls (MPVs <20%: $P = 0.10$; MPVs <5%: $P=0.08$), although this was not maintained for the MPVs <5% when the data were log transformed due to non-normality in the data. In general, we did not find an increase in MPVs in either individuals with CL/P or their non-cleft relatives. Larger samples are needed to verify these results. NIH grants R01-DE016148, P50-DE016215.

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## 2509/F/Poster Board #25

**Stormorken syndrome: Confirmatory report in a French family.** *G. Morin[1], A. Hazard[1], B. Roméo[2], B. Demeer[1], A.G. Le Moing[1], J.C. Capiod[3], E. Bourges-Petit[4], H. Sevestre[5].* 1) Clinical Genetics, Amiens University Hospital, France; 2) Pediatric Pneumology, Amiens University Hospital, France; 3) Hematology, Amiens University Hospital, France; 4) Pediatric Cardiology, Amiens University Hospital, France; 5) Pathology, Amiens University Hospital, France.

In 1985, Helge Stormorken reported a new syndrome associating thrombocytopathia, muscle fatigue, asplenia, miosis, migraine, dyslexia and ichthyosis. This affection segregated in a Norvegian family with an apparent autosomal dominant transmission on 4 generations. The physical characteristics were documented in a teen-aged boy and his mother: small stature, deep set eyes, high and arched forehead, permanent miosis without efficiency of mydriatic drugs and decreased darkness vision. A bleeding tendency was demonstrated, including hematomas, frequent nose bleedings but no major hemorrhagic accident. Hematologic investigations exhibited consistently prolonged bleeding time, normal coagulation, presence of giant platelets and various abnormal forms of red cells with Howell-Jolly bodies in the peripheral blood. The spleen was absent in both patients explaining the post-splenectomy aspect of the red cells. The muscular defect consisted in limitation during physical exercise and an abnormally increased duration of muscle response. Ichthyosis was mainly located in the extremities. Both patients suffered from headaches with photophobia. Dyslexia was early diagnosed for the affected son, responsible of learning disability, but less severe for his mother. From that time, this disease was only reported in a Japanese mother and daughter, who both presented muscle weakness, moderate increasing of creatine kinase rates and thrombocytopenia. Muscle biopsy showed fibre necrosis and regeneration, variation in fibre size, and tubular aggregates in approximately 5% of the fibres. We report the observation of a young boy and his father. At 17 days of life the baby presented an urticaria-like eruption on the face and the lower limbs. Blood count revealed thrombopenia (48000/mm3) but the myelogram was normal. During the hospitalization, he presented an episode of supraventricular tachycardia requiring a treatment with acebutolol and amiodarone. The father had small stature (157cm), high pitched voice, chronic eruption of the arms and shoulders partially regressive after anti-mycosic treatment, and a severe but reactive miosis responsible of impairment of darkness vision. He also presented asplenia and moderate thrombocytopenia (97000/mm3). At the age of 2 years and half, a less severe miosis became more apparent in the son. Peripheral blood examination revealed anisocytosis, the presence of macrothrombocytes, Howell-Jolly bodies and prolonged bleeding time.

## 2510/F/Poster Board #26

**Genitopatellar syndrome: A further delineation or a new association?** *L.E. Becerra[1,2], R. Valenciano[3], D. Morales[2], L. Chacón[2], E. Monroy[2], M. Díaz[1], J.J. Toscano[1], J.E. García[1], M.L. Ramirez[1].* 1) Division de Genética, CIBO-IMSS, Guadalajara, Jalisco, Mexico; 2) Centro de Rehabilitación Infantil Teletón (CRIT), Chihuahua, Chihuahua, Mexico; 3) Escuela de Medicina, CUCS-U de G, Guadalajara, Jalisco, Mexico.

Introduction. Genitopatellar syndrome (OMIM 606170) is clinically featured by the association of absent/hypoplastic patellae, genital anomalies, mental retardation, agenesis of the corpus callosum, flexion deformities of the knees and hips, club feet, and hypogonadism. Clinical report. A male, 6 years-old was sent to the Clinical Genetics due to MCA. He was product of the second pregnancy (G2, P2) of healthy non-consanguineous parents. At birth: weight 2400 g, height 44 cm, and Apgar score 9-10. The following malformations were recorded: umbilical hernia, ventricular septal defect, right cryptorchidism, hypospadias and poor swallowing. Psychomotor development was notoriously delayed. Physical examination: OFC 38.2 cm, height 116 cm, weight 21 kg. Microcephaly, turricephaly, prominent metopic, bitemporal narrowing, round face, down-slanting palpebral fissures, blepharophimosis, telecanthus, low nasal bridge, bulbous tip of the nose, submucous cleft palate, prominent upper lip, prognathism, posteriorly rotated and easily foldable ears, left preauricular pit, bilateral retroauricular notches, short neck with redundant skin back, slim thorax, holosystolic murmur of low intensity, increasing inter-nipple distance, left microorchidism, limitation to extension in upper limbs, bilateral digitalized thumbs, hyperconvexed nails, lower limbs with small and dislocated patella, popliteal folds and limitation to extension, and bilateral club foot. Karyotype was normal (46,XY). X-ray, showed in thorax a fusion costal on 6-7th, and bilateral tibia vara. MRI demonstrated pachygiria, and hypoplasia of corpus callosum. Discussion. Among differential diagnosis the following syndromes were discharged: Goldblatt syndrome (OMIM 241760), which has not coarse face, limbs contractures, and neurological migration alterations. Fryns-Aftimos syndrome (OMIM 606155) was ruled out due to the absence of polydactyly, notoriously affected nose and webbed defects on limbs and neck. Multiple pterigium syndrome (OMIM 265000) was no considered because of the lacking of cranio-neurological affectation, and distinctive expressionless face. Bibliography. Online Mendelian Inheritance in Man, OMIM. Goldblatt J; Wallis C; Zieff S. A syndrome of hypoplastic patellae, mental retardation, skeletal and genitourinary anomalies with normal chromosomes. Dysmorph. Clin. Genet 1988: 2:91-93.

## 2511/F/Poster Board #27

**MELAS syndrome: Mexican Case Report.** *R. E'Vega[1, 2], L. Sandoval[3], A. Franco[4], C. Galván[4], M. González[5], M. Islas[6], A. Rincon[7], N. Dávalos[2,7, 8, 9].* 1) Instituto de Genética Humana, UdeG, Guadalajara, Mexico; 2) Doctorado en Genetica Humana, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Mexico; 3) Genetica, Centro de Investigacion Biomedica de Occidente, Guadalajara, Mexico; 4) Carrera de Medicina, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Mexico; 5) Neuropediatria Hospital Regional "Dr. Valentin Gomez Farias" ISSSTE, Guadalajara, México; 6) Departamento de Fisiología, UdeG, Guadalajara, Mexico; 7) Instituto de Enfermedades Crónico Degenerativas, UdeG, Guadalajara, Mexico; 8) Instituto de Genetica Humana, Departamento de Biologia Molecular y Genomica CUCS UdeG; 9) Unidad de Medicina Genomica y Genetica, Hospital Regional "Dr. Valentin Gomez Farias" ISSSTE, Guadalajara, México.

**INTRODUCTION** MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) syndrome, described by Pavlakis in 1984, is a genetically heterogeneous mitochondrial disorder. It can be caused by mutation in several genes: MTTL1, MTTQ, MTTH, MTTK, MTTS1, MTND1, MTND5, MTND6 and MTTS2 It can be accompanied by central nervous system disorders such as seizures, hemiparesis, hemianopsia, cortical blindness and episodic vomiting. Phenotype is variable. Presentation occurs with the first stroke-like episode, usually at aged 4-15 years. **CASE REPORT** 15 year-old Mexican male, born in Tepic, Nayarit from non consanguineous parents. Onset at age 8 with grand mal seizures accompanied by continuous vomiting, without sphincter relaxation. Phenitoine prescription until now, presenting approximately 4 seizures each month despite the treatment. Physical exploration at age 13 reveals CP 51 cm (P 10), height 137 cm (< 3 P), weight 34kg (P 10), SS 146 cm, IS 67 cm, brachycephaly, normal auricular pavelions, wide nasal base, long philtrum, asymmetric thorax, apparent left hypoplasia, general muscular hypoplasia, lumbar hyperthricosis, and genu valgum. The rest of the physical exploration is normal. Radiography shows bilateral degeneration of femoral heads. Electromyography does not show evidence of polyneuropathy, however the diagnosis of a myopathy is not left aside due to the presence of increased polyfacetic potentials and diminished insertion potentials. Laboratorial studies reveal lactic acidosis. **DISSCUSION** Differential diagnosis with other mitochondrial encephalopyopathies such as Leigh syndrome, Kearns- Sayre syndrome, MERF syndrome and Leber optic atrophy should be considered.

## 2512/F/Poster Board #28

**DNA Resequencing and Variant Identification Using a Non-syndromic X-linked Mental Retardation (MRX) Panel.** *C. Davidson[1], F. Bartel[2], R. Santhanam[1], E. Nordman[1], B. Johnson[1], R. Padilla[1], R. Fish[1], L. Joe[1], S-C. Hung[1], A. Pradhan[1], A. Felton[1], M. Friez[2].* 1) Applied Biosystems, 850 Lincoln Centre Dr, Foster City, CA 94404; 2) Diagnostic Laboratories, Greenwood Genetic Center, One Gregor Mendel Circle, Greenwood, SC, 29646.

The prevalence of X-linked mental retardation (XLMR) is estimated to afflict approximately 1/1000 males. To date, there have been approximately 90 X-linked genes implicated in causing XLMR with the majority of these genes being associated with syndromal MR (MRXS). A smaller set of genes on the X chromosome have been associated with nonsyndromal MR (MRX) where the only discernible feature is mental retardation/intellectual disability. Significant overlap between these two sets of genes indicates that syndromal and nonsyndromal can both be caused by alterations in many of the XLMR genes. From a clinical perspective, proper diagnosis of males with nonsyndromal XLMR is more straightforward when a positive family history exists. Unfortunately, many undiagnosed males with MRX exist, and the absence of an X-linked pedigree makes the identification of their underlying etiology much more difficult. Indicative of this difficulty is that MRX accounts for roughly 2/3 of the total number of XLMR cases. To test for MRX, the Molecular Diagnostic Laboratory at the Greenwood Genetic Center has designed a resequencing panel consisting of 95 amplicons encoding the exons and intron junctions of nine X-linked genes (*ACSL4*, *ARX*, *FTSJ1*, *GDI1*, *IL1RAPL1*, *JARID1C*, *OPHN1*, *PQBP1*, and *ZNF41*) based on their precedent for being the most common MRX genes in the literature at the time of selection. To demonstrate the advantages of a new capillary electrophoresis (CE) instrument, nineteen blinded probands suspected of having MRX were directly sequenced using the Greenwood MRX resequencing panel. Variant detection in the nine genes across the nineteen samples was performed and no truncating mutations were identified. The gene with the most frequent non-synonymous SNPs was *ZNF41*; two non-recurrent (I125R and D397E) missense variants and one recurrent (D315E) missense variant were identified in *ZNF41*. A non-recurrent missense variant (R1546Q) was identified in *JARID1C* and a recurrent missense variant (V39I) in *OPHN1*. Bioinformatic analysis predicted all identified non-synonymous SNPs to be benign changes with minimal impact on respective protein's function. Further, a single non-recurrent in-frame indel was identified in the gene *ARX* (A111del)-.The MRX Resequencing Panel coupled to the new Fast Resequencing Workflow highlights the advantages of using CE for DNA resequencing and variant identification across a large number of samples and genes.

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## 2513/F/Poster Board #29

**Novel FLNB mutation in a child with complex congenital heart disease and skeletal features of atelosteogenesis, type III.** *G.A. Bellus[1], B. Kahn[2], R.S. Lachnan[3], D. Krakow[4], S. Gulley[1].* 1) Genetic & Metabolic Services, Dep. Pediatrics University of Colorado, Aurora, CO; 2) Fetal Maternal Medicine, Dept. Obstetrics & Gynecology University of Colorado, Aurora CO; 3) Ahmanson Dept Pediatrics, Cedars-Sinai Research Instittute, Dept Radiology, David Geffen Sch Medicine, UCLA, Los Angeles, CA; 4) Ahmanson Dept Pediatrics, Cedars-Sinai Research Instittute, Depts of Orthopedic Surgery, Huamn Genetics Obstetrics & Gynecology, David Geffen School of Medicine, UCLA, Los Angeles, CA.

Mutations in FLNB are known to cause at least five clinically distinct disorders: autosomal dominant Larsen syndrome (LS), Atelosteogenesis, type I (AOI), Atelosteogenesis, type III (AOIII), Boomerang dysplasia (BD) and Spondylocarpotarsal synostosis syndrome (SCTSS). LS, AOI, AOIII and BD have overlapping skeletal features, vary in clinical severity ranging from LS (least severe) to AOI and BD (most severe) and are caused by FLNB missense mutations that occur in highly conserved regions of the FLNB protein. The majority of FLNB mutations that cause AOI, AOIII and BD are localized to exons 2 and 3 that comprise the CH2 domain of the N-terminal actin binding region. Mutations causing LS occur predominantly within the filamin B beta sheet repeats 14 and 15 (exons 28 and 29). However, this association is not exclusive and there is overlap between phenotypes and regional localization of FLNB mutations. Congenital heart disease is not considered to be a feature of AOI, AOIII or BD but some cardiac features (mitral valve prolapse, aortic dilation, and atrial and ventricular septal defects) have been reported in LS patients. We present a case of a new-born male who presented with cleft palate, facial dysmorphism, complex congenital heart disease (pulmonary atresia with hypoplastic R heart physiology) and skeletal findings consistent with AOIII. Sequencing of FLNB revealed a novel mutation (4912G>T / Val1638Phe) within exon 29. FLNB Val1638 is highly conserved among species and this mutation is highly likely to be pathologic. This patient represents the first report of complex congenital heart disease in a patient with an AOIII phenotype and suggests that FLNB beta sheet repeat domains play some role in cardiac embryogenesis.

## 2514/F/Poster Board #30

**Frontometaphyseal dysplasia: severe phenotype in two sisters.** *I. Furquim[1], M. Passos-Bueno[2], S. Robertson[3], C. Kim[1], D. Bertola[1].* 1) Unidade de Genética, Instituto da Crianca, Sao Paulo, Brazil; 2) Instituto de Biociências, USP, São Paulo, Brazil; 3) Departament of Paediatrics and Child Health, Dunedin School of Medicine, Dunedin, New Zealand.

Frontometaphyseal dysplasia (FMD) belongs to a group of overlapping dysplasias called the otopalatodigital syndrome (OPD) spectrum disorders, that comprises besides FMD, otopalatodigital (OPD) syndromes type 1 and 2, and Melnick-Needles syndrome (MNS). The skeletal dysplasia observed in these conditions consists of varying degrees of undertubulation of the long bones, cortical irregularities and campomelia. Extra skeletal malformations observed are CNS abnormalities, cleft palate, cardiac defects, omphalocele, mixed deafness and obstructive uropathy. The common molecular basis is mutations in FLNA , the gene encoding filamin A. Mutations reported to date are missense or small in-frame deletions, that are exert a gain-of-function effect. Most pedigrees are consistent with X-linked inheritance. In males, severity ranges from mild to more severe presentations and females exhibit variable expressivity. Here we report on two sisters affected with FMD presenting with prominent supraorbital ridges, hypodontia, mild joint contractures of the fingers and normal intelligence. Radiological studies showed undermodeled long bones and pronounced supraorbital hyperostosis. The father had cutaneous syndactyly of the third and the fourth fingers of the left hand and the second and the third toes of his left foot. X-ray revealed mildly undermodeled long bones. A daughter from a previous relationship had similar facial appearance. The molecular analysis of the FLNA gene in both affected sisters disclosed a novel heterozygous c.6611C>T mutation which leads to p.P2204L. Nevertheless, this gene alteration was not found in blood leukocytes from the father. The residue is conserved in all three filamins, which suggests that this mutation is responsible for the phenotype observed in the sisters. Contrary to what is frequently observed in FMD, in which females are usually less severely affected than related affected males, in the family here reported the father had a very mild phenotype. This presentation is consistent with mosaicism for the causative mutation in the father, which has been described in the overlapping OPD and Melnick-Needles syndromes. Examination of other tissues, besides blood, in the father will be performed to confirm this hypothesis.

## 2515/F/Poster Board #31

**Geleophysic dysplasia is a clinically and genetically heterogenous disease.** *P. Piccolo[1], J. Hicks[2], V. Fano[3], S.A. Caino[3], M.G. Obregon[3], M. García-Barcina[4], J. Tolmie[5], S.K. Law[6], L. Potocki[1], R. Sutton[1], B. Lee[1,7], C. Bacino[1], N. Brunetti-Pierri[1].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; 2) Department of Pathology, Baylor College of Medicine, Houston, TX, USA; 3) Hospital J. P. Garrahan, Buenos Aires, Argentina; 4) Unidad de Genética, Hospital de Basurto Avda, Bilbao, Spain; 5) Institute of Medical Genetics, Yorkhill Hospital, Glasgow, UK; 6) Jules Stein Eye Institute, University of California, LA; 7) Howard Hughes Medical Institute, Houston, TX, USA.

Geleophysic dysplasia (MIM 231050) is a rare autosomal recessive disorder characterized by short-limb dwarfism, brachydactyly, and a 'happy-looking' facial appearance. It is frequently associated with cardiac valvular disease although the incidence and natural history of the heart complications remain unclear. Mutations in the *ADAMTSL2* gene resulting in a dysregulation of TGF-β signaling have been recently recognized as responsible for this condition. We have screened for *ADAMTSL2* mutations ten cases of geleophysic dysplasia diagnosed on the basis of clinical and radiological findings. Pathogenic mutations were found in only two cases suggesting that additional and yet unknown gene(s) are involved in the pathogenesis of the disease. Short stature and brachydactyly were present in most of the cases while laryngeal stenosis, cardiac disease, and Perthes disease were variably present. Taken together, these findings show a significant clinical variability and are consistent with a broad disease spectrum. In 3 of 4 cases in which skin fibroblasts were available, we detected by electron microscopy cytoplasmic lysosome-like inclusions which have been previously reported as a characteristic finding of the disease. In summary, the clinical and molecular findings in our patients show that geleophysic dysplasia is a clinically and genetically heterogenous condition.

## 2516/F/Poster Board #32

**Absence of choroidal neurofibromas, a suggestive sign for multiple café-au-lait spots unrelated to NF1?** *K. Fukai[1], T. Oshimo[1], Y. Imai[2], K. Shiraki[3], M. Ishii[1].* 1) Dept Dermatology, Osaka City Univ, Osaka, Japan; 2) Dept Orthopaedic Surgery, Osaka City Univ. Osaka, Japan; 3) Dept Ophthalmology, Osaka City Univ. Osaka, Japan.

The presence of multiple café-au-lait spots is most often associated with neurofibromatosis type 1 (NF1), but can be also part of the manifestation of McCune Albright syndrome, ring chromosome syndrome and Watson syndrome. However, some of the patients with multiple café-au-lait spots lack any of the associated anomalies of the above and thus are called familial multiple café-au-lait spots (MCAL). The etiology of MCAL is still unknown, but three linkage analyses of MCAL excluded the locus of NF1. Here, we describe a patient with multiple café-au-lait spots without choroidal abnormalities. A 28-year-old woman with café-au-lait spots was referred for evaluation as NF1. She had more than six large café-au-lait macules on her chest, abdomen, back, and extremities. Small pigmented freckles were also present on the trunk and the extremities, sparing on the axillaes. No skin tumors were found. X-ray and MRI examination showed no abnormalities of the skull, the spine, and long bones of the extremities, the spinal code, and the central nervous system. Ophthalmological examination revealed no Lisch nodules, and no choroidal abnormalities. She had no family history of pigmentary disorders or neurofibromatosis. The patient is an intelligent and attractive lady, and has no associated signs for systemic disorders. We concluded that the spots are highly probably not related to NF1. The frequency of choroidal neurofibromas in NF1 was once thought to be rare. However, the development of infrared light examination with a scanning laser ophthalmoscope allowed ophthalmologists to examine choroid non-invasively, and Yasunari et al studied 17 cases with NF1 and reported that the frequency was as high as 100%. Furthermore, Nakakura et al. examined 14 NF1 patients and found that the choroidal abnormalities were detected in all of the 14 case, 28 eyes. Inversely, choroidal abnormalities suggestive for neurofibromas were not observed in non-NF1 patients so far. Considering the extremely high specificity and sensitivity of the choroid neurofibroms for NF1, choroidal neurofibroma may be included in the future diagnostic criteria for NF1. The test is non-invasive and rapid, and thus the patients with multiple café-au-lait spots should be tested for possible choroidal neurofibromas.

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## 2517/F/Poster Board #33

**NOTCH3 GENE MUTATIONS IN TWINS WITH CADASIL.** *R. Mazzei[1], C. Ungaro[1], F.L. Conforti[1], D. Guidetti[2], A. Magariello[1], A. Patitucci[1], G. Di Palma[1], A. Gambardella[1,3].* 1) ISN-CNR, C.da Burga, Mangone (CS), Cosenza, Italy; 2) UOC di Neurologia, Ospedale Guglielmo da Saliceto, Piacenza, Italy; 3) Institute of Neurology, University "Magna Graecia", Catanzaro, Italy.

Background: CADASIL is an autosomal dominant disorder leading to cognitive decline and dementia. Mutations in the NOTCH3 gene, encoding a transmembrane protein involved in cellular signalling and cell differentiation, are responsible. These highly stereotyped mutations are located within the 22 exons, encoding for the 34 Epidermal Growth Factor (EGF)-like repeats of the extracellular domain of the Notch3 receptor, all mutations resulting either in a gain or loss of a cysteine residue. Therefore it has been suggested that the unpaired cysteine residues, generated by these mutations may cause aberrant interaction of the Notch3 receptor with its ligand. Objective: In the present study we examined the NOTCH3 gene exons in two couples of twins from two different families with clinical and radiological findings consistent with CADASIL. Patients and method: After clinical examination the patients underwent MRI investigation, and then they were analyzed for mutations in the NOTCH3 gene using the DHPLC analysis and direct sequence. Conclusion and discussion: In the first couple of dizygotic twins, DHPLC analysis revealed a variant profile in exon 11. Sequencing of the exon 11 showed a nucleotide change that lead an amino acid substitution at position 578 (R578C), resulting in a gain of a cysteine residue. In the second couple of monozygotic twins a variant DHPLC profile was found in the exon 10. Subsequently, the sequencing analysis showed a nucleotide change leading a gain of a cysteine residue in position 528 (G528C). Despite carrying the same mutations, the two couples of twins showed a variable clinical phenotype. The remarkable discrepancies in the clinical manifestation of the disease, also noticed in twins, suggests a strong influence of other inheritability and environmental factors.

## 2518/F/Poster Board #34

**SOS1 gene mutation in a patient with a Costello-like phenotype.** *D. Bertola[1], A. Brasil[1,3], A. Jorge[2], A. Malaquias[2], L. Wanderley[3], L. Albano[1], I. Furquim[1], A. Pereira[3].* 1) Pediatrics, Instituto da Crianca, Sao Paulo, Brazil; 2) Endocrinology, HCFMUSP, Sao Paulo, Brazil; 3) Molecular Biology Laboratory, InCor, HCFMUSP, São Paulo, Brazil.

Costello syndrome (CS) is a developmental syndrome of RAS/MAPK pathway desregulation, encompassing failure to thrive, dysmorphic craniofacial features, cardiac and neurological abnormalities and a predisposition to papillomata and malignant tumors. It has been suggested that CS should be a diagnostic label that applies only to those patients with a HRAS gene mutation, which is responsible for the great majority (<80%) of the cases. Nevertheless, a Costello-like phenotype was described in patients presenting KRAS gene mutations. So far, mutations in SOS1 gene are responsible for Noonan syndrome (NS) and, possibly, for some cases of cardiofaciocutaneous syndrome. In the former, the patients commonly show ocular ptosis, curly hair and hyperkeratotic skin and lower frequency of mental retardation, when compared to the ones with PTPN11 mutation. We report on a 24 year-old patient with a clinical diagnosis of CS. He presented with severe developmental delay/mental retardation, behavioral problems, failure to thrive, relative macrocephaly, ventriculomegaly, coarse facial features, short neck, pectus exacavatum, congenital heart disease, cryptorchidism, repaired inguinal hernia, darker skin compared to other family members, keratosis pilaris and markedly loose skin, with deep palmar and plantar creases and important hyperextensibility. Molecular studies of the RAS/MAPK pathway genes disclosed a c.806T-C (p.M269T) in the SOS1 gene. Analysis of the HRAS and KRAS genes were normal. This SOS1 gene mutation was previously described in two typical NS patients. Contrary to what is currently recognized that SOS1 gene is involved primarily in typical NS patients, our report emphasizes that some atypical findings, such as, striking loose skin and an unusual cardiac involvement, could be part of the spectrum of SOS1 gene mutations. Moreover, although is common sense that HRAS is the main gene in CS, in the absence of mutations in this particular gene, KRAS gene and, now, SOS1 gene should be analyzed in these patients. The underlining genetic mechanism has implications in the follow-up of these individuals, once those presenting HRAS gene mutations has a higher risk of tumor development. Supported by FAPESP grants - 08/50184-2.

## 2519/F/Poster Board #35

**Severe facial deformity in an adolescent girl with a novel variant in POLH gene causing Xeroderma Pigmentosum.** *A. RAAMS[2], N. LEBOEUF[1], H. SHUHAIBER, HANS H.[3], E. GUZMAN[3], M. GARZON[1], N. JASPERS[2], K. ANYANE-YEBOA[3].* 1) DEPARTMENT OF DERMATOLOGY, COLUMBIA UNIVERSITY MEDICAL CENTRE, NEW YORK, NY; 2) DEPARTMENT OF GENETICS ERASMUS MEDICAL CENTRE,ROTTERDAM, NETHERLANDS; 3) DEPARTMENT OF PEDIATRICS, COLUMBIA UNIVERSITY MEDICAL CENTRE, NEW YORK,NY.

Xeroderma pigmentosum (XP) is an autosomal recessive disease characterized by sun sensitivity, early onset of freckling and subsequent neoplastic changes on sun-exposed skin. We describe an 18 -year -old El Salvadorian female with a reported history of XP complaining of progressive severe facial pain and difficulty swallowing. She reported a history of progressive vision loss with complete blindness for one year. She lost her ability to eat over the days because of progressive destruction of her maxilla and mandible.The patient was diagnosed clinically with XP at the age of seven, when she developed a skin cancer on the right cheek which was treated with an unknown non-surgical method. The patient's family history was remarkable for an affected 12 -year -old brother, two cousins alive with symptoms of disease and one who died at the age of 13 with presumed disease. Her entire central face was destroyed by tumor, with displacement of the right globe and consumption of the left. CT scan confirmed the presence of a massive deformity of the facial anatomy, encompassing the ocular structures with associated osseous and sinus destruction. Genetic studies revealed a normal nucleotide excision repair capability and moderate cellular UV-sensitivity compatible with a diagnosis of XP variant. In the POLH (polymerase eta) gene and cDNA, we found an insertion in exon 6 at codon 225: a C is changed into TT causing a frameshift. Apparent homozygosity in both cDNA and genomic sequences and absence of heterozygote polymorphisms in introns 4-7 are compatible with parental consanguinity.The POLH gene is located on chromosome 6p21.1- 6p12 spanning 40 kb of DNA; it comprises 11exons covering the entire coding sequence .The corresponding mRNA, 2432 bp in size, has an open reading frame of 2139 bp. Since the discovery of POLH, a considerable number of mutations of this gene have been described to be associated with the XPV ( Xeroderma Pigmentosum phenotype). Our case underscores the severity of symptoms associated with this newly designated variant XP21NY causing a frameshift mutation at codon 225. Further studies are currently underway to genetically test other family members being affected. More studies are needed to correlate this genotypic variant with the severity of thE phenotypic presentation, affecting several family members at the same time.

## 2520/F/Poster Board #36

**Correlation of a novel FBN1 mutation (c.5225 -2 A>C) with isolated ectopia lentis in a Chinese patient.** *J. Du, S.F. Li, L.Y. Li, G.X. Lu.* Reprod & Stem Cell Eng, Central South University, Changsha, HN., China.

Mutations in FBN1 gene causes Marfan syndrome (MFS;OMIM 154700) as well as other fibrillinopathies such as isolated ectopia lentis(EL; OMIM 129600), MASS syndrome(OMIM 604308), Weill- Marchesani syndrome(OMIM 608328) and Shprintzen-Goldberg syndrome(OMIM 182212). In this study, we aimed to analyze the correlation between FBN1 mutation and isolated EL in a Chinese patient with isolated EL. The 10-year-old boy suffered from myopia since he was 5. His naked visual acuity in the left and right eye were 0.2 and 0.1, respectively, and best corrected visual acuity,0.5 and 0.2, respectively. The slit lamp examination showed that his left lens dislocated to nasal side and right lens to upper nasal side. The ultrasonographical diagnosis showed that the axial length of the left eye was 22.66mm, and that of the right eye was 22.59mm with bilateral vitreous and retina appearing normal. The ratio of the patient's arm span to height was 147/146(<1.05). The patient was not found other skeletal features or cardiovascular disorders. His parents were nonconsanguineous and in good health. Five microliter peripheral blood was collected in EDTA from the patient and his parents, and the genomic DNA was extracted from the leukocytes by the standard method. The 65 exons and flanking intronic sequence of FBN1 gene were amplified by PCR. Mutation screening of the PCR products was performed using denaturing high-performance liquid chromatography (dHPLC) and those PCR products with heteroduplex chromatogram were followed by further DNA sequencing. The dHPLC result showed a heteroduplex chromatogram of the 42th exon PCR product in the patient but not in his parents. The subsequent sequencing analysis showed that the patient carried a heterozygous mutation, c.5225 -2 A>C in the splice 3' acceptor site of the 41 th intron in FBN1 gene, while his parents didn't have this mutation. As we know, the mutation c.5225 -2 A>C has not been reported in the previous studies. The alteration of AG to CG in the splice site between the 41 th intron and the 42th exon would lead to frameshift mutation and form a truncated fibrillin-1 protein, which causes abnormal suspensory ligament development and imbalant tensile strength, and finally results in displacement of lens. In summery, our data indicated that the novel mutation, c.5225 -2 A>C in FBN1 gene might be an etiological cause of isolated EL. The study also provided a basis of genetic counseling and the further prenatal diagnosis.

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## 2521/F/Poster Board #37

**Characterization of disease phenotypes in a multi-generational family segregating a MECP2 A140V mutation.** *J. Gorski, R.M. Troxell, J.L. Kussmann.* Div. Medical Genetics, Dept Child Health, Univ Missouri School of Medicne, Columbia, MO.

Mutations in the gene encoding methyl-CpG-binding protein 2 (MECP2) result in Rett syndrome, a severe neurodevelopmental disorder predominantly affecting females. In males, MECP2 mutations cause a variety of disease phenotypes including severe neonatal encephalopathy, severe hypotonia and mental retardation. MECP2 A140V mutations are somewhat unique in not causing Rett syndrome in females but causing neurodevelopmental disease in hemizygous males. Several reports have documented the association of A140V MECP2 mutations in males with neurodevelopmental abnormalities; however, the reported number of affected individuals is small and the phenotype remains poorly defined. Here, we report a four generation family that segregates the A140V MECP2 mutation in six affected males and four female carriers. Each affected individual carries a MECP2 C>T transition at nucleotide 419 resulting in an A140V missense mutation in the MECP2 methylated DNA-binding domain. Affected males ranged from 3 to 43 years of age. Younger affected males display hypotonia, seizures, tremors, developmental delays, and aggressive and mild autistic-like behavior. Older males display mental retardation, a progressive parkinson-like disorder, spastic quadriparesis, and macro-orchidism. Carrier females were not mentally retarded. However, they did display a variety of neurological and psychiatric problems including depression, obsessive-compulsive disorder, attention-deficit hyperactivity disorder, learning difficulties, and memory loss. To our knowledge, this is the largest kindred to be described segregating the MECP2 A140V mutation. We believe that these cases confirm the previous observation that MECP2 A140V mutations adversely affect hemizygous males, and expand our understanding of the disease phenotype in both hemizygous males and heterozygous females.

## 2522/F/Poster Board #38

**Recurrent erythema nodosum in a patient carrying a mutation in the Mediterranean fever gene (MEFV).** *M. Michelson-Kerman[1,2], C. Vinkler[1,2], I. Nezer[1], D. Lev[1,2].* 1) Inst Med Genetics, Wolfson Medical Ctr, Holon, Israel; 2) Maccabi Health Sevice, Wolfson Medical Ctr, Holon, Israel.

Erythema nodosum (EN)is a painful disorder of the subcutaneous fat, and it is the common type of panniculitis. The disorder is characterized by tender, nodular lesions located on the anterior aspects of the lower extremities. The process is considered to be an immunologic response and may serve as a marker for systemic diseases such as inflammatory bowel disease, tuberculosis, sarcoidosis, bacterial or deep fungal infection and cancer. Certain antibiotics, oral contraceptives and pregnancy may also be associated. The condition is usually self-limiting and specific treatment is seldom needed in uncomplicated cases. We present an unusual recurrent course of erythema nodosum in a 35 year old woman who is a carrier of a mutation in the MEFV gene. A 35 year old, generally healthy, non-pregnant, woman from Sephardic Jewish ancestry, had suddenly developed moderately painful palpable nodules on the anterior aspects of the lower extremities. Diagnostic evaluation included normal blood count, erythrocyte sedimentation rate and normal liver functions. C-reactive protein, antistreptolysin O-titer, blood cultures and tuberculosis test were negative. Chest radiography was normal. The symptoms exacerbated despite treatment with nonsteroidal anti-inflammatory drugs. Clinical improvement appeared after two courses of corticosteroid therapy,which is unusual for common EN course. Mutational analysis of the MEFV gene revealed a heterozygous E148Q mutation. Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurrent attacks of fever with serosal inflammation. The MEFV gene encodes the protein pyrin that plays an important role in modulating the innate immune response. MEFV mutations have been identified primarily in patients from Mediterranean populations and in Israel the carrier state is as high as 1 in 5. Although the clinical spectrum and genetic alteration in FMF is variable,erythema nodosum has never been described as a presenting sign of FMF especially in patients with a heterozygous mutation. Heterozygous mutations in the MEFV gene have been described in association with certain diseases such as Henoch-Schonlein purpura, rheumatoid arthritis and other autoinflammatory disorders and are are considered to be modifying factors. We suggest that MEFV, and particularly the E148Q mutation, is an important susceptibility factor and an independent modifier for the clinical manifestation of erythema nodosum in our case.

## 2523/F/Poster Board #39

**Lipomatosis familial benign cervical (BCFL). A familial case report.** *EJ. Ramirez[1,2,3], SE. Totsuka-Sutto[1,3], TA. García-Cobían[1], L. García-Benavides[1], D. Román-Rojas[4], EG. Cardona-Muñoz[1].* 1) Unidad de Investigación Cardiovascular, CUCS, Departamento de Fisiología, Univ Guadalajara; 2) Dpto de Genética, Instituto Jalisciense de Cirugía Reconstructiva, Secretaria de Salud Jalisco; 3) Instituto de Genética "Dr. Enrique Corona Rivera", CUCS; 4) Doctorado en Farmacología, CUCS, Univ. de Guadalajara. Gudalajara, Jalisco, México.

Lipomatosis familial benign cervical (BCFL) (151800 OMIM) is a rare disease characterized by the presence of multiple, symmetric, non encapsulated lipomas in the face, neck, shoulders, supraclavicular and deltoid region, abdomen, groin, and buttocks. There are multiple synonyms for this disorder such as Lipodystrophy cephalothoracic, Multiple symmetrical lipomatosis, Madelung's disease and Launois-Bensaude syndrome because they described this nosologic entity in 1888 and 1889. It seems more frequent in countries around the Mediterranean Sea. Is usually described in adults from 30 to 60 years old, with 1 in 25,0000 incidence and a male-to-female ration of 15-30:1. Most cases have no hereditary pattern, familial occurrence has been reported and autosomal dominant mode of inheritance has been postulated. The ethiology remains unknown but more of 90% have associated alcoholism and they have features of metabolic syndromes such as diabetes mellitus, hyperlipidemia and hyperuricemia. We describe a 48-years old male, who was referred to plastic surgery by multiple symmetric lipomas in face and neck. At physical examination showed normal weight and height, neck collar of fat, lipomatosis on face and shoulders, muscle cramps and extensor plantar reflexes. Eight relatives were found with variable affectation. Our patient and his relatives presented a typical fatty tissue accumulation. The present case show a family with BCFL supported on the clinical data (lipomas in the face, neck and shoulders) and nuclear magnetic resonance in the patient and different grade of severity of affectation in all eight family members. Male-to-male transmission wasn't observed but the autosomal dominant inheritance cannot be discarded.

## 2524/F/Poster Board #40

**A Recurring Mutation G12S in HRAS causes Variable Ocular Phenotype in Costello Syndrome.** *S. Shankar, K. Rauen.* Medical Genetics, Dept of Pediatrics, Univ California San Francisco, San Francisco, CA.

Costello syndrome is a rare developmental disorder characterized by failure to thrive, neurocognitive delay, distinctive facial features, cardiac involvement, musculoskeletal and cutaneous features. These patients have about 15% lifetime risk for developing malignant tumors. Costello syndrome is caused by missense mutations in the gene *HRAS*, with the Gly12Ser substitution accounting for >80% of all individuals with a known mutation. *HRAS* belongs to the Ras oncogene family encoding products of the Ras/MAPK signal transduction pathway. Dysregulation of the Ras/MAPK pathway results in a number of syndromes (referred to as RASopathies) with significant phenotypic overlap. A number of ocular features have been described in RASopathies. We sought to analyze the ocular features in a cohort of 35 mutation positive Costello individuals, 32 harboring the G12S mutation, two with the G12A mutation and one with the G13C mutation. We analyzed available medical information and parental reports for these patients. The age group of the patients ranged from 2 to 37 years. Ocular involvement was reported in 33/34 (97%) patients; 1 patient had not yet had an eye exam and another had no reported eye issues. Significant ocular features included strabismus 16/34 (48%); refractive errors 19/34 (55%) with myopia (14/19) occurring significantly more than hyperopia (2/19), type of refractive error unknown (3/19); nystagmus 12/34 (35%) and optic nerve anomalies in 4/34 (0.12%). One individual with G12A reported nystagmus and the second individual with G12A had nystagmus, myopia and esotropia. The individual with G13C reported nystagmus, and optic atrophy. Although, the remainder of the 31 patients had identical *HRAS* mutation G12S, the ocular features were variable. The high occurrence of myopia, nystagmus and strabismus suggests that *HRAS* plays an important role in human eye development and in ocular alignment. Variation in the phenotype caused by the same HRAS mutation, G12S suggest the role of other modifying factors such as genetic or environmental. Routine ophthalmic evaluation is recommended to improve the visual outcome by early interventions. In addition, a number of investigations on therapeutics targeting the Ras/MAPK pathway are underway. Further characterization of the ocular manifestations in a larger number of mutation positive Costello patients is important to clearly define the ocular phenotype, and to develop potential treatment strategies.

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## 2525/F/Poster Board #41

**Branchio-Oto-Renal syndrome: a review of the phenotypic spectrum based on 18 patients.** *J. Hogue[1], L. Hudgins[2], E. Chen[1], L. Bird[3], A. Slavotinek[1].* 1) University of California San Francisco; 2) Stanford University; 3) University of California San Diego.

Branchio-Oto-Renal syndrome (BOR; OMIM 113650) is a condition characterized by branchial clefts, hearing loss, and renal anomalies. *EYA1* mutations are the most common etiology. The *EYA1* product is part of the Six-Eya-Dach network where a DNA-binding protein binds Eya1 enabling transcriptional activation. Consistent with this, mutations in *SIX1* and *SIX5* have also been found in BOR patients. Given *EYA1*'s function in a network, we hypothesized that manifestations of BOR may be broader than previously reported and that there may be a genotype-phenotype correlation.

We reviewed 18 patients with BOR. Hearing impairment was the most common manifestation (14/18, 78%). Branchial arch anomalies, preauricular pits, and outer ear anomalies were each seen in 72% of the patients. Renal anomalies were present in 50%. Other anomalies were facial asymmetry (4/18, 22%), ear tags (3/18, 17%), inner ear anomalies (3/18, 17%), palate abnormalities (2/18, 11%), and lacrimal duct stenosis (2/18, 11%). Anomalies found in single patients included ankyloglossia, congenital hip dysplasia, eye pterygium, thyroid nodules, torticollis, vocal cord paralysis, and mental retardation.

*EYA1* mutations were found in 3 of 5 patients. Two mutations, p.R407Q and p.D429G, reportedly cause classic BOR, and a c.1604delAAA was found in a male with BOR, abnormal ossicles and a facial palsy. An additional patient was found to have a deletion including *EYA1* by array CGH.

Two of our patients had findings outside of the usual BOR phenotypic spectrum. A 38 year old woman had congenital hip dysplasia, an eye pterygium, and a goiter in addition to common BOR features and had the p.R407Q *EYA1* mutation. A 19 year old man had intellectual impairment, short stature, cleft lip and palate, hypertelorism, an upper eyelid coloboma, a broad neck, and single transverse palmar creases in addition to common BOR manifestations. The cause of his BOR has not yet been determined, with normal *EYA1* sequencing.

In conclusion, BOR is a genetically heterogeneous condition. We have described the features of 18 patients with a clinical diagnosis of BOR and include anomalies not previously associated with BOR.

## 2526/F/Poster Board #42

**Phenotypically different presentations in 2 patients resulting from RECQL4 gene compound-heterozygous mutation.** *A. Nakatomi[1], K. Yoshioka[2], Y. Ushiroda[1], T. Hasuwa[1], H. Kuniba[1], K. Nakashima[1], H. Motomura[1], K. Motomura[1], A. Kamitamari[2], Y. Nakashita[2], N. Okamoto[4], T. Kondoh[3], H. Moriuchi[1].* 1) Pediatrics, Nagasaki University , Nagasaki city, Nagasaki prefecture, Japan; 2) Pediatrics,Sasebo General Hospital,Sasebo city, Nagasaki prefecture,Japan; 3) Division of Developmental Disability, the Misakaenosono Mutsumi Developmental, Medical, and Welfare Center, Isahaya city,Nagasaki prefecture, Japan; 4) Department of Planning and Research, Osaka Medical Center and Research Institute for Maternal and Child Health,Izumi city,Osaka.

Baller-Gerold syndrome (BGS; OMIM #218600) is a congenital condition characterized by craniosynostosis and radial dysplasia or hypoplasia. RECQL4 gene, the only causative gene of BGS, has also been associated with Rothmund-Thomson syndrome (RTS; OMIM #268400) and RAPADILINO syndrome (OMIM #266280), both of which have some clinical features overlapping with those of BGS. We detected RECQL4 gene compound heterozygous mutations in 2 patients who had considerably different clinical features. The first patient is an 11-month-old boy. He was born at overterm of 42 weeks and 2 days of gestation with birth weight of 1984g as the first child of unrelated parents. He had severe intrauterine growth retardation(IUGR), coronary craniosysnotosis,bilateral radial defect, absence of both thumbs, midfacial hypoplasia, cleft palate, low-set ears, auditory ossicle hypoplasia, imperforate anus, rectourethral fistula,left renal aplasia and congenital heart defect. He also presented growth delay and motor retardation at 11 months of age. Thus, he had typical clinical presentations for BGS. Direct sequencing of his RECQL4 gene revealed compound heterozygous mutations: c.423delG, p.K141fsX38 in exon 5 and c.1569delC, p.S523fsX34 in exon 9. The second patient is a 9-year-old boy. He was born at preterm of 32 weeks and 3 days of gestation with birth weight of 924g as the first child of unrelated parents.He had severe IUGR, hypoplasia of his right-sided thumb, midfacial hypoplasia,hypertelorism, sparse eyebrows, micrognathia, pear-shaped nose, and low-set ears at birth. He never had craniosynostosis. At age 4, he was referred for short stature and hyperpigmented skin. Endocrinological and hematological tests ruled out endocrinal dysfunction and Fanconi anemia, respectively. Re-evaluation of his minor anomalies gave suspicion of BGS/RTS complex condition. Direct sequencing of his RECQL4 gene revealed compound heterozygous mutations: c.1136G>A, p.W379X in exon 6 and c.1569delC, p.S523fsX34 in exon 9. Thus, although these 2 cases had similar mutations on the RECQL4 gene, their clinical presentations were considerably different. RECQL4 gene abnormality has been associated with several clinical conditions that are considered as different clinical entities with partially overlapping features. However,it may be more appropriate to regard all these conditions as one clinical entity resulting from the same gene mutations.

## 2527/F/Poster Board #43

**Late presentation of autosomal dominant congenital hyperinsulinism due to GCK gene mutation.** *M.A. Potter[1,2], L. Brick[2], J. Vandermeulen[2].* 1) Dept. of Laboratory Medicine, Hamilton Health Sciences, Hamilton, Ontario, Canada; 2) Dept. of Pediatrics, Hamilton Health Sciences, Hamilton, Ontario, Canada.

Congenital hyperinsulinism (CH) usually presents in the neonatal period with symptoms of hypoglycemia. Both autosomal recessive and autosomal dominant modes of inheritance have been described for many of the genes that have been implicated in CH. Alterations in the GCK gene (OMIM 138079), coding for glucokinase, have been shown to be a rare cause of hyperinsulinism. Mutations in this gene more commonly are associated with congenital diabetes, with heterozygous mutations causing MODY2 and homozygous mutations causing the more severe permanent neonatal diabetes. Here we describe a 1-year-old male who presented with hypoketotic hypoglycemia associated with an intercurrent illness. Initial blood-work showed the glucose to be 1.6 mmol/l with insulin 53 pmol/l (43-194 pmol/l) and growth hormone <1 mmol/l. Sustained hypoglycemia on intravenous glucose was associated with continued inappropriate insulin secretion, low growth hormone and absence of ketones (beta-hydroxybutyrate <0.02 mmol/L). Urine organic acids and plasma acylcarnitines were normal. Family history revealed that the child's father had been admitted to hospital from 3 months of age to 2 years of age for "low blood sugar problems" that were medically managed. The father is obese and has migraines when he doesn't eat regularly. Some of his siblings report symptoms consistent with fasting hypoglycemia as well. Work-up for growth hormone deficiency and hyperinsulinism in our patient was undertaken, and therapy was started with both growth hormone and diazoxide. MRI of the pituitary was normal and no pathogenic mutations were found in the genes more commonly associated with congenital hyperinsulinism (ABCC8 and KCNJ11), although a polymorphism (p.A1369S) in the ABCC8 gene was found that may be associated with increased insulin secretion in pregnancy. Because of the suggestive family history and relatively mild hyperinsulinism, sequencing of the GCK gene was also performed. A previously undescribed c.1a-84C>G mutation was found in the child and his father. The patient responded well to oral diazoxide and subcutaneous growth hormone and continues management with these medications. This case illustrates two important findings: one, milder congenital hyperinsulinism can present with symptoms in association with metabolic stress, mimicking a fatty acid oxidation defect, and two, possible associated growth hormone deficiency should be clinically evaluated and managed in cases of CH.

## 2528/F/Poster Board #44

**Somatic and Germline Mosaicism in individuals with Rubistein-Taybi Syndrome may not be as rare as we thought.** *A.C. Tsai[1], N.C Lee[2], Y.S Chien[2], W.L. Hwu[2], E. Spector[3], P.W. Chiang[3].* 1) Sect Clinical Gen & Metabolism, Childrens Hosp, Denver, B300, Denver, CO; 2) Department of Genetic Medicine and Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan; 3) DNA Diagnostic Lab. University of Colorado at Denver Health Sciences Center.

Rubinstein-Taybi syndrome (RSTS) is a rare autosomal dominant genetic disease and is characterized by mental retardation and distinctive facial and other dysmorphic features. CREBBP and EP300 are the only genes currently known to be associated with RSTS. Mutations in CREBBP and EP300 were identified in approximately 50% and 3% of RSTS patients, respectively. Most of the identified mutations were assumed to be de novo mutations and the recurrence rate in a family was mentioned to be low. However, our clinical observations contradict the assumed low recurrence rate. Of the eight "classic" families seen in our genetic clinics, three families have more than one affected child from apparently normal parents. Family one was found to have one novel small 4 nucleotide deletion (6122-6125delCCAT; ref. seq. NC_000016.8) in exon 31 in two affected sisters but both parents were normal, highly suggesting the mechanism of germ-line mosaicism. Family two consists of one affected boy with one novel nonsense mutation (R1360X:c4078C>T; ref. seq. NC_000016.8) in exon 24, and an apparently normal father with low level mosaicism indentified in the in blood, oral mucosa and sperm cells; most significantly in the blood sample. Family three consists of two affected paternal half brothers; both brothers have classic facial and cognitive features as well as broad thumbs and great toes and the father is apparently normal with normal intellectual function. However, the family is not available for molecular analysis. Our study concurs with the previous study by[Bentivegna et al., 2006] that somatic mutations of CREBBP may not be uncommon. When the somatic mutations happen post-zygotically will lead to a spectrum from a low level mosaicism carrier (such as the father in family II) to a RSTS patient with almost complete RSTS phonotypical findings. We hypothesize this be the reason why the mutation could only be found in 50% of the affected individuals because the mosaicism was not readily detected in the blood. Our study, as seen in family I and III, also demonstrate germ line mosaicism might not be as low as the estimated 0.1%Larger scale analysis of RSTS families will be performed to confirm our hypothesis. Testing parental samples other than blood can be considered.

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## 2529/F/Poster Board #45

**Supernumerary teeth associated with monogenic disorders.** *E. Severin, C. Albu, D. Albu, D. Stanciu.* "Carol Davila" Univ Med Pharm, Bucharest, Romania.

Background and aims: Monogenic disorder such as cleidocranial dysplasia is often associated with supernumerary teeth (ST) but teeth formed in excess to the normal dental number in beta-thalassemia are not a frequent feature. The aims of the study were to characterize the clinical features, both dental and non-dental, of patients and then to explore molecular genetic basis of ST in these patients. Subjects and Methods: the study was achieved by ascertaining 3 patients from Orthodontic Department. Full medical and dental history was obtained. Physical, oral and radiographic examinations were performed. Peripheral blood samples from each patient were used to isolate DNA for molecular investigations. Results: Two unrelated Caucasian female patients had multiple supernumerary teeth in association with cleidocranial dysplasia. The analysis of the entire RUNX2/CBFA1 coding sequence by PCR amplification identified the mutations R225W which is typical for CCD. RUNX2 gene is involved in tooth morphogenesis and its mutation has pleiotropic effects which can explain association of several congenital anomalies. One Caucasian male patient expressed in permanent dentition a single supernumerary tooth in the posterior maxilla (paramolar) associated with beta-thalassemia. His co-twin was concordant for beta-thalassemia but discordant for dental phenotype. HBB gene has no influence on tooth formation or discordant paramolar. The association of unrelated features, beta-thalassemia with ST, may represent the action of different causes on different developmental field by coincidence. Conclusions: the study showed that not all cases with ST had similar dental pattern or same etiology. It is of interest to remark that mutation of a single gene has pleiotropic effects including syndromic supernumerary teeth but actually no specific mutation was found providing isolated supernumerary tooth.

## 2530/F/Poster Board #46

**Variable Expression of Neurofibromatosis 1 in Monozygotic Twins.** *E.K. Schorry[1], M.B. Rieley[1], D.H. Viskochil[2], D.A. Stevenson[2], B.T. Tinkle[1].* 1) Div Human Genetics, Cincinnati Childrens Hosp, Cincinnati, OH; 2) Univ of Utah, Div of Medical Genetics, Salt Lake City, UT.

Monozygotic (MZ) twinning, which occurs in 1/250 live births, has historically been used as a valuable tool in studying genetic traits. MZ twins who are discordant for specific traits are thought to demonstrate non-genetic etiologies for those traits, as their genes are presumably identical. Recent studies have shown that MZ twins are not as genetically identical as originally assumed. Differences in copy number variants and in methylation patterns have been shown within pairs of MZ twins, raising speculation that these differences may contribute to discordancies of phenotype of MZ twins. We have studied a group of MZ twins with NF1 as a tool to understanding mechanisms behind the variable expression of this complex disorder. We collected data on 10 sets of multiples with NF1: 9 sets of MZ twins and 1 set of MZ triplets, ranging in age from 4-18 years. Features evaluated included numbers of café-au-lait spots and cutaneous neurofibromas; plexiform neurofibromas; optic nerve glioma and other CNS tumors; skeletal complications; and learning disabilities (LD). MZ twins were generally highly concordant in numbers of café-au-lait spots (90% concordance rate) and cutaneous neurofibromas (70% concordance). IQ scores were within 10 points for all twin pairs tested, and similar patterns of LD were noted. However, the twin pairs showed significant discordance for presence and location of tumors, particularly plexiform neurofibromas (20% concordance) and MPNST (discordant in one pair), as expected if post-natal second-hit events were responsible for these features. Optic pathway gliomas showed only 55% concordance, implying a combination of modifying genetic factors as well as stochastic factors. Skeletal complications were of particular interest. There was high concordance rate (80%) for presence or absence of pectus deformities of the chest. Two sets of multiples were concordant for scoliosis, but differed greatly in the degree of curvature, presence of dystrophic features, and need for surgery. This study has helped to document which features and complications of NF1 are likely influenced by modifying genes and which are related to stochastic events and other yet-to-be elucidated factors. Future studies may investigate modifying genes and epigenetic changes within these twins to look for other potential etiologies of the variable expression of NF1.

## 2531/F/Poster Board #47

**CONGENITAL MYASTHENIA DUE TO CHAT-MUTATIONS IN TWO FAMILIES WITH THE FETAL HYPOKINESIA SEQUENCE.** *J. van den Ende[1], R. Mercelis[2], L. Mahieu[3].* 1) Dept Med Gen, Univ Antwerp/ University Hospital, Antwerp, Belgium; 2) Dept. of neurology, University Hospital, Antwerp, Belgium; 3) Neonatal Intensive Care Unit, University Hospital, Antwerp, Belgium.

The fetal hypokinesia sequence is a condition at birth in which multiple congenital joint contractures are present, generally resulting from lack of intra-uterine fetal movement. This can be the consequence of a wide variety of conditions, and for the sake of recurrence risk it is very important to distinguish the genetic causes. Among these are neurological causes (90%), myopathies and congenital myasthenia syndromes (5-10%), and connective tissue disorders, like diastrophic dysplasia. We present several cases belonging to two families, born with the same condition of multiple contractures and a lethal course, in which thorough investigation revealed 2 mutations in the ChAT gene, responsible for autosomal recessive Congenital Myasthenia. The ChAT gene encodes choline acetyltransferase, which catalyzes the reversible synthesis of acetylcholine from acetylCoA and choline at cholinergic synapses. This is the first gene in which mutations cause a presynaptic congenital myasthenia syndrome (CMS). In literature patients were described with CMS due to Chat mutations, who showed episodic apnea, often leading to death in the neonatal period. In survivors anticholinesterase drugs can partly prevent the crises that recur with infections, excitement or overexertion.

## 2532/F/Poster Board #48

**Partial agenesis of the pancreas associated with mutation in hepatocyte nuclear factor-1 beta (HNF1β).** *H. Gregory[1], E. Edghill[2], J. Minton[2], S. Ellard[2], J.C.S. Dean[1].* 1) Department of Medical Genetics, NHS Grampian, Aberdeen, Scotland, United Kingdom; 2) Molecular Genetics Laboratory, Royal Devon & Exeter NHS Foundation Trust, Barrack Road, Exeter, United Kingdom.

A female aged 48 presented with abdominal pain, diarrhoea and weight loss. She was found to have mildly deranged liver function tests and pancreatic insufficiency. Investigations demonstrated partial agenesis of the pancreas, with absence of the body and tail. Abdominal ultrasound also showed multiple small cortical renal cysts. Regular monitoring shows continuing normal fasting blood glucose. She responded well to treatment with pancreatic enzyme supplementation.Family history included one male sibling with insulin dependant diabetes diagnosed age 30, father with extrahepatic bile duct carcinoma and paternal uncle with maturity onset diabetes. DNA testing of hepatocyte nuclear factor -1 beta (HNF1β) revealed a missense mutation, R235Q, in exon 3, c.704G>A. The mutation was also found in the male sibling with diabetes, who was subsequently found also to have cortical renal cysts. This mutation is associated with the Renal Cysts and Diabetes syndrome (RCAD). Features include maturity onset diabetes of the young (MODY), renal cysts and failure, liver dysfunction and uterine abnormalities. It has also previously been associated with pancreatic atrophy and exocrine pancreatic failure. The findings in this family support previous observations of the wide phenotypic variability of the RCAD syndrome.

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## 2533/F/Poster Board #49

**Hereditary Disorders with Clinical Overlap with Marfan Syndrome: A Practical Guide for Clinicians.** *M. Murphy-Ryan, A. Psychogios, N.M. Lindor.* Mayo Clinc, Rochester, MN.

**Background and Purpose:**Underlined Text Nearly all clinicians have a basic knowledge of Marfan syndrome, the prototypical genetic disorder of connective tissue. Clinical features involve the musculoskeletal, cardiovascular, respiratory, ophthalmologic, and cutaneous systems. There is a newly emerging and complex clinical and molecular differential diagnosis to be considered for patients evaluated for any one feature of Marfan syndrome. The aim of this project is to consolidate new clinical and genetic information in accessible matrices to facilitate the diagnostic evaluation of individuals with suspected disorders of connective tissue due to overlap with features of Marfan syndrome. **Methods:**Underlined Text Each of the clinical features of Marfan syndrome, as specified in the Ghent Criteria, were subjected to Pub Med and OMIM literature searches to identify other Mendelian syndromes or clinical entities that shared that feature. One table was constructed that contains the clinical features of Marfan syndrome and additional key features of all identified syndromes and a second table was constructed that contains the clinical entities and the molecular bases for those, if known, many of which are molecularly heterogeneous. **Summary:**Underlined Text Thirty three different clinical entities and 35 different genes or gene loci are identified that might overlap with one or more of the presenting features in Marfan syndrome. The clinical entities include Abdominal Aortic Aneurysms, Arterial Tortuosity Syndrome, Bicuspid Aortic Valve with Thoracic Aortic Aneurysm, Camurati-Engelmann Disease , CATSHL Syndrome, Congenital Contractural Arachnodactyly, Cutis Laxa, (AD, AR Type I, II, X-L), Familial Ectopia Lentis, Ehlers-Danlos Syndrome (Arthrochalasia, Cardiac Valvular, Classical, Dermatosporaxis, Kyphoscoliotic, Vascular, TenascinX types), Familial Thoracic Aneurysm, Homocystinuria, LH3 Deficiency Syndrome, Loeys-Dietz Syndrome Types I and II, Lujan Syndrome, MASS Phenotype, Mitral Valve Prolapse Syndrome, Persistent PDA with Familial Thoracic Aneurysm , Shprintzen-Goldberg Syndrome, Stickler Syndrome, and Weill-Marchesani Syndrome. The matrices constructed from this project may be of clinical use to geneticists as well as clinicians who encounter patients with apparent alterations affecting connective tissue.

## 2534/F/Poster Board #50

**Restrictive dermopathy with massive thrombosis - a previously uncreognized finding?** *A. Hinek[3], H.Y.B. Chung[1,2], P. Shannon[4], R. Teitelbaum[2], D. Chitayat[1,2].* 1) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, Ontario, Canada; 2) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Ontario, Canada; 3) Division of Cardiovascular Research, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; 4) Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada.

Restrictive dermopathy (RD) is a lethal genodermatosis characterized by IUGR, tight and rigid skin, prominent superficial vasculature, epidermal hyperkeratosis, typical facial features, sparse/absent eyelashes and eyebrows, thin dysplastic clavicles, pulmonary hypoplasia and arthrogryposis. It is caused by LMNA or, more frequently, ZMPSTE24 mutations. We report 2 siblings with RD and ZMPSTE24 mutations. Case report: The mother is 28y G2P1. The couple was 1st cousin of Pakistani origin. Family history was unremarkable. The 1st pregnancy resulted in IUD at 27w, preceded by decreased fetal movement, oligohydramnios and IUGR at 24w. Autopsy was inconclusive and G-banding was not possible. Placenta showed chronic deciduitis, subchorionic hematoma and fetal thrombotic vasculopathy. The 2nd pregnancy was complicated with GDM treated with insulin. She was also on heparin for previous miscarriage. IPS was negative. Serial ultrasounds were normal. She had decreased fetal movement with poor biophysical profile at 28w and delivered a stillborn by emergency CS. Autopsy revealed absent eyelashes, sparse eyebrows, flat nasal bridge with vertical indentation, poorly defined alae nasi, thin and poorly defined philtrum, protruding tongue, retrognathia with a midline cleft chin. The skin was pink and had tight shiny texture. There were severe multiple flexion contractures with fixed thumbs, camptodactyly, absent distal IP creases and dorsiflexed feet. Skeletal survey showed hypoplastic clavicle and scapulae. Internal examination showed left adrenal infarction with propagation of thrombus obstructing the IVC, organizing venous thrombi in the contralateral kidney, and acute microthrombi in the brain. Bilateral perinephric and germinal matrix haematoma were noted. Chromosome analysis showed 46, XY. Skin histology was consistent with RD. Sequencing of the ZMPSTE24 gene showed a homozygous mutation resulting in premature protein termination, p.Glu237Stop. Discussion: Coagulopathy is not well described in RD. From literature review, only 1 case showed evidence of coagulopathy with lenticulostriate vasculopathy and multiple old, calcified, organized thrombi and intimal fibrosis in the abdominal aorta [Chiang, et al, 2008]. Further studies are required to see if the coagulaopathy in RD is the result of vasculopathy or platelet abnormalities and may provide further insights into the function of ZMPSTE24 in coagulation.

## 2535/F/Poster Board #51

**Multi-site assessment of hypertension prevalence in short stature skeletal dysplasia.** *J. Hoover-Fong[1,2], J. McGready[3], J. Leadroot[1,2], G. Oswald[1,2], D. Miller[1,2], I. Berkowitz[2], M. Ain[2], T. Yost[2], W. Mackenzie[4], C. Ditro[4], K. Rogers[4], J. Hecht[5], D. Rimoin[6], K. Schulze[3].* 1) Greenberg Center for Skeletal Dysplasias, McKusick-Nathans Institute of Genetic Medicine, Baltimore, MD; 2) Johns Hopkins University, Baltimore, MD; 3) Bloomberg School of Public Health, Baltimore, MD; 4) AI duPont Hospital, Wilmington, DE; 5) University of Texas, Houston, TX; 6) Cedars Sinai Medical Center, Los Angeles, CA.

**Background:**Hypertension (HTN) is systolic blood pressure(SBP)≥140 mmHg, diastolic blood pressure (DBP)≥90 mmHg or use of anti-HTN medication[1].HTN is a leading cause of cardiovascular (CV) mortality, and HTN prevalence among US average stature adults is 24%[2].30% of hypertensive adults are unaware of their HTN, 42% aware are not treated, and 69% treated are not controlled.HTN prevalence is unknown in the short stature skeletal dysplasia population.However a 42 year follow-up of 793 achondroplasia subjects revealed 10 X greater CV mortality and 10 year reduced median survival compared to average stature[3]. Accurate blood pressure(BP) measurement is needed for basic healthcare and to understand CV disease-related mortality risk. **Methods:**Subjects were recruited from 3 clinic sites and Little People of America events.BP was measured with an automatic oscillometric device (Dinamap, GE Healthcare) and commercially-available short-long BP cuff (Critikon, GE Healthcare) on the upper extremity.A single representative BP from each subject was used for analysis.Anthropometry, medications, geographic region, and exercise were also assessed.**Results**:325 subjects with 26 skeletal dysplasias enrolled. 274(84%) had 8 diagnoses including achondroplasia (192), diastrophic dysplasia (22),Kniest/SED (18), pseudoachondroplasia (17), hypochondroplasia (9), OI (6), acromesomelia (5), or cartilage hair hypoplasia (5); 18(5.5%) were undiagnosed.265 were adults >18 years (108 M, 40.8±11.6 yrs; 157 F, 39.6±13.4 yrs) and 60 were children (22 M,38 F;2-17 yrs). In adults, SBP was ≥140 in 87 (33.0%), DBP was ≥90 in 42 (15.8%), and 41 (15.5%) took anti-HTN or cholesterol-lowering agents; 113 short stature adults (42.6%) met ≥ 1 HTN criteria.In hypertensive (n=113) vs normotensive (n=150) adults, BMI (kg/m2) was 38.2±9.5 vs 33.3±7.3 and wt was 58.3±17.1 kg vs 48.5±12.2 kg, (both p<0.00001).In a subset, 45% reported moderate to vigorous exercise, with a trend of lower wt, BMI and BP in exercisers vs sedentary.Preliminarily, regional differences parallel average stature US obesity and HTN trends. Discussion:In this adult short stature cohort, HTN prevalence was nearly double the general US population.Though meeting HTN criteria, only a fraction were treated. Aggressive BP screening with a well-fitted cuff, treatment, and monitoring is a healthcare priority. [1]US Joint National Committee on High Blood Pressure, 2000; [2]Gu et al, Annals of Epidemiology, 2008; [3]Wynn et al, AJMG, 2007.

## 2536/F/Poster Board #52

**Spinocerebellar ataxia type 7: a rare cause of infantile nephrotic syndrome.** *M.T. Gabbett[1, 2], B. Holland[1].* 1) Genetic Health Queensland, Royal Brisbane & Women's Hospital, Brisbane, Australia; 2) School of Medicine, The University of Queensland, Brisbane, Australia.

Spinocerebellar ataxia type 7 (SCA7) is caused by an expanded trinucleotide repeat in the ATXN7 gene. The hallmark features of SCA7 are progressive cerebellar ataxia and a pigmentary macular degeneration. However large trinucleotide expansions in ATXN7 can present as a very different and rare clinical phenotype. We report on a two year old girl with a suspected inborn error of metabolism who died after an illness characterised by profound nephrosis and progressive hypotonia. Her half brother was diagnosed with SCA7 six years after her death. It was subsequently apparent that the two year old died from very rare complications of SCA7. It was proven that the two year old had a large trinucleotide expansion in ATXN7. We present this case to illustrate that SCA7 can present as nephrotic disease in infancy. This is only the second reported case of SCA7 presenting with nephrosis.

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## 2537/F/Poster Board #53

**Germ line WTX gene defect cause osteopathia striata with sclerosing skeletal dysplasia but does not lead to tumour development in affected individuals.** *S.G.M. Frints[1, 2], W. Van Hul[3], B. Perdu[3], F. De Freitas[3], K. van Roozendaal[1], I. Krapels[1], M. Schouten[1], J. Nijhuis[4], C.T.R.M. Schrander-Stumpel[1,2].* 1) Clinical Genetics, Maastricht University Medical Center, Maastricht, The Netherlands; 2) School of Oncology, Growth and Development, GROW, University of Maastricht, The Netherlands; 3) Department of Medical Genetics, University and University Hospital of Antwerp, Antwerp, Belgium; 4) Department of Gynecology & Obstetrics, Maastricht University Medical Center, Maastricht, The Netherlands.

Introduction: Osteopathia striata with cranial sclerosis (OSCS)(OMIM 166500) is a rare sclerosing bone dysplasia marked by linear striations in the metaphyseal region of the long bones and pelvis in combination with cranial sclerosis due to an increased osteoblast functioning. This condition is X-linked dominant with mostly mildly affected women but severely affected men, usually leading to lethality. Other clinical findings of OSCS include: macrocephaly, frontal bossing, ocular hypertelorism, broad nasal bridge, hearing loss, abnormalities of the palate and rarely cardiac, intestinal and genitourinary malformations. Family report: We present 20-year clinical genetic follow-up data of a woman with OSCS and her affected offspring. A WTX pathogenetic variation: c.337delG was identified in both. X-inactivation studies, using AR exon 1 methylation sensitive PCR showed random X-inactivation in both affected women. Discussion: Jenkins et al., Nat Genet, 2009, identified several WTX (Wilms tumour on the X-chromosome) gene defects in OSCS patients. Wtx is a component of the β-catenin destruction complex and negatively regulates the canonical wnt/ β-catenin signalling pathway. Somatic WTX gene defects are present in a subset of Wilms tumours and than show 100% skewed X-inactivation pattern in the tumour. As previously shown, WNT signalling is known to play an anabolic role in bone formation by osteoblasts. In mouse, wtx expression nicely correlates with the sclerotic skeletal phenotype seen in the affected female carriers. Conclusion: Further studies are needed to reveal how this WTX tumour suppressor gene can explain the pathogenesis of the intriguing bone phenotype of OSCS without apparently increasing risk for tumour formation.

## 2538/F/Poster Board #54

**Cerebello-Olivary degeneration of Holmes, further evidence from a new family.** *O. Caluseriu[1], L. MacDonald[1], J.N. Scott[2], R. Casey[1], O. Suchowersky[1].* 1) Dept Med Gen, Alberta Children's Hosp, Calgary, AB, Canada; 2) Dept of Diagnostic Imaging, Foothills Hosp, Calgary, AB, Canada.

Described more than 100 years ago by G. Holmes, cerebellar ataxia with hypogonadotropic hypogonadism represents a rare autosomal recessive entity and pathogenesis remains elusive. We describe a new family with variable expressivity in phenotype. The proband is a 30 year old male, son of first cousins of Caucasian origin. He presented with 2-year history of progressive slurred speech, short term memory loss, and depression. Visual complaints were absent, but he described decreased libido and erections. Previous medical history revealed endocrinological evaluation at 16 years of age for delayed puberty. He had delayed motor and speech milestones in infancy, but completed high school. No other affected family members were reported. On examination, the patient looked younger than stated age but there were no dysmorphic features. Development was Tanner stage 5 with small testes and slight gynecomastia. Neurological exam revealed mild limb hypotonia, diminished reflexes, ataxic gait, dysarthria and horizontal nystagmus. He had intention tremor in both arms and legs, dysdiadochokinesia, and dysmetria. Normal genetic and metabolic investigations included: chromosomes, molecular testing for Fragile X, FRDA, SCA 1, 2, 3, 6, 7 and 8, plasma amino- an organic acids, hexosaminidase, galactocerebrosidase, glucosidase, arylsulfatase A, methylmalonic acid, homocysteine, and VLCFA. Testosterone was low and thyroid function normal. Brain MRI showed patchy bihemispheric white matter T2 hyperintensities, diffuse cerebellar atrophy, and small pituitary gland, confirming the above diagnosis. In a family counseling meeting, it was noticed that his 29 years old sister had a wide-based gait and history of primary amenorrhea. A brain MRI demonstrated similar but milder abnormalities. Further familial investigations are planned, including homozygosity mapping.

## 2539/F/Poster Board #55

**Confirmatory report of Megarbane autosomal recessive oto-facial syndrome.** *M. Mathieu[1], G. Morin[1], B. Demeer[1], A. Hazard[1], B. Devauchelle[2], C. Kolski[3], B. Deschepper[4], T. Attie-Bitach[5], A. Receveur[6], H. Copin[6].* 1) Clinical Genetics, Amiens University Hospital, France; 2) Stomatology, Amiens University Hospital, France; 3) Otorhynolaryngology, Amiens University Hospital, France; 4) Radiology, Amiens University Hospital, France; 5) Genetics Department, Necker Hospital, Paris, France; 6) Cytogenetics, Amiens University Hospital, France.

In 2005, Megarbane et al reported two sisters from a Libanese consanguineous family (their parents were first cousins), with a new oto-facial syndrome. These patients presented microcephaly, dysmorphic features, very dysplastic low-set ears, malformation of the middle ear and short stature. In addition, one of the patients had a posterior cleft palate, and the other an oesophageal atresia. Because of the recurrence in sibs and the parental consanguinity an autosomal recessive mode of inheritance was suggested. We report a similar observation concerning an 18-year-old boy. This patient was the second of three children from non consanguineous parents. There was no remarkable familial history. He presented a mandibulo-facial dysostosis with microcephaly, extremely rudimentary and low-set helix of ears, absence of the external auditory channel, long nose with low columella, short philtrum, everted lower lip and small chin. A low implantation of the thumbs, with ankilosis of the right one, was noticed. The patient also presented a mild mental retardation and severe conduction deafness. The CT scan revealed hypoplastic middle ear cavity with absence of aeration and underdeveloped auditory ossicles. At the age of 18, the microcephaly persisted (-3 SD), stature was normal (171cm) but stayed inferior to the familial height. Chromosomal investigations (standard karyotype and array-CGH) were normal. The molecular screening of the CHD7 gene responsible of the CHARGE association was negative. We compare this observation with other known oto-facial syndromes and especially the familial observation of Megarbane. We discuss the importance of the oesophageal atresia and of the posterior cleft palate in this entity. The fact that both sexes were concerned by the disease, (boy in our observation and girls in Megarbane's patients) is compatible with the previously suggested autosomal recessive mode of inheritance.

## 2540/F/Poster Board #56

**SURGICAL CORRECTION OF PSEUDOTRIPHALIA ASSOCIATED WITH ANORECTAL MALFORMATION AND HYPOSPADIAS. A CASE REPORT FROM THE HOSPITAL PARA EL NINO POBLANO, MEXICO.** *F. Cuellar-López[1], J.M. Aparicio-Rodriguez[2,6], M. Barrientos-Perez[3], M.L. Hurtado-Hernandez[4], M.I. Ortega-Molina[5].* 1) Pediatric Urology; 2) Medical Genetics; 3) Endocrinology; 4) Cytogenetics; 5) Pediatric Surgery, Hospital Para el Nino Poblano, Puebla; 6) Estomatology, Benemerita Uiversidad Autonoma de Puebla, Mexico.

INTRODUCTION. Diphallia or penile duplication is a very rare congenital anomaly. We report in this study a pseudotripahalia were a total excision of ventrally located pseudopenis and excision of left phallus was performed. The case is being reported in view of its rarity and the successful surgical correction. CASE REPORT. A 2.8-years-old male child was studied with anorrectal malformation, double penis and extra pseudo penis. Examination of the external genitalia showed normal-appearing testis within each of the separated hemiscrotums. The dorsal penis had a penoscrotal hypospadias. Normal urethra and bladder could be easily catheterized were cystourethrogram revealed normality without no more internal tissues duplication. Therefore, the urethra opened through the normal bladder neck. There was no pelvic diastasis or other systemic anomalies. Karyotype was normal, showing a chromosome formula 46XY, normal male. SURGICAL PROCEDURES. The first-stage surgery was aimed at correcting the anorectal malformation by sigmoidostomy and anorrectoplasty. Then, after colostomy was finished, cystourethrogram was performed finding penoescrotal hypospadias, with the presence of three phallus. the upper phallus or pseudophallus full of lipid material, without any fistula, both of the other two phallus, the left one with a hypoplasic urethra without any bladder comunication and the rigth phallus with a normal urethra, verumontanum completely normal. Both, pseudophallus and left no communicated phallus were surgically excisionated. DISCUSSION. Less than 100 cases of penile duplication have been reported worldwide, and it is believed that a dyphalia with a third pseudophallus has no been reported yet. Therefore, the patient in this study had three phallus, and hypospadias, with 2 normal testes, cystourethrogram revealed a single bladder with a normal urethra and diagnosed as normal chromosomic male. Abnormality was corrected with excision of ventrally located pseudopenis and excision of left phallus, and rotation of right phallus. True diphallia has been considered to be a rare condition. Surgical correction should be individualized with the aims in order to obtain a proper urinary continence, urinary stream, and erection for a better quality of life for the future of the patient. REFERENCES. Gyftopoulos K, Wolffenbuttel KP, Nijman RJ. Clinical and embryologic aspects of penile duplication and associated anomalies. Urology 2002;60:675.

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## 2541/F/Poster Board #57

**Patient with 17p11.2 deletion and clinical manifestations of Smith-Magenis syndrome with vermis hypoplasia, abnormal breathing pattern and cerebellar signs.** *C. Vinkler[1], M. Hasan[2], A. Singer[3], M. Michelson-Kerman[1], D. Lev[1].* 1) Inst Med Gen, Wolfson Med Ctr, Holon, Israel; 2) Metabolic-Genetic-Neurologic Clinic Wolfson Medical Center, Holon, Israel; 3) Institute of Medical Genetics Barzilai Medical Center, Ashkelon, Israel.

Smith-Magenis Syndrome (SMS) is a complex disorder characterized by variable mental retardation, sleep disturbance, craniofacial and skeletal anomalies and specific behavioral phenotype. The prevalence is around 1: 15,000-25,000. It is generally a sporadic disorder caused by an interstitial deletion involving chromosome 17p11.2 containing the RAI1 gene. It may also be caused by a mutation in the RAI1 gene. Various brain malformations have been previously described in SMS patients, involving primarily the ventricles and the cerebral cortex. Joubert syndrome (JS) is a rare autosomal recessive disorder comprised of mental retardation, hypotonia, truncal ataxia and either episodic hyperpnea or atypical eye movements or both. Todate at least 7 genes are known to be associated with this disorder none of them is located on the short arm of chromosome 17. The combination of SMS and Joubert-like phenotype, has been previously reported in one patient. We report a second case associating SMS with vermis hypoplasia and some clinical signs of JS. This 5 year old girl, presented with mental retardation, heart defect (three VSD's), dysmorphic features typical of SMS, including midfacial hypoplasia, brachycephaly and brachydactyly. She also had abnormal breathing pattern (alternating tachypnea), hypotonia, wide-based gait, truncal ataxia and vermis hypoplasia. all of which, may be clinical signs of JS. A "molar tooth sign" on brain MRI, is an obligatory criterion for the definition of JS and this sign was not found in our patient's brain MRI. CGH array revealed a 3.12-3.14Kb deletion on chromosome 17p at (p11.2p12) encompassing the SMS "critical region" and extending somewhat beyond it, both on its telomeric and centromeric boundaries. Seven protein coding genes are known to be located outside the "SMS region" in this particular case, non of which seem a good candidate gene to cause hypoplasia of the vermis. One explanation for this unusual clinical presentation may be the presence of a JS gene in proximity to the "SMS region". However, hypoplasia of the vermis although rare in this syndrome, may be still caused by genes within the "SMS critical region". Clinical signs of JS in patients with SMS who also have vermis hypoplasia, may therefore reflect a wider phenotype in these rare cases, which overlaps with the phenotype of Joubert syndrome. We suggest that brain MRI should be carried out in SMS patients with Joubert-like phenotype.

## 2542/F/Poster Board #58

**Detection of new candidate genes for congenital eye anomalies using whole genome oligo array CGH and high-throughput resequencing.** *G. Raca[1,2], C.A. Jackson[1], B. Wartman[3], L.A. Schimmenti[3].* 1) UW Cytogenetic Services, State Laboratory of Hygiene, Madison, WI; 2) Department of Pathology, University of Wisconsin-Madison; 3) Department of Pediatrics and Ophthalmology, Institute of Human Genetics and Developmental Biology Center, University of Minnesota.

Deletions and duplications in genomic DNA are common mutations causing congenital anomalies and monogenic diseases. High resolution whole genome array CGH therefore represents a powerful diagnostic tool and gene identification strategy. Additionally, the recent emergence of widely available high throughput sequencing platforms has enabled searching for disease-associated point mutations across greater genomic intervals and in larger numbers of individuals. Our study uses a combination of whole genome oligo array CGH and high throughput candidate gene resequencing to search for new genes causing congenital eye anomalies. Array CGH was used to survey the genomes of patients with coloboma, anophthalmia, and microphthalmia for deletions and duplications affecting genes involved in eye development. Nineteen patients were tested using a 2 million probe array from NimbleGen. One patient was found to have a 5.8Mb duplication affecting the Prader/Willi Angelman Syndrome critical region at 15q11.2q13.1. This abnormality has not been reported in association with eye anomalies, but could explain other clinical features present in our patient, including developmental delay and behavioral problems. Four additional deletions were detected at the following candidate chromosomal regions: 13q31.3, 8q23.1q23.2 and 7q34. The 13q31.3 deletion, detected in a patient with bilateral iris coloboma, was ~240 kilobases in size, and included only one known gene, glypican (*GPC5*). Interestingly, coloboma, microphtalmia and anophtalmia have previously been reported in association with deletions of the 13q31-q33 region. Additionally, a drosophila ortholog of the *GPC5* gene, *dally*, is known to affect cell division patterning in developing eye. To test our patients for base changes in genes associated with eye development, we designed a custom NimbleGen Sequence Capture array with more than 100 candidate genes, and tested it in combination with 454 GS FLX resequencing on two samples with known mutations in the PAX2 gene. Future studies to support the role of candidate genes within deleted loci or identified through resequencing efforts as the basis for eye malformations will include: 1) studying effects of their inactivation on eye development in zebrafish, and 2) surveying for mutations in additional patients. Ultimately, our study will not only detect new genetic causes of eye anomalies, but also develop methods for more comprehensive and efficient clinical diagnostics.

## 2543/F/Poster Board #59

**Characterization of the 2q31.1 microdeletion syndrome: role of the HOXD gene cluster in limb malformations.** *M. Artigas-Lopez[1], A. Bengoa-Alonso[1], RC. Narvaiza-Martínez[1], A. Pérez-Juana[1], B. Nieva[2], ME. Querejeta[2], MA. Ramos-Arroyo[1].* 1) Dept Genetics, Hosp Virgen del Camino, Pamplona Navarra, Spain; 2) Dept Genetics, Policlínica Donostia, Donosti, Spain.

We present a patient with a 2q31.1-2q31.2 microdeletion (6 Mb) detected by array-based Comparative Genomic Hybridation (array-CGH), which included the HOXD gene cluster, and study the phenotype-genotype correlation of published cases with overlapping deletions identified by array-CGH, FISH and/or microsatellite analysis. Patients with microdeletions of 2q31.1 share with our case a distinct phenotype characterized by moderate mental impairment, postnatal microcephaly and growth retardation, craniofacial dysmorphic features and characteristic limb anomalies of the hands and feet. Four patients also had genital anomalies. The minimum overlapping deletion consists of a 1.6 Mb segment containing 10 genes. Interestingly, this segment does not include the HOXD13 gene, a gene known to cause synpolydactyly (SDP). However, all cases with the common deletion present limb defects compatible with SDP or split-hand/split-foot malformation (SHFM). SDP defects could be explained by haploinsuficiency of HOXD13 gene or disruption of a long-range regulatory element for the HOXD genes. A locus for SHFM-V has been proposed to be located in a 5 Mb interval centromeric to EVX2 (2q31-q32), suggesting that SHFM-V may be caused by haploinsuficiency of the 5'HOXD, EVX2 or DLX1 and DLX2 genes. However, most patients with a deletion of the HOXD cluster, EVX2 and/or DLX2 genes show limb anomalies compatible with SPD, not with SHFM. It is therefore likely, that severe limb phenotypes may be caused by the existence of a regulatory cascade that involves other genes or epigenetic factors in addition to mutations or haploinsuficiency of the HOXD cluster, EVX2 and/or DLX2 genes.

## 2544/F/Poster Board #60

**Paternally inherited ABCC8 mutations: The underlying cause of prolonged hyperinsulinism in Beckwith-Wiedemann Syndrome?** *L.E. Brick[1], J. Holland[2,3], J. Vandermeulen[2,3], K. McAssey[2,3], M.A. Potter[4].* 1) Department of Genetics, McMaster Children's Hospital, Hamilton, Ontario, Canada; 2) Division of Pediatric Endocrinology, McMaster Children's Hospital, Hamilton, Ontario, Canada; 3) McMaster University, Hamilton, Ontario, Canada; 4) Pathology & Molecular Medicine, Hamilton Regional Laboratory Medicine Program, McMaster University Medical Centre, Hamilton, Ontario, Canada.

Beckwith-Wiedemann Syndrome (BWS) (OMIM 130650) is a congenital overgrowth syndrome that is clinically and genetically heterogeneous. Approximately 50% of infants with BWS have hyperinsulinism and associated hypoglycemia. In most cases, the hypoglycemia is mild and transient, resolving within the neonatal period. However, in rare cases, the hypoglycemia may be more severe, requiring aggressive management and intervention. The mechanism for prolonged hypoglycemia in this subset of patients is unclear. We present a case of an infant diagnosed with BWS, associated with prolonged hyperinsulinism and hypoglycemia. The baby girl was born at 39 weeks gestation and presented with macrosomia, subtle nevus flammeus, right ear lobe crease and persistent hypoglycemia. A diagnosis of BWS was confirmed with molecular genetic studies, demonstrating mosaic paternal uniparental disomy (UPD) of chromosome 11p15. Given this patient's uncharacteristically prolonged hypoglycemia, with failure of response to diazoxide and octreotide in conventional doses, additional genetic causes of hyperinsulinism were investigated. The patient was found to be heterozygous for a novel variant (c.3400-2A>G) at a splice acceptor site in the ABCC8 gene, residing at chromosome 11p15. Family studies showed this mutation to be paternally inherited. In focal congenital hyperinsulinism an inactivating mutation is inherited from the father and the resulting disease is due to loss of heterozygosity of the maternal allele. Thus, given the paternal UPD status of this patient at the 11p15 locus, it is presumed that the patient is mosaic for a homozygous ABCC8 mutation affecting the pancreas. [18F] DOPA PET scanning confirmed a 3cm focus in the head of the pancreas, consistent with loss of heterozygosity in this area and focal hyperinsulinism. This novel case highlights a possible mechanism for severe and prolonged hyperinsulinism and hypoglycemia in BWS.

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## 2545/F/Poster Board #61

**Facial dysmorphism, distal arthrogryposis and developmental delay in sisters: an additional family with Chitayat-distal arthrogryposis syndrome.** *C. Cytrynbaum, J. So, G. Yoon, R. Weksberg, D. Chitayat.* Division of Clinical & Metabolic Genetics, The Hospital for Sick Children, Toronto, Canada.

In 1990 Chitayat et al. reported a sister and brother with distal arthrogryposis, mental retardation and a characteristic facial appearance. Three subsequent reports of similarly presenting patients supported an autosomal recessive inheritance pattern, with Wortmann et al. (2007) referring to the condition as Chitayat syndrome. We report two sisters with the same condition born to healthy, non-consanguineous parents of Dutch descent. The older sister presented prenatally with polyhydramnios, ventriculomegaly, renal pelvic dilatation, bilateral clubfeet and was large for gestational age (LGA). Postnatally, she was noted to be LGA and had facial dysmorphisms, including a "boxy" skull, high forehead, deep-set eyes, broad nasal root, low-set ears, a small mouth with down-turned corners and retrognathia. She was hypotonic, had tapering fingers with flexion contractures resulting in clenched fists, scoliosis, bilateral talipes, atrial septal defect and abnormal esophageal motility. She was subsequently noted to have severe global developmental delay and required G-tube feeding. She showed decreasing growth velocity with preserved head circumference to age 3 years. Metabolic and genetic investigations, including karyotype and BAC microarray, were normal. Her sister was born after an uneventful pregnancy with reportedly normal fetal ultrasounds. Postnatally, she was found to be LGA with dysmorphic features similar to her sister (square-shaped head with frontal bossing, deep-set eyes, broad nasal bridge, low-set ears, a small mouth with down-turned corners and retrognathia). She was hypotonic with tapering fingers, camptodactyly of the fingers, clenched fists, limited extension of the knees and high arches of the feet with hammer toes. She had feeding difficulties and required G-tube placement at 3 weeks of age. The constellation of findings in the sisters presented here overlap with those found in the brother and sister first reported by Chitayat et al. (1990). The sisters reported by us here further delineate the clinical manifestations of this condition and support its autosomal recessive mode of inheritance.

## 2546/F/Poster Board #62

**Cornelia de Lange Syndrome: What causes the mild phenotypes?** *G. Gillessen-Kaesbach[1], A. Dalski[1], M. Albrecht[1], V. Bernard[1], J. Eckholt[1], R. Löffler[1], A. Rauch[2], C. Zweier[2], D. Wieczorek[3], F. Kaiser[1].* 1) Inst Humangenetik, Univ Schleswig, Luebeck, Germany; 2) Inst Humangenetik, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany; 3) Inst Humangenetik, Univ Duisburg-Essen, Essen, Germany.

The Cornelia de Lange Syndrome (CdLS) is a multisystem developmental disorder. Affected individuals show growth and cognitive retardation, reduction defects of the upper limbs, gastroesophageal dysfunction, cardiac, ophthalmologic and genitourinary anomalies along with hirsutism and characteristic facial features. In addition to a wide range of clinical signs, various behavioural problems like self injurious, aggressive, and self restraining behaviour as well as autism-like features have been associated with CdLS. Intellectual development ranges from low normal intelligence to profound MR. Clinical experience has established the existence of two different phenotypes (severe and mild) which was already suggested by van Allan in 1993. In 2004, two independent groups reported that CdLS is due to mutations in the NIPBL gene (5p13.1) which are present in about half of patients with a CdLS phenotype. The mutational spectrum included missense, nonsense, splice site and frameshift mutations, deletions seem to be very rare. Subsequently, SMC1A on the X-chromosome and SMC3 on 10q25 have been identified to be causative for a smaller proportion of patients with a milder phenotype of CdLS. As a consequence there is still a considerable percentage of patients without a detectable molecular etiology. We were able to perform a clinical and molecular study in 66 patients (32 males and 34 females) with a CdLS phenotype ranging from mild to severe. In 21 (32%) of these cases we detected a mutation in the NIPBL gene, whereas in 45 (68%) patients we neither identified a mutation in the NIPBL gene nor in the SMC1A gene. Missense mutations and splice site mutations in the NIPBL gene frequently seem to be associated with a milder phenotype. Within our cohort we identified four new splice site mutations. All four patients are considered to be mildly affected. In addition, by CGH array analysis we identified a 3 generation family in which we detected a small deletion on chromosome 9 in a mildly affected boy and his affected mother. Many of the genes within this region do have a direct functional connection with sister chromatid cohesion genes. Mutational analysis in these genes are in progress. Beside SMC1A and SMC3 we speculate that other cohesion related genes may play a causative role for the milder phenotype in CdLS. Our present results contribute to this hypothesis.

## 2547/F/Poster Board #63

**Mutation spectrum and genotype-phenotype correlations in patients with Marfan syndrome: identification of forty novel mutations in the FBN1 gene.** *C. Hung[1], S. Lin[1,2], D. Niu[3], Y. Su[1,2].* 1) Department of Medical Genetic, National Taiwan University Hospital, Taipei, Taiwan; 2) Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan; 3) Department of Pediatrics, Taipei Veterans General Hospital, Taipei, Taiwan.

Background: Marfan syndrome (MFS) has been associated with approximately 562 mutations in the FBN1 gene. Mutation scanning of the FBN1 gene with DNA direct sequencing is time-consuming and expensive because of its large size. The aim of this study was to establish a national database of mutations in the fibrillin-1 (FBN1) gene that cause MFS in the Taiwanese population using high resolution melting analysis (HRM). Materials and Methods: In this study, we screened 294 patients from 157 families for the presence of FBN1 mutations using PCR/HRM. A total of 75 PCR amplicons (179-301 bp, average 256 bp) which covered the complete coding regions and splicing sites were evaluated on the 96-well Lightcycler system. Results: We identified 56 mutations in 62 of the 157 (40%) families including 49 single-base substitutions (36 missense mutations, 7 nonsense mutations, and 6 splicing sites), 1 small insertion, 4 small deletions, 1 small indel (insertion and deletion), and 1 large deletion (Exon 36). When family history was taken into consideration, the mutation detection rate rose to 91% (29 of 32). We further investigated the phenotypic data and found that one third (47 of 157) of the families fit the Ghent criteria for MFS. Based on that data, the mutation rate was 98% (46/47). Among the 56 mutations found in this study, 40 (71%) have not been registered in the Human Gene Mutation Database (HGMD). Conclusions: This is the first study of the mutation spectrum of MFS in a cohort of patients in Taiwan. Our results support the use of high-resolution melting analysis as an alternative method for the diagnosis of Marfan syndrome as well as its suitability for high-throughput mutation scanning of other large genes.

## 2548/F/Poster Board #64

**Rubinstein Taybi-like Phenotype in Patient with Distal 4q and Proximal 2q Chromosomal Microarray Abnormalities.** *L. Loomba-Albrecht[1], D. Styne[1], A. Hata[2], S. Boyd[2].* 1) Pediatric Endocrinology, UC Davis Medical Center, Sacramento, CA; 2) Pediatric Genetics, UC Davis Medical Center, Sacramento, CA.

Objective: We report a patient with dysmorphic features, developmental delay, recurrent pilomatrixomas and hypopituitarism. Chromosomal microarray analysis revealed a gain in copy number on proximal chromosome 2q and a loss in copy number on distal chromosome 4q. We postulate that a gene or genes located at these sites may be involved in the CBP/EP300 pathway, given that the patient has multiple features consistent with Rubinstein Taybi syndrome (RSTS). Patient and Methods: Patient is a 13 year old male with a medical history notable for developmental delay, myopia, strabismus, right cryptorchidism, hypopituitarism and recurrent pilomatrixomas. On physical exam, the patient was noted to have many findings typical of those seen in Rubinstein Taybi syndrome, including prominent forehead, a low frontal and posterior hairline, low-set and posteriorly rotated ears, mild ptosis, arched eyebrows, large beaked nose, partial mild syndactyly of the fingers and broad great toes and thumbs. Results: A high resolution karyotype revealed a 46, XY karyotype. Chromosomal microarray analysis revealed a gain in copy number on proximal chromosome 2q and a loss in copy number on distal chromosome 4q. Genetic testing specific for RSTS has not yet been performed. Discussion/Conclusions: Two causal genes have been identified in RSTS, but mutations in these genes account for fewer than 55% of cases. A loss of function mutation in CREBBP or CBP, which encodes the cAMP-response-element binding protein CREBBP, accounts for roughly 50% of cases. Loss of function mutations in EP300, encoding p300, account for far fewer. CREBBP and p300 are highly homologous genes known to function dually as transcriptional co-activators and histone acetyltransferases. Additional as yet unknown mutations are likely. We report the case of a male patient with Rubinstein-Taybi like features with a gain in copy number in the proximal long arm of chromosome 2 and a loss in copy number in the distal long arm of chromosome 4 by chromosomal microarray analysis. We postulate that one of these loci may be implicated in some patients with RSTS with normal EP300 or CBP genes. Of note, this is the first report of panhypopituitarism in a patient with apparent RSTS. Further genetic testing, including parental testing, is planned.

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## 2549/F/Poster Board #65

**A heterogeneous duplication at 10q24 associated with ectrodactyly malformations in a Chinese family that concomitance with seizures.**

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OBJECTIVE: Ectrodactyly or split hand-split foot malformation (SHFM) is a human limb malformation characterized by hypoplasia/aplasia of the central digital rays and variable fusion of the remaining digits. To date, at least five loci have been identified, including SHFM1 (7q21), SHFM2 (Xq26), SHFM3 (10q24), SHFM4 (3q27) and SHFM5 (2q31). Our aim in this study was to identify the disease-causing genetic alteration of ectrodactyly in a new Chinese SHFM family with a high risky of epilepsy. METHODS: Three affected individuals were examined physically and radiologically. Moreover, two affected individuals with seizures were examined by magnetic resonance imaging (MRI) and electroencephalogram (EEG). Haplotype analysis was performed to locate the causative gene, then quantitative PCR (qPCR) and long range PCR were employed to determine copy number of the alleles and to fine map the breakpoints. RESULTS: Photographs showed that of the affected individuals, 2 females present with only camptodactyly index fingers in both hands with dystrophic nails resulting in a typical monodactyly anomaly, while 1 male had no fingers in both hands. Besides, all affected individuals had no toes in both feet. In addition, 2 affected individuals suffered from seizures attacked. The male attacked tonic-clonic seizures and the epileptiform activities were found in his EEG. MRI showed that he had regional cerebromalacia and cortex atrophy of the right parietal lobe and a arachnoid cyst of right temporal lobe. Seizures of the female were related to sleep, except 2 times witnessed tonic-clonic seizures during the day time. MRI showed that she had a ventriculus sinister cerebri cyst. The potential haplotype shared by all affected individuals was detected only in the markers from SHFM3 locus. QPCR showed the normalized relative copy number was around 1.5, which indicated there was a genomic duplication in that region. Furthermore, the breakpoint positions were cloned by long range PCR. Sequence and blat analysis of the chimeric sequence indicated that the duplication region spanned 488,859bp in length and encompassed the entire LBX1, BTRC, POLL, DPCD, and FBXW4 genes. So the rearrangement may be caused by a NHEJ repair or a simplified FoSTeS event according to the junctions sequence. CONCLUSION: The heterogeneous duplication at 10q24 encompassed the entire LBX1, BTRC, POLL, DPCD, and FBXW4 genes is associated with ectrodactyly malformations concomitance with seizures.

## 2550/F/Poster Board #66

**Toward a clinical and molecular delineation of Acrocallosal syndrome.**

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Acrocallosal syndrome (ACLS) is inherited in an autosomal recessive pattern and is characterized by polydactyly of the hands and feet, mental retardation, craniofacial abnormalities, and agenesis of the corpus callosum. Clinical variability of the condition has been observed in the course of mapping studies on five published cases along with two additional cases. The neuroradiological findings among these cases suggest that there appears to be three distinct groups. A review of 67 published cases also shows that there is no consensus in the terminology of corpus callosum involvement, which is a key manifestation for differential diagnosis. In addition to the clinical variability of ACLS, this disorder can overlap with the severe end of the Greig cephalopolysyndactyly syndrome contiguous gene syndrome and one patient with acrocallosal syndrome has been found to have a c.2800G>C, p.Ala934Pro mutation in *GLI3*. The exons of *GLI3* were sequenced in five published ACLS cases and no pathologic variants were detected. Zoom in custom array CGH analysis of *GLI3* was also performed to exclude the GCPS contiguous gene syndrome but no deletions or duplications in *GLI3* were identified. Whole genome 380K genotyping of the five published cases was also performed. These data were analyzed for areas of overlapping homozygosity, reasoning that the high incidence of consanguinity in the communities from where the cases originated would be associated with identity by descent. No homozygous regions of significant size were detected. Our conclusion is that ACLS is more heterogeneous than previously believed. We suggest that new, more specific clinical criteria may be necessary for ACLS to improve clinical care, refine recurrence risk estimates, and facilitate mapping studies.

## 2551/F/Poster Board #67

**Array-CGH analysis to identify novel microdeletion/duplication syndromes and to extend the clinical phenotype associated with susceptibility regions.**

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We investigated 332 patients with mild to severe mental retardation associated to facial dysmorphisms and/or congenital anomalies. Forty two cases (13%) were considered positive using the following criteria: i) de novo non polymorphic rearrangements (16/332 or 5%); ii) either inherited (15q11.2q13.2) or de novo rearrangements of known syndromes (22/332 or 6.6%); iii) either inherited or de novo rearrangements in susceptibility regions (15q13.3 and 16p11.2) (5/332 or 1.5%). The de novo non polymorphic rearrangements include: del2q24.3q31.1, del2q31.2q32.3, del3q27.3q29, del6p25.3p25, del6q24.3q25.1, del6q27, dup7q11.23, del7q22.1q22.3, del7q36.1q36.2, del8q22.3, dup8q22.3, del9q31.1, del14q12, dup17q12, del21q22.11q22.12 and a complex rearrangement of a del9p24.1p24.3 and a dup17p13.3. The group of known syndromes includes a case of 1p-, the shortest 4p- and 6q- known in the literature, two cases of Williams-Beuren syndrome, a case of a AS/PWS and two cases of AS/PWS reciprocal duplication, three cases of Potocki-Lupski, two cases of Smith-Magenis, four cases of 22q11 deletions and two cases of 22q11 duplication, one case of 22q13 deletion, two cases of dupXq28. Susceptibility regions include three cases with dup15q13.3, one with dup16p11.2 and one with del16p11.2. Parental inheritance has been verified in two cases. The patient carrying dup15q13.3 inherited from normal father shows hypotonia, mental retardation, recurrent infections, epilepsy and cerebral anomalies. The patient carrying dup15q13.3 inherited from normal mother shows mental retardation, behavioral disorder, cleft palate and skeletal anomalies. The third patient shows autistic traits, cerebral anomalies and recurrent infections. The patient with del6p11.2 shows recurrent infections, behavioral and phonological disorders. His mother does not present the deletion while analysis of his father and brothers which present dyslexia is ongoing. The patient with dup16p11.2 shows mental retardation, epilepsy and EEG anomalies. Latest data indicate a strong correlation of these two region with a pathological phenotype not only regarding autism and epilepsy but also variable clinical outcome. An accurate search of the literature allowed to identify patients with overlapping rearrangements delineating novel microdeletion/duplication syndromes or extending the phenotype associated with susceptibility regions.

## 2552/F/Poster Board #68

**Whole gene duplication of the PQBP1 gene in Renpenning syndrome.**

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Renpenning syndrome is a well-described X-linked multiple congenital anomaly and mental retardation syndrome [OMIM 309500]. Classic clinical features include mental retardation, microcephaly, dysmorphic features, short stature, small testes, and lean body build. Renpenning syndrome is caused by mutations in the PQBP1 gene. Missense mutations, insertions, deletions, and duplications within the gene have been well-described. We present a 46 year old male with typical clinical features of Renpenning syndrome. He has moderate mental impairment, seizures since infancy, short stature, and dysmorphic features. He had previously been verbally communicative and did not demonstrate significant behavioral issues. More recently, however, our patient has become uncommunicative, displaying little and unclear speech. He now exhibits memory and recognition loss, is uncooperative, and is sometimes aggressive, self-abusive, and incontinent. Testes volume has not been assessed. Of note, our patient is normocephalic. The reason for his regression within the past 3 years is unclear. A CT scan showed cerebral atrophy present at 14 years of age. Chromosome analysis and Smith-Magenis FISH analyses were normal. 500K SNP microarray analysis revealed a 4.7 Mb duplication at Xp11.22-p11.23. Multiple ligation probe analysis (MLPA) of the PQBP1 gene contained within this duplicated region confirmed a duplication of the entire PQBP1 gene. Multiple other genes are duplicated within this 4.7 Mb region and may contribute to our patient's phenotype. To our knowledge, this is the second report of a whole gene duplication of the PQBP1 gene responsible for a Renpenning phenotype. Bonnet et al. (2006) reported a male with the above-described duplication (5 Mb) who had moderate mental impairment, autistic-like behavior, short stature, and mild dysmorphic features. This duplication was de novo. Maternally-inherited Xp duplications have previously been described and skewed X-inactivation has been reported. Our patient's mother is deceased and a DNA sample is not available. His sister does not carry this duplication.

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## 2553/F/Poster Board #69

**From Heterotaxy to VACTERL-H syndrome - The clinical variability of ZIC3-related disorders.** *S. Keating[3], H.Y.B. Chung[1,2], L. Shaffer[4], D. Chitayat[1,2].* 1) Department of Pediatrics, Division of Clinical and Metabolic Genetics, Hospital for Sick Children,Toronto, Ontario, Canada; 2) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Ontario, Canada; 3) Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada; 4) Signature Genomic Laboratories, Spokane, Washington, USA.

BACKGROUND: The ZIC3 gene functions as a transcription factor in early stages of left-right body axis formation. Mutations in ZIC3 gene cause a variety of clinical manifestations including isolated congenital heart disease (CHD), heterotaxy & other midline CNS, urogenital & hindgut malformations. We report a four generation family with X-linked heterotaxy associated with a deletion of the ZIC3 gene at Xq26.3. METHODS AND RESULTS: The proband was a 31y G2P1 woman of Italian descent and her husband was 33y and of the same descent. The couple was healthy, non-consanguineous and had a 3y daughter with a small VSD. The family history revealed a 4 generation pedigree consistent with X-linked pattern of multiple congenital anomalies. The maternal grandmother had a brother and two sons who were born with imperforate anus, developed cyanosis and died shortly after birth. Autopsy on an affected maternal uncle showed normal situs, hydrocephalus, vertebral abnormalities and anal atresia, which was compatible with VACTERL-H. His karyotype was 46, XY. The couple was seen during their 2nd pregnancy. Fetal ultrasound at 19w showed a male fetus and the stomach was not well visualized. A subsequent ultrasound at 23w revealed abnormal cardiac anatomy, bilateral enlarged kidneys, right-sided stomach, and polyhydramnios. Fetal echocardiography showed DORV, left atrial isomerism, interrupted IVC, unbalanced AVSD, and hypoplastic left ventricle. The pregnancy was terminated at 23.5w and the autopsy showed in addition imperforate anus. The karyotype was 46,XY. Oligoarray (SignatureChipOS) detected a 1.4Mb deletion in Xq26.3 including the ZIC3 gene. FISH analysis revealed that the proband, her daughter, sister, mother and grandmother were all carriers of this deletion. Echocardiography done on them showed no cardiac abnormalities and abdominal ultrasounds were normal. CONCLUSION: VACTERL-H is a genetically heterogeneous condition and some of the X-linked cases were found to be caused by a mutation in the FANCB gene associated with Fanconi anemia B. However, early reports pointed out there is higher incidence of rotational CHD, lung lobation defects and spleen anomalies in patients with VACTERL-H when compared to VACTERL, suggesting a potential linkage between VACTERL-H and the left-right patterning defects in embryogenesis. Our report support the suggestion that some of the cases with VACTERL-H syndrome may be caused by a mutation in the ZIC3 gene.

## 2554/F/Poster Board #70

**Associated malformations in patients with esophageal atresia.** *C. Stoll, Y. Alembik, B. Dott, M.P. Roth.* Genetique Medicale, Faculte de Medecine, Strasbourg, France.

Esophageal atresia is a common type of congenital malformation. The etiology of esophageal atresia is unclear and its pathogenesis is controversial. Because previous reports have inconsistently noted the type and frequency of malformations associated with esophageal atresia, we conducted this study in a geographically well defined population, evaluating the birth prevalence of esophageal atresia and associated malformations ascertained between 1979 and 2003 in 334,262 consecutive births. Of the 99 patients with esophageal atresia, 46 (46.5%) had associated malformations. These included patients with chromosomal abnormalities (8 patients,8 %); non chromosomal recognized syndromes 4 patients, including one each CHARGE syndrome, Fanconi anemia, Fryns syndrome, and Opitz G/BBB syndrome; associations including VACTERL (10 patients), and one schisis; one oculo-auriculo-vertebral spectrum; one malformation complex, a sirenomelia, and non syndromic multiple congenital anomalies (MCA) (21 patients, 21 %). Malformations of the cardiovascular system (24 %), urogenital system (21 %), digestive system (21 %), musculoskeletal system (14 %), and central nervous system(7 %) were the most common other congenital malformations occurring in patients with esophageal atresia and nonsyndromic MCA. We observed a high prevalence of total malformations and specific patterns of malformations associated with esophageal atresia which emphasizes the need to evaluate all patients with esophageal atresia for possible associated malformations. The malformations associated with esophageal atresia could be classified into a recognizable malformation syndrome or pattern in 25 out of 46 patients (54 %).

## 2555/F/Poster Board #71

**Analysis of microsatellite DNA markers in the diagnosis of Williams-Beuren Syndrome.** *R. Dutra[1,2], P. Pieri[1,2], F. Macaferri[2], R. Honjo[1], D. Bertola[1], L. Albano[1], C. Kim[1].* 1) Dept of Genetics, Instituto da Criança - FMUSP, São Paulo, Brazil; 2) Laboratory of Medical Investigation - LIM36 - FMUSP, São Paulo, Brazil.

Introduction: The Williams-Beuren syndrome (OMIM 194050) is caused by a hemizygous contiguous gene microdeletion at 7q11.23. Supravalvular aortic stenosis, mental retardation, overfriendliness, renal and dental abnormalities comprise typical symptoms in WBS. The commonest deletion includes 1.5 Mb, but atypical deletions of 1.8 Mb have also been reported. Deletions arise as a consequence of misalignment of the repetitive sequences - Low Copy Repeats (LCR) during meiosis followed by unequal crossing over. Although fluorescence in situ hybridization (FISH) is widely used, microsatellite DNA markers are considered highly informative and easy handling for confirmation of clinical suspicion of the syndrome. Purpose: To detect the microdeletion and the parental origin using three microsatellite DNA markers (D7S1870, D7S489 and D7S613) in the diagnosis of WBS. Subjects and Methods: For the study 99 patients with clinical diagnosis of WBS and their parents were included. Inclusion criteria were the presence of dysmorphic craniofacial abnormalities associated with cardiovascular abnormalities and behavioral characteristics or hyperacusis. FISH was previously performed in 21 patients. For the molecular study, the polymerase chain reaction (PCR) with three markers was used for amplification of the corresponding regions of interest. The samples were submitted to electrophoresis in a polyacrylamide denaturing gel (with 7.5% urea) and the results visualized with silver staining. Results: Among the markers studied, the D7S1870 was the most informative (80.8%) followed by D7S613 (76.8%) and D7S489 (71.7%). For 84 patients, DNA from both parents were available, for 12 patients we could not retrieve the father's DNA and only 3 patients presented only with their fathers. Except for 2 patients for whom father's DNA were not available, for all the 97 patients it was possible to determine the presence of the deletion, found in 86 (88.7%) patients, or its absence, found in 11 (11.3%) patients, even in those 13 cases for which the analysis included only one of the genitors. The deletion was proven to be maternal in 48/86 patients (55.8%) and paternal in 38/86 patients (44.2%). All the patients with positive FISH analysis were concordant with the microsatellite DNA markers results. Conclusion: This study reinforces the usefulness of microsatellite DNA markers in the detection of microdeletions to confirm the clinical diagnosis of WBS. (Supported by FAPESP 2008/55391-6).

## 2556/F/Poster Board #72

**Genodermatology, a multidisciplinary approach: Overview of five years collaboration.** *M. Vreeburg[1], Y.J.H.A. Detisch[1], C.T.R.M. Schrander-Stumpel1[1,3], M.A.M. van Steensel[2,3], D. Marcus-Soekarman[1].* 1) Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, the Netherlands; 2) Department of Dermatology, Maastricht University Medical Center, Maastricht, the Netherlands; 3) Research Institute Growth & Development (GROW), Maastricht University, Maastricht, the Netherlands.

Since diagnosis of rare genetic syndromes is based on pattern recognition and requires specific expertise, as is the case with many skin diseases, we joined forces starting a multidisciplinary out-patient clinic "Genodermatology" in March 2004. Our goal was to evaluate patients with diagnostic questions in the combined field of dermatology and clinical genetics by the dermatologist and the clinical geneticist together. Patients were referred by either a dermatologist or a clinical geneticist (or other specialists) and were seen by both specialists and a genetic counselor. In a 5 years time-period 245 patients were counselled. Five patients were lost in follow up. The medical history of the patient and the pedigree were recorded. Clinical examination, followed by karyotyping, DNA-analysis, metabolic investigation and/or a skin biopsy (if indicated) were performed. Four main groups could be discerned, based on diagnosis: a. Oncogenetic syndromes (n= 58) b. hypermobility/ connective tissue disorders (n=50) c. disorders involving the X-chromosome (n=18) d. miscellaneous (n= 114) . Medication was prescribed by the dermatologist if indicated or referral to the department of dermatology if special treatment was needed, was arranged. In case of a hereditary condition, genetic counselling was offered to family members. Our results indicate that combined efforts are worthwhile to offer these specific patients. Moreover, in various cases light could be shed on the pathogenetic mechanisms leading to a particular skin disorder.

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## 2557/F/Poster Board #73

**Cardio-Facio-Cutaneous Syndrome: Clinical and Molecular Diagnosis in the NICU.** *S. Jamal[1], Z. Wang[1,2], J. Milunsky[1,2,3].* 1) Ctr Human Genetics, Boston Univ Sch Medicine, Boston, MA; 2) Dept of Pediatrics, Boston Univ Sch Medicine, Boston, MA; 3) Dept of Genetics and Genomics, Boston Univ Sch Medicine, Boston, MA.

Cardio-Facio-Cutaneous (CFC) syndrome [MIM 115150] was first described by Reynolds *et al*. (1986) in eight unrelated individuals with multiple congenital anomalies, mental retardation, and characteristic facial features. Since its original description, 100-200 additional patients have been reported. In 2006, mutations in genes within the RAS-MAP kinase pathway were implicated as causative of CFC (Rodriguez-Viciana *et al*.; Niihori *et al*.). The CFC phenotype is known to be reminiscent of Noonan syndrome and Costello syndrome, and the differential diagnosis can be difficult, particularly in infancy. We describe a 3.5 month old male (ex 30 4/7 weeker; at birth paternal age was 51 years and maternal age was 38 years) with a clinical and molecular diagnosis of CFC. C-section delivery was required secondary to breech presentation and NRFHT. His birth weight was 1610g, birth length was 38.5cm, and birth OFC was 29cm. He developed hyperbilirubinemia requiring phototherapy for five days. On physical examination, he was noted to have a broad nasal bridge, epicanthal folds, downslanting palpebral fissures, low set ears with prominent upturned lobules, a broad short neck with an excess nuchal fold, wide low set nipples, and hypotonia. A renal ultrasound revealed a right duplex kidney. On DOL 29, given the patient's dysmorphic features and valvar pulmonic stenosis (gradient of 30mm Hg), RAS-MAP kinase conditions were queried. Molecular testing for CFC revealed a heterozygous reported mutation (T241P) in exon 6 of the *BRAF* gene. At 3.5 months (CA: 5 weeks) a repeat echocardiogram also detected mild LVH, possibly indicating HCM. In addition, the patient had sparse scalp hair, very sparse eyebrows, and sparse eyelashes. Since the molecular elucidation of CFC, *BRAF* mutations are known to be the most common, accounting for approximately 75-80% of patients. The T241P *BRAF* mutation was first described by Nava *et al*. (2007) in one patient, and again by Shulz *et al*. (2008) in two unrelated patients. We describe a premature male infant patient with CFC who further contributes to the clinical phenotype of *BRAF*-related CFC. To our knowledge, our patient may be the earliest postnatal molecularly confirmed case of CFC. Syndromes involving the RAS-MAP kinase pathway need to be considered in a neonate with pulmonic stenosis and dysmorphic features. Molecular testing is useful for confirmation of diagnosis and enables better anticipatory guidance.

## 2558/F/Poster Board #74

**Spondyloepiphyseal dysplasia-bracydactyly and distinctive speech: a propos of a new male case.** *N.Y. Nunez-Reveles[1], J. Sanchez-Corona[2], G. Castaneda-Cisneros[3], J.E. Garcia-Ortiz[4], J.R. Corona-Rivera[1], N.O. Davalos-Rodriguez[1], G. Perez-Garcia[5], D. Garcia-Cruz[1].* 1) Instituto de Genetica Humana "Dr. Enrique Corona Rivera", Departamento de Biologia Molecular y Genomica, CUCS, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico; 2) Division de Medicina Molecular, Centro de Investigacion Biomedica de Occidente, CMNO, IMSS, Guadalajara, Jalisco, Mexico; 3) Servicio Neurocirugia, Hospital de Especialidades, UMAE, CMNO, IMSS, Guadalajara, Jalisco, Mexico; 4) Division de Genetica, Centro de Investigacion Biomedica de Occidente, CMNO, IMSS, Guadalajara, Jalisco, Mexico; 5) Servicio de Genetica, Hospital Civil "Fray Antonio Alcalde".

Introduction. Spondyloepiphyseal dysplasia-brachydactyly and distinctive speech (SED-BDS), also called Fantasy Island syndrome, or Tattoo Dysplasia OMIM N° %671717 was first described by Cantu et al in 1991, although the first affected case was studied by J.R. Gorlin, an anecdotic report of a father and son by J. Hall and C.L. Johnson reported five more affected individuals. Clinically and radiologically is characterized by short stature with acral shortness, distinctive face, mild blepharophimosis, upslanted palpebral fissures, abundant eyebrows and eyelashes, thick and abundant hair and coarse voice; and radiologically by brachymetacarpalia, brachymetatarsalia and brachyphalangia of all fingers and toes, short and broad long bones with normal morphology and small pelvis. Case Report. The propositus aged 43 years old, was the product of the 3rd pregnancy from non-consanguineous parents; prenatal and delivery antecedents are unknown. He is an actor in a taurine show since 32 years ago. At physical examination his height was 130 cm (more than -3 SD), weight 36 kg (more than -3 SD), OFC 53.3 cm (-1.5 SD), armspan 134.5 cm, lower segment 55.5 cm, upper/lower segment ratio 1.34, total hand length 14 cm (more than - 3SD), foot length 19 cm (more than -3 SD). Clinically he showed disharmonic short stature, blepharophimosis, abundant eyebrows, upslanted palpebral fissures, depressed nasal bridge, long philtrum, large mouth with thick lower lip, round chin; short and wide neck; abundant and thick scalp hair; short thorax, rhizo-meso-acromelic shortness in the four extremities, limited pronosupination, short and stubby hands and feet with generalized brachydactyly; coarse voice was evident. The X-ray examination revealed cuboid-shaped vertebral bodies and lack of lumbar lordosis, hypoplastic pelvis, short and broad tubular bones, brachymetacarpalia and brachymetatarsalia, brachydactyly of all fingers and hypertrophy of the first ray on hands and feet. Conclusions. We report a new sporadic male case with (SED-BDS), corresponding to the 14th known case in the literature.

## 2559/F/Poster Board #75

**Infantile Convulsion Choreoathetosis Syndrome in a Hong Kong Chinese Family.** *C.W. Fung, H.Y.B. Chung, P. Ng, M. Zhao, W.L. Yang, V.C.N. Wong.* Department of Paediatrics & Adolescent Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam Road, Hong Kong.

Background: Infantile convulsion choreoathetosis syndrome (ICCA) is inherited as an autosomal dominant trait. Seizures appear at 3-12 months, and are partial and may secondarily generalize. Seizures soon stop but paroxysmal choreoathetosis starts between 5-9 years. Genetic studies reveal evidence of linkage to the pericentromeric region of chromosome 16. No causative gene has been found to date. The SLC5A11 gene, encoding a sodium/glucose cotransporter and located in 16p12-p11 region, is postulated to be a candidate. However, no pathogenic mutation was found so far in families with ICCA. We report a Chinese family with ICCA syndrome. Method and Result: CWP was a 30-month-old boy who presented with 3 episodes of generalized tonic clonic convulsions, which may or may not be provoked by upper respiratory tract infection, between 6 and 7 months old. Physical examination and EEG was unremarkable. He was treated with low-dose valproate and remained seizure free since then. At 30 months, he had mild developmental language delay. His maternal 1st cousin, CWH, was a 16-year-old boy. He had multiple generalized tonic clonic seizures associated with upper respiratory tract infection at 11 months old. He was put on a week course of anticonvulsant then remained seizure-free. Since the age of 10, he had recurrent episodes of choreoathetosis involving all the 4 limbs. He had normal intelligence with normal neurological examination. The diagnosis of paroxysmal kinesigenic dyskinesia (PKD) was subsequently made. Once diagnosed, low-dose carbamazepine induced remission. 4 other family members had infantile convulsions with complete remission after few years of age. They did not have PKD. 2 more had transient PKD without infantile convulsion. They were not on treatment. Direct sequencing of the 15 coding exons of SLC5A11 gene from CWP, his mother and his normal sister did not show any coding mutations or splicing junction changes, indicating that this gene may not be involved in this family. Conclusion: Our family shows typical features of ICCA syndrome. Accurate diagnosis is very important as it is amenable to low-dose anticonvulsant treatment and in general of good prognosis. In agreement with other studies, our family may not be related to SLC5A11 gene mutation. Further linkage study is in progress.

## 2560/F/Poster Board #76

**Clinical aspects of Noonan syndrome in one Polish family with mutation p.L613V in *RAF1* gene.** *E. Ciara, A. Tańska, M. Kugaudo, D. Jurkiewicz, M. Gajdulewicz, A. Jezela-Stanek, K.H. Chrzanowska, D. Piekutowska-Abramczuk, M. Borucka-Mankiewicz, P. Kowalski, A. Barczyk, E. Popowska, M. Krajewska-Walasek.* Department of Medical Genetics, The Children's Memorial Health Institute, Warsaw, Poland.

Noonan syndrome (NS) is an autosomal dominant disorder, characterized by short stature, facial dysmorphism, a wide spectrum of congenital heart defects, chest deformity, scoliosis, hearing loss, motor and neurocognitive delay, various coagulation defects and lymphatic dysplasias. After 2006, mutations in the *RAF1* gene were reported to cause Noonan syndrome, in addition to the *PTPN11*, *SOS1*, *KRAS* genes, and now we can find the etiology of the disease in approximately 60-70% of NS cases. All these genes encode proteins of the Ras-MAPK signaling pathway. Mutations in the *RAF1* gene are rare and were detected in 3-17% of individuals clinically diagnosed with NS. To date, nearly 20 various missense mutations were identified in *RAF1* in NS patients. Most of them occurred *de novo*; only one substitution was of familial origin. In this study we report on a Polish family with an affected mother and three children (twins and their older sister) who have inherited Noonan syndrome caused by *RAF1* gene mutation. The family was refereed to our department 15 years ago for the evaluation of congenital heart defects and short stature. The observed heart anomalies were as follow: valvular and supravalvular pulmonary stenosis, hypertrophic cardiomyopathy in a twin boy, patent foramen ovale in a twin girl and valvular pulmonary stenosis in their older sister, in whom also personality disorders were diagnosed. The physical examination revealed the distinctive facial dysmorphism in all of them. It was severely expressed in the twins and resembled their mother's phenotype (curly, coarse hair, hypertelorism, low nasal bridge, posteriorly rotated low-set ears, low posterior hairline and short neck). No developmental delay was noted, twins had borderline development while the oldest patient's IQ score was very high. The patients' mother had short stature, no heart abnormality and high IQ score. In all 4 cases, the analyses of the *RAF1* gene (by SSCP or HRM and sequencing technics) revealed a nucleotide substitution c.1837C>G leading to p.Leu613Val missense mutation. This mutation is localized in the region of the *RAF1* gene coding for CR3 kinase domain and results in increased CRAF kinase activation. So far only two cases with the p.Leu613Val mutation in NS and one in LEOPARD syndrome have been described. We compare the clinical data of our family patients with the previously reported cases. The study was supported by MNiSW Project PB 0056/B/P01/2008/35 and by CMHI project 190/08.

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## 2561/F/Poster Board #77

**WAGR Syndrome: Oral and Craniofacial Characteristics.** *D.L. Domingo[1], N. Tomona[1], K.M. Danley[2], M.K. Tune[2], J.C. Han[2].* 1) Clinical Research Core, NIDCR/NIH, Bethesda, MD; 2) DEB, SWHR, NICHD / NIH Bethesda, MD.

BACKGROUND: WAGR syndrome is a rare genetic disorder caused by heterozygous contiguous gene deletions of variable size on chromosome 11, and is characterized by Wilms tumor, aniridia, genitourinary anomalies, mental retardation, and, in those with deletion of the brain-derived neurotrophic factor gene, hyperphagia and obesity. OBJECTIVE: As part of a comprehensive phenotype-genotype study, we characterized clinical features involving the oral-craniofacial structures. METHODS: Nine WAGR patients (2 males, 7 females; 8-38 yrs, median 17 yrs, mean 19 yrs) were prospectively examined. Clinical, radiographic, and cephalometric findings were noted. RESULTS: Patients in permanent dentition (n=7) universally exhibited an anterior openbite and/or crossbite(s). Three presented with bilateral crossbites, 2 left unilateral, and 3 anterior. Unusual palatal morphology was highly prevalent, manifested as hypertrophic alveolar bone plates (n=9; 100%) and narrow palatal arches (n=8; 89%). In addition, a midline sagittal thick fibrotic palatal bridge was present in the oldest 6 patients (14-38 yrs) (67%). Among those who could cooperate with visualization of the oropharynx (n=7), deep surface fissures extending from the hard palate-soft palate junction were universally manifested. Cephalometric measurements (n=8) yielded the following: 1) upper pharyngeal constriction (n=8; 100%; range: 6-13 mm; norm: 15-20 mm); 2) shorter anterior cranial base (n=8; 100%; range: 44-70 mm; norm: 73-75 mm); and 3) protrusion of upper and/or lower lips (n=7; 88%). Assessment of hyoid bone position (n=7) yielded: 1) longer distances between the hyoid and base of mandible (range: 23-25 mm; norm: 10-14 mm) in 6 patients (86%); and 2) longer distances between hyoid and mandibular symphysis (range: 44-52 mm; norm: 40-44 mm) in the same six patients (86%), half of whom were obese. CONCLUSION: Our study identified novel features involving the oral-craniofacial tissues. The high prevalences of oral tissue irregularities and dental crossbites highlight developmental aberrations involving the maxillary apparatus. The inferior-posterior positioning of the hyoid bone is consistent with the measured pharyngeal constriction and may reflect co-existing obesity or could be an additional phenotype. By contributing to the clinical picture of WAGR syndrome, our preliminary findings may yield further insight into this complex condition.

## 2562/F/Poster Board #78

**A novel mutation in the SMC1A gene in a boy with Cornelia de Lange and severe clinical manifestations.** *F.J. Ramos[1,3], M.P. Ribate[1,2], C. Gil[2], M. Ciero[2], B. Puisac[2], M. Arnedo[2], J.C. de Karam[2], A. Luis-Díaz[2], J. Pie[1,2].* 1) Dpto. Pediatría, Facultad de Medicina, Universidad de Zaragoza, Zaragoza, Spain; 2) Laboratorio de Genética Clínica y Genómica Funcional, Dpto. Fisiología, Facultad de Medicina, Universidad de Zaragoza, Zaragoza, Spain; 3) Servicio de Pediatría, Hospital Clínico Universitario -Lozano Blesa- , Zaragoza, Spain.

Cornelia de Lange Syndrome (CdLS; OMIM 122470 and 300590) is an inherited multisystemic developmental disorder characterized by distinctive dysmorphic craniofacial features, growth and cognitive impairment and limb malformations. Mutations in two major genes (NIPBL and SMC1A) of the cohesin complex and its regulators have been found in about 60% of affected patients. To date, 11 different mutations in 14 unrelated individuals have been reported in the X-linked SMC1A gene. Here we present a 3 year-old male with mild CdLS facial appearance, microcephaly, postnatal growth retardation and moderate psychomotor retardation with absence of speech. He had no major limb defects, except for a bilateral brachyclinodactyly of the 5th finger and a 2-3 syndactyly in the feet. He also had congenital heart defect (ASD and PDA) and severe gastroesophageal reflux that caused Sandifer syndrome with cyanotic “seizure-like” episodes of up to a minute of duration. He was found to have a novel sporadic mutation in the SMC1A gene (p.R711Q) that altered a highly conserved residue and was not detected in his parents or in 50 control individuals. The mutation was located at the SMC coiled-coil domain and we hypothesize that it may affect its angulation within the proteína. This case underscores that, despite most mutations in the SMC1A gene generally cause a mild external phenotype, there are mutations that may result in severe physical and cognitive impairment. This work was in part supported by a grant from the Ministerio de Sanidad y Política Social of Spain (Ref. PI061343) and from the Gobierno de Aragón (Ref. B20).

## 2563/F/Poster Board #79

**SECKEL SYNDROME ASSOCIATED TO RENAL TUBULAR ACIDOSIS: TWO CASES REPORT AT THE HOSPITAL PARA EL NIÑO POBLANO, MEXICO.** *F.L.F. Hernandez[1], R.J.M Aparicio[2,3].* 1) Nephrology; 2) Medical Genetics, Hospital para el niño poblano, Mexico; 3) Estomatology, Benemerita Universidad Autonoma de Puebla.

INTRODUCTION. The Seckel syndrome or microcephalic primordial dwarfism is a congenital hereditary recessive disorder supposed to be caused by defects of genes on chromosome 3 and 18. One form of Seckel syndrome can be caused by mutation in the gene RAD3-related protein (ATR) which maps to chromosome 3q22.1-q24. This gene is central in the cell’s DNA damage response and repair mechanism. The main symptoms include severe mental retardation (more than half of the patients have an IQ below 50), achondroplasia, microcephaly, pancytopenia, cryptorchidism, low birth weight, dislocations of pelvis and elbow, unusually large eyes, low ears and small chin. CASE 1. A one and a half years old Male patient was estudied in 2005 at the Hospital due to low birth weitght, deshidratation and weight losses. He was diagnosed as Seckel syndrome with tubular acidosis, treated with sodium bicarnonate. Fenotipicallly, microcephaly, a bird-headed profile, unilateral ocular strabism, cryptorchidism and mental retardation. CASE 2. A one years old female patient was also estudies at the Hospital in 2005 due to low birth weitght, deshidratation and weight losses. She was diagnosed as Seckel syndrome with tubular acidosis, treated with sodium bicarnonate and metabolic acidosis with a normal GAP anion. Fenotipicallly, microcephaly, a bird-headed profile and and mental retardation. Both patients with normal Kidney structure reported with a renal ultrasound. CONCLUSIONS. Both patients were diagnosed as Seckel syndrome. However, both were treated with Sodium Bicarbonate, due to their association to Renal Tubular Acidosis. It would be important to find this renal alteration pathogenesis with this specific syndrome. REFERENCES. 1.- Cruz M, Bosch J. Atlas de síndromes pediátricos. EPAX 2003. Barcelona. Pág.68-69. 2. -Borglum AD., and cols. A new Locus for Seckel syndrome on chromosome 18p11.31-q11.2. Eur J Hum Genet 2001;9:753-757. 3. - Faivre L, and cols. Clinical and genetic heterogeneity of Seckel syndrome. Am J Med Genet 2002; 112:379-383.

## 2564/F/Poster Board #80

**Complex deletion/duplication rearrangement involving the X chromosome in a male with novel phenotype combining elements of Langer mesomelic dysplasia and severe rhizomelic shortening.** *R. Mendoza-Londono[1], L. Dupuis[1], P. Babyn[2], D.J. Stavropoulos[3].* 1) Division of Clinical & Metabolic Genetics and; 2) Department of Diagnostic Imaging and; 3) Department of Pediatric Laboratory Medicine; The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada.

Langer-type mesomelic dysplasia (MIM 249700) is a recessive skeletal dysplasia that results from haploinsufficiency for *SHOX*, located in the pseudoautosomal region of chromosomes X and Y. It is characterized by severe mesomelic shortening and micrognathia. Radiologic findings include: mesomelia with short and broad ulna and radii, marked shortening of the tibia and rudimentary fibula. The rest of the skeletal elements are usually not involved. We present the radiologic and cytogenetic findings in a four month old boy who was identified during the prenatal period to have rhizomelic shortening. On assessment at 4 months of age he had mildly dysmorphic features given by a broad nasal bridge, micrognathia and plagiocephaly. His extremities were significantly short. His radiographs showed asymmetric marked bilateral shortening of the femurs with a triangular shape. The tibias and fibulas were poorly modeled and had moderate shortening. The humerii, radius, ulna and radii were markedly short and bowed. Both hands had ulnar deviation. X-rays of the parents demonstrated bilateral Madelung deformities in the mother. Cytogenetic analysis revealed a male karyotype with an unbalanced X chromosome harboring a terminal deletion with breakpoint at Xp22.33. The deleted region is replaced by an additional copy of chromosome region Xq28 to Xqter [46,Y,der(X)(qter->q28::p22.33->qter).ish der(X)(pter-,qter++)]. Array CGH with an oligoarray platform confirmed the terminal deletion/duplication. The deletion involves 62 oligonucleotide probes (0.749 Mb) and results in the loss of the *SHOX* gene on the X chromosome. The terminal duplication involves 136 oligonucleotide probes (5.898 Mb) and results in the gain of one copy of approximately 108 genes. Molecular testing for *SHOX* mutations in the other allele is underway Our patient presents with a novel phenotype including features of Langer mesomelic dysplasia as well as atypical findings including severe rhizomelic shortening. His phenotype is likely the result of haploinsufficiency and excess dosage for genes located in the area of the deletion/duplication. Further delineation of his rearrangement is underway to allow the identification of genes that may be critical for the development of the limb’s proximal segment.

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## 2565/F/Poster Board #81

**A PATIENT WITH NEONATAL PROGEROID (WIEDEMANN-RAUTENSTRAUCH) SYNDROME AND COMPLEX CHROMOSOMAL REARRANGEMENT (CCR).** *A. Singer[1], K. Devriendt[2], D. Lev[3], C. Vinkler[3].* 1) Dept Clinical Gen Unit, Barzilai Med Ctr, Tel Aviv, Israel; 2) Kliniekhoofd, Centrum Menselijke Erfelijkheid, Leuven Belgium; 3) Institute of Medical Genetics Wolfson Medical Center, Holon, Israel.

The neonatal progeroid syndrome (NPS), or Wiedemann-Rautenstrauch (WRS), is a very rare autosomal recessive disorder. Approximately 30 patients with WRS have been reported. The clinical presentation is characterized by an appearance of premature aging, general absence of subcutaneous fat, prenatal growth deficiency, sparse scalp hair, eyebrows, and eyelashes, relative macrocephaly, persistent anterior fontanelle and natal teeth and mental retardation. We present a girl with a clinical diagnosis of NPS with deletion-duplication in chromosome 17. She is the first child of unrelated couple from Ukraine. Pregnancy was uneventful until shortly before 34w gestation when IUGR was diagnosed and early delivery was induced. Birth weight was 1230 gr, length was 37 cm, and head circumference was 28 cm. She had congenital glaucoma in her right eye and two natal teeth.. She underwent trabeculotomy and teeth excision. She had a small PDA and ASD. At the age of one year her height, weight and head circumference were 4SD below the average for her age and she was developmentally delayed. She still had widely open sutures and a permanently open fontanelle . Karyotype is: 46,XX. At the age of two years her weight, head circumference and height were around -6SD below the average for her age. She had prominent scalp veins, facial dysmorphism sparse eyebrows and hair and significant developmental delay. Brain MRI showed a Chiari 1 malformation, mild ventricular dilatation, and white matter changes. Hearing and vision were normal. Growth hormone and basal metabolic evaluation were normal. Array based genomic hybridization was done: 6q24.1(RP11-631F7)x1;17p13.3(RP11-216P6?RP11-233O10)x1;17p13.1(RP11-243K12?RP11-404G1)x3. Molecular karyotyping by means of BAC array with 1 Mb resolution revealed several chromosomal defects in this patient. (1) a submicroscopic microdeletion was detected on the long arm of chromosome 6. This microdeletion was inherited from the patient's father. (2) Both a deletion and a duplication were detected on the short arm of chromosome 17. These defects were confirmed by qPCR and occurred de novo. This is the first case of a complex chromosomal rearrangement associated with neonatal progeroid syndrome. Our data illustrate the important role for high-resolution investigation of apparently normal karyotype in patients with unexplained developmental delay and/or other clinical features.

## 2566/F/Poster Board #82

**Clinical and cytogenetic characterization of a familial duplication 16p11.2 using microarray-CGH technology. A new syndrome?** *A. Iglesias.* Dept Pediatrics, Div Genetics, Beth Israel Medical Ctr, New York, NY.

Duplications on chromosome 16p11.2 are rare, and have been considered non-pathogenic. However, recent reports have found them associated with an increase susceptibility for autism in about 1% of the cases. However, no evidence of developmental delay and/or dysmorphic phenotypes have been reported. A familial 16p11.2 duplication associated with a variable clinical presentation on the proband, her brother and her mother is described clinically and cytogenetically. The proband is a 26 month-old female, born at term after a pregnancy complicated for maternal seizure disorder that was well controlled. Delivery was vaginal. Birth weigh was 2.4 kg, length 45 cm and head circumference 32 cm. She was small for gestational age. Without complications she was sent home on day 2. By 9 months her development was delayed. On exam she was unable to sit without support. Language was also delayed. Weight was on the 5th percentile, length was normal and head circumference was 41 cm. Positive findings included microbrachicephaly, closed fontanels, hypertelorism, epichantal folds, downslanting palpebral fissures, and small nose with depressed nasal bridge, smooth philtrum, bowed upper lip and cupped ears. Axial hypotonia with normal strength and normal reflexes were found. Chromosome were normal, 46,XX. However, a microarray-CGH showed a duplication that at least 250 kb at band 16p11.2. Further evaluation of the proband at 13 months, showed no changes in her phenotype and mild progress in development. Maternal studies showed the same duplication. Moreover, the mother had a history of learning disability as a child and thereafter. In addition, she shares similar facial dysmorphic features with the proband. At the time of the maternal diagnosis, she was 7 months pregnant. She delivered a full term boy without complications. Microarray-CGH analysis showed the same duplication 16p11.2. On physical examination, the boy showed similar facial dysmorphic features, but normal development and growth by 2 months of age. He is currently on follow-up. To our knowledge, this is a unique report of a family carrying a duplication 16p11.2 associated with distinct physical dysmorphic features and developmental delay/learning disabilities; although the expression of the phenotype seems to be variable. Continuing follow-up of this family as well as further cases will give insights into this new syndromic condition associated with 16p11.2 duplication. .

## 2567/F/Poster Board #83

**A Case of XY Sex Reversal and Nail-Patella Syndrome Due to an Interstitial Deletion of Chromosome Region 9q33.3-9q34.11.** *D. Kostiner[1], C. Beattie[1], P. Jacky[2], D. Quigley[2], L. Jenkins[3].* 1) Dept. of Clinical Genetics, Kaiser Permanente PC, Portland, OR; 2) Dept. of Cytogenetics, Kaiser Permanente PC, Portland, OR; 3) TPMG Regional Genetics Laboratory, San Jose, CA.

We present the case of an XY female with mental retardation, small stature, skeletal defects and nail anomalies to add to the limited literature on 9q microdeletions involving the NR5A1 and LMX1B genes. The sex reversal in this pt. was known prenatally because genitals appeared female by ultrasound, but amniocentesis (performed for IUGR and club feet) revealed a 46,XY karyotype. The result was confirmed postnatally. Additional work-up revealed 11 pairs of ribs, absent ovaries, normal uterus, normal kidneys, and oddly-formed, hypoplastic nails of the thumbs and second digits. Smith-Lemli-Opitz was excluded with normal 7-dehydrocholesterol testing. Campomelic dysplasia was suspected, but sequencing of the SOX9 gene showed no mutation. At age 4 years, a CGH Array test was done. The result showed a 3.67Mb deletion from 9q33.3-9q34.11. Parental studies confirmed that the deletion was de novo. The deleted region contains at least 48 annotated genes, including the NR5A1 gene, reported to be associated with XY sex reversal, and the LMX1B gene, reported to be associated with nail-patella syndrome (although usually when mutated, not deleted). The literature reveals only a few other cases of 9q deletions involving these genes, but additional studies, such as "smallest region of overlap" (SRO) analysis, might further delineate the phenotypic features of these genes and others in the region.

## 2568/F/Poster Board #84

**Phenotypic comparison of Williams syndrome and its reciprocal duplication syndrome reveals significant differences in dysmorphic features, speech, cognitive ability, and type of anxiety.** *C. Morris[1], C. Mervis[2], S. Velleman[3], A. John[2], A. Currier[2], C. Rios[1], K. Kimberley[1], N. Crawford[2].* 1) Dept Pediatrics, Genetics Div, University of Nevada School of Medicine, Las Vegas, NV; 2) Dept. Psychology, University of Louisville, Louisville, KY; 3) Communication Disorders, University of Massachusetts, Amherst, MA.

Williams syndrome (WS) is caused by deletion of 25 genes on chromosome 7q11.23 mediated by nonallelic homologous recombination. Chromosome microarray technology resulted in detection of the reciprocal duplication of the region (Dup7). The study's purpose is to delineate the Dup7 phenotype, compare it to WS, and identify traits likely sensitive to gene dosage. Method: Physical, cognitive, language, and speech assessments were performed on 17 children and 6 adults with Dup7 and on 2 children with longer duplications including HSP27. Performance was compared to that of participants with WS. Results: Dup7: 11 males, 14 females; 11 cases sporadic, 14 familial. Dysmorphic features: prominent forehead (75%), high broad nose (70%), long columnella (52%), short philtrum (36%), facial asymmetry (95%). Birth defects were rare, including one person each with ASD/VSD, hydrocephalus, sagittal craniosynostosis, and severe micrognathia. Two had club feet. About 60% had ADHD, and 82% had anxiety (social phobia, separation anxiety). Mean standard scores on most measures were higher for the Dup7 group than the WS group. While the WS cognitive profile includes severe weakness in visuospatial construction, participants with DUP7 had DAS-II Spatial Cluster and VMI standard scores averaging ~20 points higher than for WS. Verbal standard scores averaged ~10 points higher than for the WS group. A particularly striking characteristic was current or former difficulty with speech; despite generally strong receptive language, 92% had severe speech delay and 95% had problems with motor speech and/or oral-motor movements. Some had symptoms of Childhood Apraxia of Speech. The combination of delayed speech and shyness/anxiety led to an inappropriate diagnosis of autism in some young children. Discussion: The facial phenotype of Dup7 is subtle, but recognizable. GTF2IRD1 has been implicated in the facial asymmetry in WS, and may be copy number sensitive, since it is a trait shared by the Dup7 group. Both groups share anxiety as a behavioral problem, but specific phobia is seen in WS, while separation and social anxiety are found in Dup7. The strong differences in speech and cognitive abilities between children with WS and those with Dup7 suggest that one or more genes in the WS region are dosage sensitive and that these genes, in transaction with other genes and the environment, are important for speech and cognitive development.

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## 2569/F/Poster Board #85

**Haemolytic uraemic syndrome in a patient with 46XX,del(10)(p13).** *F. Gök[1], S. Vurucu[2], S. Kozan[3], D. Gül[3].* 1) Pediatric Nephrology, Gulhane Military Medical Faculty, Ankara, Ankara, Turkey; 2) Department of Pediatric Neurology, GATA Medical Faculty, Ankara, Turkey; 3) Department of Medical Genetics, GATA Medical Faculty, Ankara, Turkey.

The patients with short arm deletion of chromosome 10 show high phenotypic variability, and until now more than 50 cases have been reported. We present a 5-year-old girl with a partial monosomy involving chromosome segment 10p13→pter. The proposita, first product of a second cousin marriage was referred with complaints of oliguria and hematuria persisting about 5 days followed by fever, vomiting and diarrhoea at her 16th month. She had hypocalsemic convulsion at her 3rd month. In addition to growth retardation, craniofacial features were remarkable: mild dysmorphism, microcephaly, trigonocephalic head, frontal bossing, hypertelorism, short and down-slanting palpebral fissures, epicanthus, ptosis, strabismus, high and broad nasal bridge with anteverted nostrils, small low-set pinnae, asymmetric facial appearance with right gum hypertrophy and skin hyperpigmentation in the left preauricular region , low-set and underdeveloped ears, prominent upper lip, micrognathia, high palate, and short neck. Widely spaced nipples, a gap between toes 1-2 and mild cutaneous syndactyly of toes 2-3 bilaterally and congenital hip dislocation at left were also present. Right kidney was absent. The diagnosis of haemolytic uraemic syndrome (HUS) was made on the basis of thrombocytopenia, microangiopathic haemolytic anaemia and acute renal failure, associated with diarrhoea. The karyotype was 46,XX,del(10)(p13). The karyotypes of parents were normal. We here suggest that haemolytic uraemic syndrome may be a part of the 10p13 deletion syndrome. Renal abnormalities should also be considered in cases of postnatal growth and mental retardation together with craniofacial dysmorphism.

## 2570/F/Poster Board #86

**Further characterization of a microdeletion syndrome involving 2p15-p16.1.** *T.M. Felix[1], A.L. Petrin[2], M.T.V. Sanseverino[1], J.C. Murray[2].* 1) Servico de Genetica Medica, Hospital Clinicas Porto Alegre, Porto Alegre, RS, Brazil; 2) Department of Pediatrics, University of Iowa, Iowa City, IA, USA.

Screening of the whole genome for submicroscopic abnormalities in patients with multiple congenital anomalies and mental retardation (MCA/MR) using new technologies such as array-CGH or single nucleotide polymorphism (SNP) arrays has increased the number of previously unrecognized cytogenetic abnormalities. Recently, the use of array CGH contributed to the description of a new microdeletion syndrome involving the region 2p15-p16.1 (Rajcan-Separovic et al., 2008). The authors described two unrelated patients with a 4.5 and 5.7 Mb deletion respectively presenting with microcephaly, ptosis, short palpebral fissures, broad and high nasal root, camptodactyly, cortical dysplasia and mental retardation. A third case was described presenting with a 570 kb deletion in the 2p15 region (Chabchoub et al., 2008). We report a patient with mental retardation, prenatal and postnatal growth deficiency, microcephaly, ptosis, a high and broad nasal root and camptodactyly. Analysis of Affymetrix genome-wide human SNP array 6.0 showed a 3.35Mb deletion in 2p15-p16.1. Studies of selected SNPs in the deleted area in the proband and her parents showed Mendelian errors compatible with a paternal origin of the deletion. Based on the three cases described previously in the literature we narrowed down the critical region responsible for the 2p15-p16.1 microdeletion syndrome to the position 59,200,000 and 61,700,000. The common deleted area included several known genes with different functions. One of those genes is CCT4, which assists in the folding of actin and tubulin. We suggest that haploinsufficiency of CCT4 gene may play a role in the camptodactyly of 2p15-p16.1 syndrome and may be a good candidate gene for still unknown arthrogryposis syndromes.

## 2571/F/Poster Board #87

**Microdeletion 1q21.1: A locus newly found to be associated with wide phenotypic variability and reduced penetrance.** *T. Jewett[1], C. Haldeman-Englert[2], M.N. Berry[1], L. Terry[1].* 1) Dept Pediatrics, Med Gen, Wake Forest Univ Sch Med, Winston-Salem, NC; 2) Div Hum Gen, Children's Hospital of Philadelphia, Philadelphia, PA.

1q21.1 is a region rich in segmental duplications and low-copy repeats, predisposing to rearrangements at this site. Recurrent copy number abnormalities at this locus have recently been reported in association with numerous outcomes ranging from a normal phenotype to neuropsychiatric disorders to multiple congenital anomalies and/or intellectual disability. We present 5 individuals in 2 families with overlapping microdeletions of 1q21.1, each of whom has a distinctive phenotype, demonstrating the difficulty in predicting natural history. MK is a 2 4/12 year old girl who was evaluated in genetics clinic initially at 7 months of age due to congenital bilateral ptosis of the eyelid requiring surgical correction as well as laryngomalacia, gastroesophageal reflux, and mild developmental delays. Family history disclosed that MK's mother had ptosis of the left eyelid in childhood, microcephaly, and a history of learning difficulties warranting special education classes. MK's mother has a female paternal first cousin who is healthy and cognitively normal whose daughter, TS, has multiple anomalies including microcephaly, bilateral complete coloboma of the eye, postaxial polydactyly of the hands, and delayed development. Genetic testing has revealed that both girls and their mothers have a similar submicroscopic 1q21.1 deletion using array comparative genomic hybridization (CGH). SH is a 2 10/12 year old boy who was first evaluated by a medical geneticist at 8 months of age during his third hospital admission for failure to thrive. Physical exam on SH showed all growth parameters <3%, hypoplastic midface with epicanthal folds and telecanthus, broad nasal bridge and full nasal tip, and mild motor delays. Genetic testing revealed a submicroscopic 1q21.1 deletion using array CGH. His mother was tested for the deletion and is normal; father is unavailable for testing. The cases presented here further expand the spectrum of phenotypes associated with 1q21.1 microdeletions detected by array CGH and highlight the importance of carefully reviewing clinical and laboratory data in patients with genomic rearrangements. We will discuss the specific copy number abnormality discovered in our patients vis-à-vis those recently reported, together with possible mechanisms that account for variability and reduced penetrance.

## 2572/F/Poster Board #88

**Microdeletion of chromosome 9p21.1 associated with eye anomalies and mild developmental delays.** *M.L. Loscalzo[1,2], T.A. Becker[2], M. Sutcliffe[1,2].* 1) Dept Pediatrics, Univ South Florida Sch Med, St Petersburg, FL; 2) Ciinical Genetics, All Children's Hospital; St. Petersburg, FL.

An four year old girl presents with multiple eye anomalies including unilateral retinal detachment with chronic band keratopahy of the right eye and unilateral optic nerve coloboma on the left. This has been accompanied by mild developmental delay and regression particularly in vocal articulation. She currently is 8 ½ years old. She functions at approximately a first to second grade level. She has recently developed hypothyroidism. A routine G-banded karyotype was normal as was PAX6 DNA testing. Array comparative genomic hybridization showed an interstitial loss in copy number in the short arm of chromosome 9 at band p21.1. Parental studies ate pending. These results suggest a potential critical region for eye anomalies such as colobomas. Further investigation will be needed to investigate candidate genes in the region that may be associated with these features.

## 2573/F/Poster Board #89

**Chromosome 1q21.1 deletion: expanding the phenotype.** *J. McGaughran[1], G. Peters[2].* 1) Gen Hlth Queensland, Royal Brisbane and Women's Hospital, Brisbane, Australia; 2) Cytogenetics Dept, Children's Hospital at Westmead, Sydney, Australia.

Deletion of chromosome 1q21.1, detected using array comparative genomic hybridisation, is a newly-described molecular lesion that has a variable phenotype. The phenotype can vary from apparently unaffected carriers to individuals with intellectual impairment, congenital cardiac disease and limb anomalies. A male patient is described with a deletion of 1q21.1. He had significant short stature, ADHD and a number of dysmorphic features. He had a high narrow palate, bilateral preauricular tags, a supernumerary left nipple, small cafe au lait patch on his back and left 2/3 toe syndactyly. He also had high myopia. His features further expand the range seen in 1q21.1 deletion.

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## 2574/F/Poster Board #90

**Identification of Unrecognized Submicroscopic Chromosomal Imbalances Associated with Human Disorders of Male Urogenital Development.** *M. Tannour-Louet[1], S. Han[2], S. Corbett[1], S. Yatsenko[3], L. Meyers[3], S.H. Kang[3], S.W. Cheung[3], D. Lamb[1,2].* 1) Scott Department of Urology, Baylor College of Medicine, Houston, TX; 2) Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Congenital abnormalities of the male urogenital tract, including undescended testicles, abnormal development of the penis/urethra and ambiguity of external genitalia, are among the most common human birth defects. The etiology of these reproductive disorders remains poorly understood. By using a clinically validated comparative genomic hybridization microarray (CMA), we reveal the existence of cryptic chromosomal rearrangements that totally evaded detection by high resolution karyotype in children born with cryptorchidism, hypospadias or genital ambiguity. Imbalances were found in about 31% of the 114 patients analyzed. Anomalies were scattered across the genome but notably clustered in gene- enriched subtelomeric loci. De novo duplications and deletions events were noted on 1p36.33, 9p23p24 and 19q12-q13.11 for ambiguous genitalia and 10p14 and Xq28 for cryptorchidism and 12p13 and 16p11.2 for hypospadias. The causality of the copy number variants was supported by the presence of common chromosomal rearrangements encompassing candidate genes such as FGFR2, DMRT1 and ZEB2 in several unrelated patients. These findings should have significant consequences for clinical diagnosis and genetic counseling and will pave the way for the elucidation of the molecular mechanisms underlying the pathogenesis of congenital urogenital defects.

## 2575/F/Poster Board #91

**Deletion of the SOX5 gene at 12p12.1 is associated with autistic-like behavior and severe language delays.** *A.N. Lamb[1], J. Rosenfeld[1], D. Keelean-Fuller[2], Z. Fan[2], J. Pouncey[3], K. Stevens[3], L. Mackay-Loder[4], D. Terespolsky[4], K. Rosenbaum[5], S. Vallee[6], J. Moeschler[6], R. Dineen[7], G. Hoganson[7], S. Girirajan[8], E.E. Eichler[8], B.A. Torchia[1], L.G. Shaffer[1].* 1) Signature Genomics, Spokane, WA; 2) University of North Carolina, Chapel Hill, NC; 3) TC Thompson Children's Hospital, Chattanooga, TN; 4) Credit Valley Hospital, Mississauga, ON; 5) Children's National Medical Center, Washington, DC; 6) Dartmouth-Hitchcock Medical Center, Lebanon, NH; 7) University of Illinois, Chicago, IL; 8) University of Washington, Seattle, WA.

SOX5 is a member of the Sox (SRY-related HMG box) family of transcription factors that is involved in the regulation of chondrogenesis and in the development of the central and peripheral nervous system. SOX5 spans about 1 Mb in band 12p12.1. Although infrequent, some larger cytogenetically visible deletions of the chromosome 12 short arm have been described, but there is a lack of published submicroscopic deletion cases characterized by array CGH. We describe four patients with small deletions within the SOX5 gene - two de novo and one inherited from a phenotypically abnormal mother - and four larger deletions (5.7, 5.4, 10.5, 8.7 Mbs) that include the complete SOX5 gene. Of the four patients with the small deletion, clinical information was available for two. Both had autistic-like features with severe delays in language development. Dysmorphic features tended to be minor, with butterfly vertebrae of the thoracic spine and mild scoliosis, and small simple ears and low nasal bridge in one patient. Both had low, normal growth for wt, ht, and head circumference. The 3rd patient with a small deletion had an indication of developmental delay and failure to thrive, and was inherited from the mother (4th patient) with developmental delay (details not yet available). In the individuals with larger deletions, the language impairment was also observed with severe speech delay in three (no details on 4th yet available) and developmental delays in all. No aggressive behavior was noted in this group. Other clinical features identified in at least two individuals included mild facial features, MRI abnormalities, digital anomalies, and minor spinal anomalies. Data on deletions in the SOX5 gene from a control population of 5674 samples (Itsara et al, 2009) show only one deletion in an intron of the gene. These cases suggest that deletions of SOX5 may represent a new microdeletion syndrome with autistic-like behavior and severe language delays, and larger deletions encompassing the gene also have additional dysmorphic features.

## 2576/F/Poster Board #92

**2q23.1 microdeletion involving the MBD5 gene - large deletion associated with a relatively mild phenotype.** *HYB. Chung[1], DJ. Stavropoulos[2], R. Weksberg[1], G. Yoon[1,3].* 1) Clinical & Metabolic Gen, Hosp Sick Children, Toronto, Canada; 2) Department of Paediatric Laboratory Medicine, Hospital for Sick Children, University of Toronto, Toronto, Canada; 3) Department of Paediatrics, Division of Neurology, Hospital for Sick Children, University of Toronto, Toronto, Canada.

Background: We report a female patient with a de novo interstitial deletion in chromosome region 2q23.1-23.3 identified by array-CGH. To our knowledge, this is the 6th reported case of a patient with a deletion corresponding to the same genomic region containing the MBD5 gene, a homologue of MECP2, implicated in the pathogenesis of Rett syndrome. Case Report: The proband was born at 41 weeks to healthy non-consanguineous Caucasian parents, with a birth weight of 9lb 1oz. She had significant global developmental delay affecting gross motor, fine motor and speech. She developed seizures at 3 years, which were not controlled despite trials of multiple antiepileptics. There was history of regression at age 6, with progressive difficulties with balance, loss of fine motor skills and worsening aggressive behavior. On examination at age 7 years, 3 months, she was profoundly microcephalic (HC << 2nd percentile, 50th centile for 13 months) and of short stature (height at 3rd percentile), while her weight was at the 75th percentile. She had dysmorphic features consisting of low anterior hairline, mild synophrys, almond-shaped eyes, long nasal columella, short philtrum, thin upper lip, and small teeth. On neurological exam, speech was dysarthric but she was able to speak in complete sentences. She demonstrated repetitive hand movements but did not have the wringing movements typical of Rett syndrome. Gait was wide-based and unsteady, and she held her arms in high guard when walking. Results: Karyotype, MECP2 testing, chromosome 15 methylation studies, UBE3A sequencing, MRI/MRS brain, and metabolic studies were all normal. BAC microarray revealed a de-novo deletion at 2q23.1 to 2q23.3 with an estimated size between 3.725 - 5.632Mb. Conclusion: Published cases of 2q23.1 deletion (Vissers et al 2003, Koolen et al 2004, De Vries et al 2005, De Gregori et al 2007, Jaillard et al 2008) suggest an overlapping region of 250 kb involving the MBD5 gene is responsible for the phenotype. Our patient shares similar clinical features to those reported, including microcephaly, developmental delay, speech impairment, seizure and ataxia. She differs by having milder delay in development, with reasonably preserved speech. Interestingly, she had a larger deletion compared to the reported cases, with telomeric extension involving the CACNB4 gene. This case adds to the phenotypic heterogeneity of the newly described "Angelman-like" or "Rett-like" microdeletion syndrome.

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## 2577/F/Poster Board #93

**Situs inversus totalis in a fetus with a deletion at 7q36.2 detected on microarray analysis.** *G. Andelfinger[3], M.P. Hitz[3], S. Keating[4], J. Mercier[3], R. Teitelbaum[2], A. Richter[3], H.Y.B. Chung[1,2], D. Chitayat[1,2].* 1) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, Ontario, Canada; 2) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Ontario, Canada; 3) Department of Pediatrics, CHU Sainte Justine, Montreal, Quebec, Canada; 4) Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada.

Laterality disorders are a heterogeneous group of disorders associated with maternal diseases (maternal IDDM), maternal exposures to teratogens (retinoic acid), chromosome abnormalities and single gene disorders (ZIC3 mutation). We report on a fetus with a submicroscopic deletion at 7q36.2 with right atrial isomerism (RAI). CASE:The mother was a 30y G4P1SA2L1 and the father was 35y. Both were healthy and non-consanguineous and their family history was non-contributory. They had a healthy son and daughter and had two miscarriages. MSS was negative. Detailed fetal U/S and echocardiography at 20w showed: RAI with levocardia, L-sided liver and R-sided stomach with common atrium, unbalanced AVSD, hypoplastic LV, DORV, unobstructed aorta arising anteriorly from RV, hypoplastic pulmonic valve and branched pulmonary arteries. The pulmonary veins drained into a confluence and entered the back of the common atrium slightly L- sided. The couple decided to terminate the pregnancy. The autopsy confirmed the ultrasound findings and in addition, detected trilobed L lung, asplenia and bilateral eparterial bronchi. The fetal karyotype & FISH for 22q11.2 were normal. Microarray analysis (SignatureGenomic®) showed deletion at 7q36.2. FISH analysis showed that the father is mosaic for this deletion. CONCLUSION:Genome-wide, array-based CNV confirms the presence of the deletion, shared by affected fetus and his father. Overlapping deletions described in the Decipher database do not show the same phenotype. Based on the deleted interval in the affected fetus, all isoforms of the DPP6 gene are predicted to be haploinsufficient. Looking at the other de novo and shared CNVs, we could not identify other candidate gene directly involved in left right axis patterning. However, a de novo deletion of the KCNC3 gene was detected. Interestingly, DPP6 interacts with the potassium channels of the KV- type. Although these channels have not been described to play a role in LR patterning, several other potassium channels do play a role in LR asymmetry in model organisms. These studies propose a mechanism in which a change in potassium current at the cell membrane leading to a change in the movement of the cilia which are crucial for proper left right axis formation. Thus, the deletion of KCNC3 and DPP6 may act in an epistatic fashion, consistent with a two-hit model affecting potassium channel assembly and, subsequently, cilia movement and LR patterning.

## 2578/F/Poster Board #94

**The first reported case of the DRAGON gene deletion in human. A case with a de-novo interstitial deletion of chromosome 5q15-21.1.** *G. Brenner[4], H.Y.B. Chung[1,2], P. Shannon[3], A. Toi[5], L. Shaffer[6], D. Chitayat[1,2].* 1) Department of Pediatrics, Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, Canada; 2) Mount Sinai Hospital, The Prenatal Diagnosis and Medical Genetics Program, Toronto, Canada; 3) The Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, University of Toronto, Toronto, Otario, Canada; 4) Department of Anesthesia and Critical Care, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA; 5) The Department of Diagnostic Imaging, Mount Sinai Hospital,University of Toronto, Toronto, Ontario, Canada;; 6) Signature Genomic Laboratories, Spokane, Washington, USA.

Chondrodysplasia punctata (CDP) is an etiologically heterogenous condition caused by single gene disorders, chromosome abnormalities, maternal diseases or exposures to teratogens. We report a male fetus with rhizomelic CDP associated with deletion at 5q15-5q21.1. This segment contains the DRAGON gene, a bone morphogenetic factor co-receptor, also known as RGMb (repulsive guidance molecule b). It is postulated that its haplo-insufficiency is associated with the phenotype in this fetus. The mother (30yo, G2P0SA1L0) was referred at 19.3 weeks for abnormal antenatal ultrasound findings of short limbs, short splayed digits, brachycephaly, small cistern magna, hypoplastic inferior cerebellar vermis, micrognathia, multiple intra-cardiac echogenic foci and 2-vessel umbilical cord. There was no history of maternal disease/ exposures. The pregnancy was terminated at 21 weeks. Autopsy confirmed the ultrasound findings and in addition showed brain hypomyelination with ventriculomegaly. Biochemical studies revealed normal plasmalogen and cholesterol biosynthetic function. Radiograph showed numerous abnormal calcific stipplings and prematurely calcified ossification centers. There was rhizomelic shortening of proximal long bones, abnormal calcification/ossification in the cervical, thoracic and sacral spine and poorly formed with platyspondyly of the cervical and thoracic spine with thin ribs. The findings were in keeping with rhizomelic CPD. The Agilent 105K array (Signature Genomics) shows a de novo 3.4Mb deletion at 5q15-5q21.1. The region does not contain any OMIM disease genes and has not been reported in the DECIPHER Initiative. It contains 3 genes including the DRAGON (RGMB) gene, which is of interest as its encoding protein is a bone morphogenetic protein (BMP) co-receptor. BMPs induce ectopic bone and cartilage formation in experimental animals. They are important for skeletal patterning and are involved in the development of many tissues including bones, craniofacial structures and nervous system. As a co-receptor for BMPs, DRAGON is important in neural and neural crest patterning early in development. DRAGON is also found to be expressed in brachial arches, somites and the tail bud of developing mouse and Xenopus but its function in skeletal patterning is not well described. Since a copy of the DRAGON gene is deleted in our patient, we postulate that haplo-insufficiency of this gene is related to the pathogenesis of the CDP phenotype.

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## 2579/F/Poster Board #95

**Molecular genetic study on a new case of a fetus prenatally diagnosed with Agnathia-Otocephaly.** *F. Morin[1], J. Gekas[2], D. Kamnasaran[1,3].* 1) Laval Hospital Research Centre, Quebec, Quebec, Canada; 2) Human Genetics Research Unit, Centre de recherche de l'Hôpital Saint-François d'Assise, Quebec, Canada; 3) Department of Pediatrics, Laval University, Quebec, Canada.

Otocephaly is a severe and lethal malformation of the first and second branchial arches in the developing fetus, with a prevalence of ~ 1 to 60 out of every 70,000 pregnancies. These cases are sporadic with an etiology associated with both genetic and environmental factors. We report a new case of a fetus prenatally diagnosed with Agnathia-Otocephaly from a 24-year-old G1 P0 woman. The family history was unremarkable and the mother was healthy during the pregnancy with no use of medications or abusive substances. The fetus presented striking Otocephalic features including agnathia, astomia with a hypoplastic oropharynx cavity, synotia, and a proboscis. There was no evidence of fetal maceration. In addition, all organs were normal. Comprehensive cytogenetic analyses found a normal karyotype in the fetus (46,XX) with no evidence of deletions, duplications, inversions or complex rearrangements. We first examined the OTX2 gene, encoding a transcription factor, mapping to human chromosome 14q23, as a candidate. Mice that are heterozygous for a null allele in the Otx2 orthologue are born with Otocephalic features (Genes Dev. 1995 9(21):2646-58). Unfortunately, we were unable to identify any evidence of mutations in this gene in the fetal DNA. However, we believe that this finding is not surprising since patients born with human chromosome 14 aberrations have not yet been reported with Otocephalic clinical features. We also considered the human PGAP1 gene, which maps to human chromosome 2q33, since mice that are heterozygous for a null allele of the Pgap1 orthologue are also born with Otocephalic features (J Biol Chem. 2007 282(42):30373-80). We were however unable to screen this gene for mutations in the fetal DNA due its large size (> 27 exons encompassing >11 kb of coding sequence). In addition, we believe that the human PGAP1 gene is not associated with the phenotype in our fetus, since patients born with aberrations of human chromosome 2 have not yet been described to date with Otocephalic clinical features. We suggest that the Agnathia-Otocephaly phenotype in our fetus is due to a mutation in a yet to be identified gene. Since no gene or chromosome aberrations are yet known to be definitively associated with Otocephaly in humans, the use of ultrasonography on pregnant mothers will have to prevail as the current preferred choice for the antenatal diagnosis of these cases.

## 2580/F/Poster Board #96

**Microdeletion 2p16.3 Associated with Familial Multi-Organ Cystic Disease in the Females without Hepatic and Renal Involvement.** *C. Pan[1], C. Li[2].* 1) McGill University, QC, Canada; 2) Pediatrics, McMaster Children's Hosp, Hamilton, ON, Canada.

We report familial multi-organ cystic disease (MOCD) in a 5-generation pedigree of Spanish/German and Irish descent. Multiple individuals, aged from newborn with prenatal onset to 72 years of age are affected with cystic changes variably involving the brain, the spine, the ovary and uterine, the cervix, the breast, the stomach, the eye, and the carotid artery. The proband was a baby girl with symblepharon, periventricular cysts and ovarian cysts. Array CGH analysis revealed a 483-kb deletion of 2p16.3 involving the first four exons of NRXN1, a gene noted to be linked to autism, mental retardation, vertebral anomalies and schizophrenia by a number of studies. This same deletion was also found in her 27 year-old mother who has cystic ovaries, carotid cyst, brain cyst, mood disorder, seizure disorder and acidic sweat. She also reported to be a hyperactive and socially inadequate child. The family history is remarkable for multiple individuals clinically manifesting with migraine headaches, psychiatric (primarily mood disorders) and fertility problems associated variably with cysts involving multiple organs. They also appear to have a higher incidence of benign and malignant tumors associated with these cysts. Additionally, several affected individuals have been reported to have abnormal "acidic sweat". All affected individuals are of normal intelligence and are non-dysmorphic. Interestingly the kidneys and the liver are not known to be affected. The family history is also negative for other birth defects, recurrence miscarriages, stillbirth, dementia and sudden death. Of interest, all eight affected individuals are females spanning five generations. At least one obligate man carrier who has affected mother, sister and daughter has no clinical manifestations at the age of 51. Further investigations of male relatives are pending. In conclusion, del2p16.6 involving the NRXN1 gene in this pedigree has not been associated with autism, mental retardation, vertebral anomalies and schizophrenia. Rather, mood disorder without psychosis, and cystic changes involving multiple organs, in particular the ovaries, the breast and the brain, but not the kidneys and the liver, are linked to this particular microdeletion. Whether the penetrance of this deletion is sex-dependent or sex-influenced or not remains to be determined.

## 2581/F/Poster Board #97

**Congenital Diaphragmatic Hernia and Multiple Congenital Anomalies due to 4p duplication/Xp deletion syndrome.** *M. Dasouki[1], L. Cooley[2], A. Parthiban[1], P. Parimi[1], I. Ekekezie[2], E. Taboada[2].* 1) Dept Pediatrics, Univ Kansas Med Ctr, Kansas City, KS; 2) University of Missouri Kansas City Children's Mercy Hospital Kansas City, MO.

Neonatal congenital diaphragmatic hernia (CDH) is a serious malformation when encountered as part of a multiple congenital anomalies syndrome. Approximately 40%-50% of affected individuals have complex CDH as part of a single gene disorder or chromosomal abnormality. Various chromosomal malformation syndromes in which CDH rarely occurs include autosomal trisomies (13,18, 21) and deletion 4p16 (Wolf-Hirschhorn syndrome). Three genomic loci for diaphragmatic hernia (DIH1, DIH2, DIH3) had been mapped to chromosomes 15q26.1, 8p23.1 and 8q23 respectively. We report a Caucasian female newborn with multiple congenital anomalies that included CDH diagnosed prenatally at 32 weeks gestation. The fetus also had features of Fryns syndrome and Turner syndrome. She died at 80 days of life following surgical closure of her CDH which was complicated by ECMO support and Pseudomonas sepsis. Autopsy showed bifid uvula, short neck, short distal phalanges and dysplastic nails, hypoplastic and abnormally lobulated left lung, abnormal left hepatic lobulation, midgut malrotation, small accessory spleens, large patent ductus arteriosus, coarctation of the aorta, fibrotic & myxomatous dysplastic cardiac valves leaflets, bilateral streak gonads and small uterus. Blood chromosome and FISH analyses showed a de-novo deletion of Xp and partial trisomy of chromosome 4 from an unbalanced translocation [46,X,-X,+der(4)t(X;4)(q12;q21.3).ish der(4)t(X;4)(wcpX+,wcp4+,DXZ1-,CEP4+,XIST+). X-chromosome inactivation studies were not done. We predict that complete inactivation of the derivative chromosome 4 which retained the XIST locus would have produced the equivalent of monosomy X and Turner syndrome phenotype. The spectrum of anomalies found in this child suggests that the derivative chromosome 4 remained active. The rare occurrence of CDH in children with Wolf-Hirschhorn syndrome coupled with our observation of duplication of "4pter-q21.3" suggests that a gene locus for CDH may exist on chromosome 4 and probably within 4p16-pter. Studies of gene expression in the developing diaphragm have recently been reported (Am J Physiol Lung Cell Mol Physiol 294: L665-L675, 2008). In Silico analysis of these data may help narrow this putative "CDH" gene locus.

## 2582/F/Poster Board #98

**Genetic analysis of Midline Facial Defects with Hypertelorism patients detected two submicroscopic chromosomal rearrangements in different locations of the genome.** *E.L. Freitas[1,4], S.M. Gribble[2], M. Simioni[1], T.P Vieira[1], E. Prigmore[2], D.G. Melo[3], A.C. Krepischi-Santos[4], C. Rosenberg[4], N.P. Carter[2], V.L. Gil-da-Silva-Lopes[1].* 1) Departamento de Genética Médica, Faculdade de Ciências Médicas, UNICAMP, Campinas , SP, Brazil; 2) The Wellcome Sanger Institute, Hinxton, Cambridge, United Kingdom; 3) Centro de Ciências Biológicas e da Saúde, Departamento de Medicina, UFSCAR, São Carlos, SP, Brazil; 4) Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, USP, São Paulo, SP, Brazil.

The Midline Facial Defects with Hipertelorism (MFDH) are a rare and heterogeneous group of craniofacial disorders mainly characterized by ocular hypertelorism and bifid nose. The pathogenesis of these conditions is still unknown. We excluded monogenic disorders and weel-known syndromes in all 14 individuals in this study. They were previously investigated by clinical, dysmorphologic and neurological evaluation, skull and facial X-rays, CT and MRI of the brain, and ophthalmologic and otorhynolaringologic evaluation. They also had normal karyotype. The evaluations demonstrate facial alterations, structural and functional anomalies of the central nervous system, indicating, mainly, cortical migrations errors, perfusion variances and cerebella involvement. Based upon these observations and review of the literature, we determined an initial molecular investigation strategy. The phenotypes suggested genes related to face and CNS development such as SHH, FGF8 and PAX3 may be involved in these disorders. These genes have been reported to participate in embryological development and are associated with some syndromes with craniofacial anomalies. Therefore, the objectives of this study were to investigate the etiology of the MFDH affected individuals through SHH, FGF8 and PAX3 candidate genes study and to identify possible chromosomal anomalies using array-CGH. The SHH, FGF8 and PAX3 genes were screened by direct sequencing and to complement these studies, the whole genome tiling path array-CGH technique was performed and the results were confirmed by automatic genotyping and FISH. It was not possible to relate any punctual mutation in the studied genes associated to the malformations since none pathogenetic alteration was found. Although, two chromosomal anomalies in different locations of the genome were detected in MFDH patients: a partial monosomy 9 and partial trisomy 20 derived from a maternal translocation (9;20)(p24;p13); and a familial case with interstitial deletion of chromosome 2 region (2q36.1-2q36.3). In the latter case, the PAX3 gene was inside the deleted region, supporting the initial hypothesis that some development genes are involved in the MFDH etiology. Several CNV described in HapMap were also found. Therefore, it could be concluded that in MFDH patients, the array-CGH investigation can be useful to detect a wide range of chromosomal anomalies and the genetic counseling must be individualized.

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## 2583/F/Poster Board #99

**Interstitial Microduplication of Xq27.3 in a Male with Mental Retardation, PDD-NOS, Absent Speech, and Overgrowth.** 
*A. Battaglia[1,2], A. Novelli[3], E. Santocchi[1], L. Bernardini[3], A. Capalbo[3], R. Tancredi[1].* 1) Stella Maris Clinical Research Inst. for Child & Adolescent Neuropsychiatry, Calambrone, Pisa, Italy; 2) Division of Medical Genetics, Dept Pediatrics, University of Utah, SLC, UT, USA; 3) CSS-IRCCS Hospital S. Giovanni Rotondo & CSS Mendel Inst., Rome, Italy.

In XY males, duplication of any part of the X chromosome, but the pseudoautosomal region, leads to functional disomy of the corresponding genes. We describe a male patient with severe mental retardation, PDD-NOS with absent speech, overgrowth, and minor anomalies. Using whole genome array comparative genomic hybridization (array CGH) at a resolution of about 10 Kb (244K chip; Agilent Techonologies), fluorescent in situ hybridization (FISH), and quantitative PCR, we have identified and characterized an interstitial microduplication of Xq27.3 (approx. 0.7 Mb in size), inherited from the healthy mother. The X inactivation test showed that in the mother the chromosome X carrying the duplication was preferentially inactivated. The duplicated segment encompasses 2 RefSeq genes: SPANX-N1, encoding a sperm protein, and SLITRK2. This latter gene is a member of the SLITRK integral membrane proteins with 2 N-terminal leucine-rich repeat (LRR) domains. Most SLITRKs, including SLITRK2, also have C-terminal regions that share homology with neurotrophin receptors. SLITRKs are expressed predominantly in neural tissues and have neurite-modulating activity. In the mouse, slitrk2 suppresses neurite outgrowth, including cAMP-induced outgrowth. In situ hybridization of developing mouse brain shows broad Slitrk2 expression, mainly in the ventricular layer, subventricular zone, cortical plate, pyramidal layer of hippocampus, subicular neuroepithelium, thalamus, hypothalamus, and spinal cord. Highest SLITRK2 expression in adult is in the cerebral cortex. Of note, LRR-genes have been associated with PDD susceptibility in populations of European ancestry.

## 2584/F/Poster Board #100

**Congenital valproate syndrome or unknown epilepsy syndrome.** 
*D. Albu[1], C. Albu[1], E. Severin[1], A. Toma[2].* 1) Dept Human Genetics, Carol Davila Univ Med & Pharm, Bucharest, Romania; 2) Panait Sirbu Hospital, Bucharest, Romania.

Aim: to present an unusual case of dysmorphic newborn born from a mother with epilepsy treated with Valproate Material and method: repeated clinical examinations of the newborn were performed. A head ultrasound, a heart ultrasound and an abdominal ultrasound were performed too. In order to exclude any chromosomal abnormality, a karyotype has been made. Results: The case of a term newborn is presented. The mother was known with epilepsy, grand mal, the first episode as a teenager. She is presently in treatment with Valproate, 1500 mg/day (upper limit of safe dose) The neonate presented with a particular facies, proeminent forehead, hypertelorism, low set ears, palmar and plantar grif. The ultrasounds undertaken excluded a head, heart or internal organ malformation. The neurological exam revealed a neonate with axial and periferic hypotonia. The karyotype indicated a normal male, 46 xy. The facial and neurologic features are not consistent with congenital valproate syndrome. Conclusions: This could be a new case of a syndrome of facial dysmorphic features and neurologic deficit, occurring in a mother with neurological deficit (epilepsy), not associated with chromosomal abnormalities.

## 2585/F/Poster Board #101

**A new case of CLSD implicates genetic heterogeneity.** 
*S. KIM[1], J. Liu[1], A. Hata[1], C. Haldeman-Englert[2], E. Zackai[2], S. Hamamoto[3], R. Schekman[3], J. Kim[1], S. Boyadjiev[1].* 1) Section of Genetics, Departmento of Pediatrics, University of California-Davis Medical Center, Sacramento, CA 95817, USA; 2) Chilren's Hospital of Philadelphia; 3) Department of Molecular and Cell Biology, Howard Hughes Medical Institute, University of California, Berkeley, CA 94720, USA.

We identified a novel autosomal recessive syndrome, cranio-lenticulo-sutural dysplasia (CLSD, Boyadjiev-Jabs syndrome) due to SEC23A missense mutation in a large consanguine family. Characteristic features of this disease are late-closing fontanels, sutural cataracts, facial dysmorphisms and skeletal defects. Prominent cellular features of skin fibroblasts from these patients are secretion defect and marked distension of the endoplasmic reticulum (ER), concordant with the function of SEC23A in protein export from the ER. A second CLSD case with a heterozygous mutation M702V in a highly conserved region of SEC23A was recently identified by our group. The M702V allele was inherited from his clinically unaffected father and no maternal mutation has been identified so far. Cultured skin fibroblasts from this new patient show secretion defect of collagen and enlarged ER, suggesting that other mutations of maternal origin must contribute to this autosomal recessive disease. Indeed, the expression levels of SEC24A and SEC24B COPII components were significantly reduced in the fibroblasts of the M702V patient. These results suggest that compound deficiency of COPII components is not lethal and can synthetically cause craniofacial anomalies. Collectively, our data suggest that defective ER export is a common basis for CLSD. We have obtained parental fibroblasts and are in the process of analyzing them. We are also sequencing SEC24A and SEC24B to identify possible mutations.

## 2586/F/Poster Board #102

**Severe Non-Lethal Recessive Type VIII Osteogenesis Imperfecta: Clinical, Radiographic, Biochemical and Histological Features.** 
*J. Marini[1], W. Chang[1], F.H. Glorieux[2], T.E. Hefferan[3], F. Rauch[2], M. Abukhaled[1], P.A. Smith[4], D. Eyre[5].* 1) Bone/Extracellular Matrix, NICHD/NIH, Bethesda, MD; 2) Shriners Hospital for Children, McGill Univ, Montreal, Quebec; 3) Mayo Clinic, Rochester, MN; 4) Shriners Hospital for Children, Chicago, IL; 5) Univ Washington, Seattle, WA.

Type VIII osteogenesis imperfecta (OMIM #601915) is a recently define recessive form of lethal or severe OI caused by null mutations in *LEPRE1*, the gene encoding collagen prolyl 3-hydroxylase 1. Most type VIII OI is lethal in infancy. We present here the first complete description of two non-lethal cases of type VIII OI. The probands are two boys, a 17 yr old Pakistani who is homozygous for a c.1656C>A which creates a premature termination codon in exon 11, and a 10 yr old Sri Lankan who is a compound heterozygote for a 7 nt duplication (c.1383_1389 dup GAACTCC) and an 11 nt deletion (c.1924_1934 delCAGCCTCAGTG), which also lead to PTCs. Both probands were term babies who were SGA. They have extreme growth deficiency - the 17 yr old has the length of a 3yr old and the HC of a 9 yr old, while the 10 yr old has the length of a 14 month old and the HC of a 22 month old. They have white sclerae and normal dentin. Their extremities are rhizomelic with popcorn epiphyses. Their hands, however, have normal length for age, with short metacarpals. They have severe scoliosis with multiple vertebral compressions; the DEXA L1-L4 z-scores were -6.3 and -5.8, which is at the severest end of the OI spectrum. The dermal collagen fibrils of the probands have average diameter equivalent to matched controls, but have greater diameter variability and multiple border irregularities on cross-section. On mass spectrometry, the level of Type I collagen Pro986 hydroxylation was <5% of normal in dermis, iliac crest bone and collagen secreted by cultured fibroblasts and osteoblasts, demonstrating that there is no redundancy of P3H function in skin and bone. Serum test results were distinctive compared to other OI types, with elevations of both BSAP, an osteoblast product, and TRAP, an osteoclast product, consistent with elevated bone turnover. Iliac crest histomorphometry confirmed elevated osteoblast surface, low bone volume, extremely high bone turnover, along with elevated mineral apposition rate and faster matrix mineralization (lower MLT). Stained bone sections display the distinctive feature of a broad ostoid seam on all trabecular surfaces. In addition, osteoblast morphology is abnormal, with irregularly shaped cells piled up in several layers on the newly deposited matrix, rather than the normal monolayer of cuboidal osteoblasts. These clinical and histological features provide a diagnostic guide for clinicians.

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## 2587/F/Poster Board #103

**Familial congenital mirror movements maps to chromosome 18q21.1-q22.1.** *M. Srour[1,2], JB. Rivière[2], F. Richer[3,4], MP. Dubé[1,5], S. Chouinard[1,3], G.A. Rouleau[1,2].* 1) Department of Medicine, Université de Montréal, Montreal, Quebec, Canada; 2) Center of Excellence in Neuromics, Centre Hospitalier de l'Université de Montréal, Montreal, Quebec, Canada; 3) Unité des troubles du mouvement André Barbeau, Centre Hospitalier de l'Université de Montréal, Montreal, Quebec, Canada; 4) Université du Québec à Montréal, Montreal, Quebec, Canada; 5) Montreal Heart Institute, Montreal, Quebec, Canada.

Mirror movements are involuntary movements that occur in the contralateral homologous muscle upon voluntary unilateral activation. Mirror movements are common in early childhood, however their persistence beyond the age of 10 years is usually pathological, and can be associated with abnormalities of the corticospinal tract such as in X-linked Kallmann's syndrome, Klippel-Feil syndrome or congenital hemiplegia. We have recently described a large 4-generation French Canadian family with autosomal dominant familial congenital mirror movements with no associated neurologic abnormalities. There are no known causative genes identified for this condition. We genotyped 23 individuals, including 11 affected, using the Human cnv370-quad beadchip from Illumina. Two-point linkage analysis was carried out, and uncovered a 21 Mbp locus between markers rs1017781 (42,496,503 bp) and rs574539 (64,361,369 bp) on chromosome 18 with a maximal LOD score of 3.18. Chromosome 18q21.1-q22.1 is the first locus to be mapped for familial congenital mirror movements.

## 2588/F/Poster Board #104

**Haploinsuficiencies of FOXF1 and FOXC2 genes in a neonate with lethal alveolar capillary dysplasia and complex congenital heart disease.** *D.L. Zwick, L. Shao, S. Yu.* Children's Mercy Hospital, Kansas City, MO.

Neonatal deaths in infants account for about 67% of all deaths during the first year of life in the USA. Genetic defects are important factors contributing to neonatal deaths with or without congenital anomalies. Here we report the identification of a 1.37 Mb hemizygous deletion of chromosome 16q24.1q24.2 by microarray-based comparative genomic hybridization (aCGH) technique in a newborn boy with lethal severe alveolar capillary dysplasia with misalignment of pulmonary veins and underdeveloped alveoli, and complex congenital heart disease with hypoplastic left ventricle, pulmonary valve atresia, ventricular septal defect, overriding aorta; persistent left superior vena cava connected to the coronary sinus; patent ductus arteriosus, and atrial septal defect. There are five annotated genes (IRF8, FOXF1, MTHFSD, FOXC2, and FOXL1) identified within the 1.37 Mb deleted region. Of them, the FOXF1 gene participates in the regulation of pulmonary genes as well as embryonic lung development, as well as other organs. Knockout mice embryos (Foxf1-/-) die around E9 due to absence of vasculogenesis in the yolk sac and allantois resulting from defects in mesodermal differentiation. Half of newborn Foxf1-/+ mice die from perinatal pulmonary hemorrhage, severe defects in alveolarization and vascularization, and fusion of lung lobes and arteries resulting from haploinsufficiency of the Foxf1 gene. Haploinsufficiency of FOXF1 identified in our case represents the first observation of its pathogenic link to malformation in human beings with remarkable similarity to the phenotypes observed in the mouse model. Additionally, haploinsufficiency of the FOXC2 gene due to the deletion identified in this patient represents a novel genetic mechanism leading to lymphedema-distichiasis syndrome, an autosomal dominant disorder characterized by obstruction of lymphatic drainage of the limbs, double rows of eyelashes, venous insufficiency resulting from incompetent venous valves, and cardiac defects etc. Our observation in this case emphasizes the significance of high resolution human genomes scanning for identifications of micro-imbalances as pathogenic causes in neonates with congenital anomalies and idiopathic clinical features.

## 2589/F/Poster Board #105

**Triphalangeal and triangular thumb and preaxial polydactyly: Locus versus allelic heterogeneity.** *D. Gül[1], H. Ulucan[2,3], J.J. Johnston[2], L.G. Biesecker[2].* 1) Department of Medical Genetics, GATA Medical Faculty, Ankara, Turkey; 2) Genetic Diseases Research Branch, National Human Genome Research Institute, NIH, Bethesda, MD, USA; 3) Department of Medical Genetics, Adnan Menderes University Medical Faculty, Aydin, Turkey.

We have analyzed a large family with 36 affecteds, whose phenotypes range from triphalangeal thumb and/or preaxial polydactyly to a very unusual manifestation of anterior (preaxial) duplication. This manifestation comprises duplication of the proximal phalanx of the first digit with a third aberrant phalanx that connects the distal aspect of these two digit 1 proximal phalanges, forming a triangle with its apex at the metacarpal phalangeal joint. Thus, this aberrant third phalanx is perpendicular to the proximo-distal axis of the anterior ray. This clinical manifestation suggests a severe perturbation of anterior digit patterning with a 90 degree rotation of the digit axis. The phenotype in this family is most similar to triphalangeal thumb-polysyndactyly syndrome (TPTPS; # 174500), which manifests widely variable degrees of triphalangeal thumb, polydactyly, and syndactyly. It is transmitted in an autosomal dominant pattern with complete penetrance and variable expressivity. Syndactyly type IV (#186200) is also inherited in an autosomal dominant pattern and manifests cutaneous syndactyly and polydactyly. This latter phenotype is less similar to the family we report here. Previous reports show that both TTTPS and Syndactyly type IV are caused by point mutations in ZRS, the limb specific enhancer of *SHH*, and duplications encompassing the ZRS. Therefore we performed candidate linkage analysis of a subset of the family to the ZRS locus. Linkage was unlikely, but could not be excluded, with a crossover close to the ZRS. As the locus could not be excluded, we performed zoom in custom array CGH for that region in six affecteds but no duplications were identified. Sequencing of the ZRS revealed no significant variants. We conclude that this phenotype is unlikely to be allelic to these two ZRS-related disorders. We cannot exclude a novel mutational mechanism that affects the ZRS but it is more likely that the locus for this phenotype lies elsewhere in the genome. Linkage analysis of additional family members is underway.

## 2590/F/Poster Board #106

**Hypotonia As A Feature of Connective Tissue Disorders: Lessons from A Hypotonia Specialty Clinic.** *E. Lisi, H. Dietz, G. Oswald, R. Cohn.* Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD.

Background: The Johns Hopkins Center for Hypotonia was established in January, 2007 as a multidisciplinary clinic designed for the diagnosis and management of children with hypotonia. Because of its specialized nature, we are now able to better categorize patients based on severity of the hypotonia and associated symptoms, providing more accurate prognostic information and guidance. Methods and Results: Of the 214 patients we have seen as of May, 2009, we have noted that 17 (7.9%) have mild hypotonia and joint hyperflexibility that is more significant than would be expected for their degree of hypotonia. We have ordered an echocardiogram in 10 of 17, finding evidence of aortic enlargement in 6 of those 10 (60%). Of particular interest are two patients: Patient 1 is a 7 year old male who was evaluated in our clinic for hypotonia, pectus excavatum, and umbilical hernia. An echocardiogram revealed an aortic root size of 2.55 cm with a Z-score of 3.88. Subsequently, he had a seizure-like episode with a vascular abnormality diagnosed on head CT. Thus, we ordered genetic testing for Loeys-Dietz syndrome and found a T775G mutation in the TGFBR2 gene. Patient 2 is a 7 year old female with trisomy 21, but more significant hypotonia than would be expected for her age. She came with her karyotypically normal 4 year old sister with mild hypotonia as well. Both sisters had hyperextensibility, easy bruising, and translucent skin. Echocardiograms are pending. Both were diagnosed as have underlying connective tissue disorder, causing the sister with trisomy 21 to exhibit more significant hypotonia than typical. Conclusion: The association of hypotonia with connective tissue disorders (CTDs) has historically been underappreciated. However, we have a series of 17 patients with hypotonia, hyperflexibility, and other soft signs of an underlying CTD, and 60% of those with echocardiograms exhibit aortic enlargement. Thus, appropriate management of a hypotonic patient with hyperextensibility should involve an echocardiogram. Careful evaluation of the hypotonic child for signs of Loeys-Dietz syndrome or other known CTDs is crucial, even if another etiology such as Down syndrome is established . Finally, a new, undescribed CTD may be the underlying etiology for many of our patients with mild hypotonia and hyperflexibilitywhich seems to have a more favorable prognosis than many other described syndromes associated with congenital hypotonia.

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## 2591/F/Poster Board #107

**Two Turkish brothers with cold-induced sweating syndrome caused by a novel missense mutation in the *CRLF1* gene.** *B. Tüysüz[1], G. YeÅŸil[1], O. Kasapcopur[2], PerM. Kanppskog[3], H. Boman[3].* 1) Cerrahpasa Pediatric Genetics, Istanbul University, Istanbul, Turkey; 2) Istanbul University, Cerrahpasa Medical Faculty, Department of Rheumatology, Istanbul, Turkey; 3) Center of Medical Genetics and Molecular Medicine Haukeland Hospital, Helse Bergen HF, N-5021 Bergen, Norway.

Cold-induced sweating syndrome is a very rare autosomal recessive disorder characterized by excess sweating induced by cold exposure, reduced pain sensitivity, camptodactyly and kyphoscoliosis. It was initially described by Sohar et al. (Lancet 1978; 312:1073-4) in two Israeli sisters. Knappskog et al. (Am J Hum Genet 2003; 72: 375-83) performed genetic studies, and found out that the Israeli sisters as well as two Norwegian brothers with similar clinical manifestations were homozygous for the mutations in *CRLF1* gene. A second locus was also identified in an Australian patient who was compound heterozygous for the mutations in *CLFC1* (*CISS2*) gene on 11q13.3 (Hahn et al., J Neurol Sci 2006; 250: 62-70). Our patients (22 and 13 years old boys) were second and third child of first cousin and healthy parents. Both brothers had excess sweating induced by cold exposure, operated severe dorsal scoliosis, camptodactyly, reduced pain sensitivity, short and smooth tongue and operated cryptorchidism. Their sweating problem and scoliosis were noted at the age of approximately 12 years. Both patient' weight, height and head circumference were normal. Older brother had atypical facial features including chubby cheeks, broad nose with anteverted nostrils, short philtrum and bifid uvula. He had also chewing and swallowing problems which were arised in infantile period. His intelligence was normal and he was working as a laboratory staff. However, younger brother had similar facies to his brother but he also had severe mental retardation due to cortical atrophy and history of neonatal hypoxic ischemic encephalopathy. Dorsal severe scoliosis was noted even after the operation. Their electromyelography and blood muscle enzyme levels were normal. We considered cold induced sweating syndrome in these brothers. The coding and flanking regions of all the 8 exons of the *CRLF1* gene were amplified, sequenced and the data analyzed mainly as previously described (Knappskog PM et al. Am J Hum Genet 2003; 72:375-83). The patients were homozygous and their parents were heterozygous for a novel missense mutation in *CRLF1* c.413C>T (p.Pro138Leu). This variant is situated in a predicted (NCBI) EpoR ligand-binding domain. We presented first family with cold induced sweating syndrome from Turkey and fourth family which reported to date. Our patient findings differ from the other reported patients as they have late onset but progressive scoliosis and sweating.

## 2592/F/Poster Board #108

**Lowry-Wood syndrome: a further case and review of the literature.** *K. Becker[1], K. Weerasinghe[2], C.M. Hall[3].* 1) North Wales Clinical Genetics Service, Glan Clwyd Hospital, Rhyl, United Kingdom; 2) Department of Paediatrics, Wrexham Maelor Hospital, Wrexham, UK; 3) Department of Radiology, Great Ormond Street Hospital for Sick Children, London, UK.

Lowry-Wood Syndrome was first described in 1975 and is characterised by multiple epiphyseal dysplasia, short stature, microcephaly, variable learning difficulties and nystagmus. The two brothers originally reported later developed diffuse pigmentary retinal degeneration, with optic atrophy in the older boy aged 17 years. Six additional patients have been reported, including two brother-sister pairs. Autosomal recessive inheritance has been postulated. Our patient, an eight year old girl, presented with short stature (height <0.4th centile), borderline microcephaly (head circumference on 3rd centile), mild learning difficulties, congenital horizontal nystagmus and pale optic discs, and the typical findings of multiple epiphyseal dysplasia on skeletal survey, with a very delayed bone age of 5.4 years at 8.1 years. She is the only child of unrelated parents.

## 2593/F/Poster Board #109

**A novel autosomal recessive MR/MCA syndrome comprising short stature, brachydactyly and pectus excavatum maps to 5q23-q32.** *k. kahrizi[1], M. Garshasbi[1,2], F. Behjati[1], H.H Ropers[2], A.W Kuss[2], H. Najmabadi[1], A. Tzschach[2].* 1) Genetics Research Center , USWR, Tehran, Tehran, Iran; 2) Max Planck Institute for Molecular Genetics, Berlin, Germany.

We report on a family in which three mentally retarded siblings suffer from an apparently novel autosomal recessive MR/MCA syndrome characterized by borderline mental retardation, cataract, short stature, brachydactyly of the feet and pectus excavatum. The patients - two brothers and a sister - were born to healthy Iranian parents who were first cousins. They had one healthy brother who was of normal height. Secondary sexual characterization is missing in the affected female. The shortening of the fourth and fifth metatarsal and phalanges in all affected sibs with generalized hypodensity was reported by X-Ray. Chest X-ray showed no abnormalities. Three affected patients had history of seizure. Brain MRI revealed no brain malformations. Endocrinological profile and blood metabolites were normal in two male sibs but slightly increased of PTH and FSH in the affected female. Serum calcium level was normal but phosphorus was minimally increased. Metabolic tests and chromosome analysis revealed no abnormalities in the patients. The pedigree structure suggested autosomal recessive inheritance, and homozygosity mapping using a 250K SNP array (Affymetrix) identified one large (24 Mb) interval in 5q23.1-q32 flanked by SNP markers rs1427941 and rs10036966. The LOD score for this interval was 2.5. A second interval in 3q13.11 had a size of only 0.5 Mb (flanked by SNPs rs6791670 and rs2961221; LOD score 2.5) but contained no known genes. We therefore presume that the large and gene-rich interval in 5q harbours the causative gene defect in this family, and screening of candidate genes will eventually elucidate the underlying mutation.

## 2594/F/Poster Board #110

**Autosomal dominant 46,XY disorder of sexual development: Evidence for a novel locus.** *K.M. Boycott[1], S. Douglas[2], C. Goldsmith[1], D.E. Bulman[2].* 1) Dept Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada; 2) Ottawa Hospital Health Research Institute, Ottawa, ON, Canada.

The 46,XY disorders of sexual development (DSD) are a genetically heterogeneous group of disorders characterized by a 46,XY karyotype, ambiguous genitalia with mild to severe penoscrotal hypospadias, dysgenetic testes, reduced to no sperm production and a range of Mullerian structure development. These disorders can be inherited in an autosomal dominant, autosomal recessive, X-linked, or Y-linked manner depending on the gene involved. Mutations in the *SF1* and *DHH* genes account for approximately one-third of autosomal dominant 46,XY DSD and duplications of *DAX1* or *WNT4* are a very rare cause of this phenotype. Here we report a four-generation family with five individuals affected with a 46,XY DSD. Male-to-male transmission was observed in this family consistent with an autosomal dominant disorder. The phenotype is characterized by penoscrotal hypospadias ranging from mild to severe in three males, ambiguous genitalia in one individual later raised as male and normal appearing female genitalia in a 46,XY child with absent Mullerian structures. All of the adult affected males report significantly reduced fertility and endocrine testing indicates hypergonadotropic hypogonadism. Pathology from the gonads removed from the affected child identified infantile testis with mild Leydig cell hyperplasia and one small microscopic focus of ovarian stroma containing a few oocytes. *DHH* and *SF1* sequencing was negative. Evidence for a *DAX1* or *WNT4* duplication was absent on a chromosomal microarray (Affymetrix, 6.0 SNP). Haplotype analysis ruled out *WT1* or *SOX9* as being involved. This data suggests that the 46,XY DSD in this family is novel and further studies are underway to map and identify the gene for this developmental disorder.

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## 2595/F/Poster Board #111

**Mendelian predisposition to Herpes Simplex Encephalitis - a novel category of Primary Immunodeficiencies.** *V. Sancho-Shimizu[1], R. Perez[1], L. Lorenzo[1], SY. Zhang[2], A. Cardon[1], E. Jouanguy[2], L. Abel[1], JL. Casanova[2].* 1) Laboratory of Human Genetics of Infectious Disease, Faculty of Medicine Necker, University Paris Descartes, Paris, France; 2) Laboratory of Human Genetics of Infectious Disease, Rockefeller University, 1230 York Ave, New York, NY USA.

Herpes simplex encephalitis (HSE) is a rare disease affecting young children as well as adults due to primary infection with herpes simplex virus-1 (HSV-1). HSV-1 is a nearly ubiquitous virus commonly infecting humans however most do not develop HSE, suggesting that these children possess an underlying genetic susceptibility to HSV-1. Notably, these patients are otherwise healthy and able to mount a normal adaptive immune response to HSV-1, pointing to the likelihood that they harbor an unidentified form of primary immunodeficiency disease affecting their innate immune system. We have recently identified the first two genetic etiologies UNC93B and TLR3 deficiencies in HSE patients validating our hypothesis. We have subsequently identified at least two other patients among our cohort of HSE patients with impaired production of type I IFN following viral and double stranded RNA activation. Here we investigate these particular HSE patients using a candidate gene approach to identify other gene(s) underlying HSE by cellular and immunological phenotyping. UNC93B and TLR3 deficiencies in these patients have been excluded based on sequence analysis and immunological phenotyping. Further molecular studies have shown that these patients' cells showed a specific decrease in interferon regulatory factor-3 (IRF3) dimerization consistent with the lack of IFN production. Sequencing and expression of all known genes in the TLR3-UNC93B-IFN pathway are currently underway and we have excluded the involvement of seven genes in this pathway so far. The identification of novel genes involved in HSE will present important biological and medical implications, providing us with a better understanding of HSE pathogenesis.

## 2596/F/Poster Board #112

**A Novel Human Immunodeficiency Disorder associated with a Mutation in the T Cell Receptor $\alpha$ subunit constant gene (TRAC).** *N.V. Morgan[1], S. Goddard[2], T.S. Cardno[3], F. Rahman[1], A. Ciupek[3], A. Straatman-Iwanowska[1], S. Pasha[1], G. Anderson[4], A. Huissoon[2], W.P. Tate[3], E.R Maher[1,5].* 1) Med & Molec Gen, Univ Birmingham, Birmingham, United Kingdom; 2) Regional Department of Immunology, Heartlands Hospital, Birmingham, UK; 3) Department of Biochemistry, University of Otago, Dunedin, New Zealand; 4) MRC Centre for Immune Regulation, The Medical School, University of Birmingham, Birmingham, UK; 5) West Midlands Regional Genetics Service, Birmingham Women's Hospital, Birmingham, UK.

We describe two families with a novel autosomal recessively inherited immunodeficiency disorder characterised by increased susceptibility to infection, autoimmunity, eczema and alopecia. Genetic linkage studies mapped the disorder to 14q11.2 and a homozygous G to A substitution was identified at the last base of exon 3 immediately following the translational termination codon (c.*1G>A) in the T Cell Receptor $\alpha$ subunit constant gene (TRAC). RT-PCR analysis in the 2 affected individuals revealed loss of the last coding exon (exon 3) from the mutant TRAC transcript. The mutant TCR $\alpha$-chain protein was predicted to lack part of the connecting peptide domain and all of the transmembrane and cytoplasmic domains. Previous studies have shown these domains to have a critical role in the regulation of the assembly and/or intracellular transport of TCR complexes. We found that T cells from affected individuals did not express TCR$\alpha\beta$ complex and in vitro studies demonstrated a failure to respond to mitogens such as OKT3. This is the first report of a human TRAC mutation. Absent TCR$\alpha\beta$ expression may be associated with a surprising level of protection against infection, but an abnormal T cell population is associated with immune dysregulation and autoimmunity.

## 2597/F/Poster Board #113

**Male Infant with Congenital SMA and Xq11.1 Triplication.** *C. Saunders[1], A. Kats[1], S. Yu[1], L. Cooley[1], M. Strenk[1], J. Jones[2], M. Friez[2], H. Ardinger[1].* 1) Children's Mercy Hospitals and Clinics, Kansas City, MO; 2) Greenwood Genetics Center, Greenwood, SC.

Our patient is a male infant with a condition resembling the neonatal form of spinal muscular atrophy, including extreme hypotonia, lack of reflexes, no suck or swallow, little to no movements, and congenital contractures. He died of respiratory failure at 3 weeks of age. His autopsy showed severe muscle fiber atrophy with prevalence of myofibrillar disorganization consistent with a lower motor unit disorder. The spinal cord had rare anterior horn cells with alterations suggestive of early chromatolysis. His genetic workup for hypotonia included normal gene testing for SMA (with two copies of SMN1 present), myotonic dystrophy and methylation analysis for Prader-Willi syndrome. The karyotype was normal at the 475-725 band level. Array-CGH revealed a 745 kb triplication of Xq11.1 from 62,662,985-63,408,000, which includes 4 genes: ARHGEF9, FAM123B, ASB12, MTMR8. The family history did not identify other similarly affected relatives, and no other males are available to test. The child's asymptomatic mother also tested positive for this triplication but has skewed X inactivation (90:10). We hypothesize that overexpression of a gene in this region may cause an SMA-like phenotype. One gene of interest, ASB12, is a member of the ankryin repeat and SOCS box family of proteins, which have been shown to regulate muscle development in mice and interact with the ubiquitination pathway.

## 2598/F/Poster Board #114

**Germline mutations in the tumour suppressor WTX cause a sclerosing bone dysplasia but do not predispose to Wilms tumor.** *M. van Kogelenberg[1], Z.A. Jenkins[1], T. Morgan[1], S. Holman[1], A. Jeffs[2], R. Fukuzawa[3], A.V. Hing[4], T. Fiskerstrand[5], R.C.M. Hennekam[6], S. Mansour[7], V. Cormier-Daire[8], A.E. Reeve[3], S.P. Robertson[1], the OSCS study Group.* 1) Paediatrics and Child Health, University of Otago, Dunedin, New Zealand; 2) Pathology, Dunedin School of Medicine, Otago University, Dunedin 9054, New Zealand; 3) Cancer Genetics Laboratory, Department of Biochemistry, Dunedin 9054, New Zealand; 4) Division of Craniofacial Medicine, Department of Pediatrics, University of Washington School of Medicine, Seattle, Washington 98195, USA; 5) Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen N-5021, Norway; 6) Clinical and Molecular Genetics Unit, Institute for Child Health, London WC1N 3JH, UK; 7) South West Thames Regional Genetics Unit, St George's Hospital, London SW17 0RE, UK; 8) Département de Génétique, Hôpital Necker-Enfants Malades, Paris 75743, France.

Osteopathia striata congenita with cranial sclerosis (OSCS) is an X-linked sclerosing bone dysplasia characterized by increased bone density and craniofacial malformations in females and lethality in males. To elucidate the pathogenesis of OSCS we performed a genome wide array on DNA from a proband which presented with X-linked OSCS. We identified a >2.1-Mb *de novo* deletion at Xq11.1. One of the four affected genes, *WTX (FAM123B)*, was a candidate monogenic contributor given its role in repression of canonical WNT signalling. Screening of 25 OSCS patients demonstrated 23 premature termination and two entire gene deletions of *WTX*. Molecular studies of the mouse homolog *Wtx* showed predominantly endogenous expression patterns of in the developing skeleton and skull. An alternatively spliced transcript was detected by RT-PCR deleting the primary determinant that localizes WTX to the plasma membrane. The differential cellular functions of the WTX splice form could implicate the plasma membrane localization as a factor associated with survival in males with OSCS. The distribution of *WTX* mutations in OSCS mirror somatic *WTX* mutations identified in 11-29% of Wilms tumor, the most common solid organ tumor in children. However, OSCS individuals did not seem to have a predisposition to Wilms tumor. The lack of tumor susceptibility in OSCS patients may imply the existence of a temporal or spatial constraint on whether a *WTX* mutation will be cancer predisposing or not.

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## 2599/F/Poster Board #115

**The variable phenotype of 1q21.1 deletion syndrome in a multigenerational family.** *E. Chow[1,2], A. Thomas[3], M. Shago[4], D. Skidmore[3].* 1) Clinical Genetics Service, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 2) Departmnet of Psychiatry, University of Toronto, Toronto, Ontario, Canada; 3) Maritime Medical Genetics Services iWK Health Centre, Halifax, Nova Scotia, Canada; 4) Clinical cytogenetics laboratory, Hospital for Sick Chilren, Toronto, Ontario, Canada.

The 1q21.1 Deletion Syndrome (1q21.1DS) is a newly identified microdeletion syndrome with a variable phenotype (Mefford et al., 2008). Reported features have included cognitive, psychiatric, neurological, cardiac, and musculoskeletal. It has an estimated prevalence of ~0.4% in the general population. We will present detailed physical and psychiatric phenotypic data on a mother (proband) and daughter pair with 1q21.1DS. The grandmother is currently being tested for 1q21.1DS. The 26-year-old proband of English and East European Jewish background was identified through a psychiatric genetics service and suffers from mild mental retardation, ADHD and psychosis not otherwise specified but is medically well. Physical examination revealed short stature (<3rd percentile), small and deep-set eyes, low set ears, bossing forehead, and small hands with short fingers. She was born with mid-lumbar spinal bifida occulta with a tethered cord and a forme fruste of a meningocele. The 8-year-old daughter was born premature at 31 weeks and experienced bilateral interventricular haemorrhages. Prenatally she was noted to have two seemingly normal kidneys. Postnatally, the left kidney was noted to be dysplastic and it has now evoluted in its entirety. There have been intermittent concerns about right-sided hydronephrosis. By age 7 she was noted to have multiple minor dysmorphic features, significant hypotonia, short stature (<3rd percentile), and a moderate degree of non-progressive developmental delay. The grandmother is a 50 year-old woman with a height of 158 cm, small eyes, and bossing forehead. She has no history of congenital anomalies or major medical problems. She was a slow learner in school and suffered from depressions and anxieties (untreated) in her adult life. Prior to the birth of the proband, the grandmother had two miscarriages and a child who died at birth because of unspecified congenital anomalies involving the brain and the heart. Should a 1q21.1 microdeletion be confirmed in the mother, this will the first, detailed, three-generational report on the phenotype of 1q21.1DS. This family highlights the variability of the phenotype, even within a single family. It also highlights the high frequency of neuropsychiatric manifestations. Early assessment and intervention for neuropsychiatric manifestations are recommended for individuals with 1q21.1DS.

## 2600/F/Poster Board #116

**Cardio-Renal-Epilepsy-Digital-Oculo (CREDO) syndrome: a "new" syndrome of developmental delays and multiple malformations.** *R. Lebel, R. Miller, N. Havernick, N. Dosa, B. Braddock, F. Smith, R. Shprintzen.* SUNY Upstate Medical University, Syracuse, NY.

The non-consanguineous marriage of healthy individuals with French-Canadian and German ancestry produced three pregnancies, at maternal ages 22, 24 and 26. The first was a female (now 9 years of age) whose pregnancy was complicated by oligohydramnios and intrauterine growth restriction; at birth she was noted to have pulmonic stenosis, bicuspid aortic valve, renal dysplasia and hypotonia. Since then, she has revealed developmental delays, mixed hearing deficit, cataracts with microcornea and myopia, gastroesophageal reflux, epilepsy, microcephaly with corpus callosum agenesis, growth delays; she has been noted to have eyelid ptosis, joint laxity, fetal finger pads, and hanging columella. The second child, also subject to oligohydramnios and IUGR, was a male with more complex heart disease (bicuspid and stenotic aortic valve with coarctation, absent left pulmonic artery and large patent foramen ovale) which proved fatal at seven months of age (before he could manifest many of the features seen in his siblings). He also was found to have GERD, microcephaly, poor weight gain, renal dysplasia, and hypotonia. The third child (now five years of age) was a male whose prenatal course had been uneventful (normal fluid levels and fetal growth) but who was found to have pulmonic stenosis with bicuspid aortic valve, renal dysplasia, microcornea, epilepsy, eyelid ptosis, hanging columella, fetal finger pads, hypotonia and hyperextensible joints. All three children had normal chromosomes by routine analysis and by subtelomere FISH. The first and third children had normal findings on Signature Genomics oligo-microarray analysis. All three children had a characteristic and highly similar facial gestalt. We think this family represents a previously unreported autosomal recessive delays-malformation syndrome, and we offer it for consideration as the CREDO (Cardio-Renal-Epilepsy-Digital-Oculo) syndrome.

## 2601/F/Poster Board #117

**Multiple Intestinal Atresia with Immunodeficiency: Gene Expression and SNP Genotyping.** *K. Chen[1], S. McGhee[1], M.I. Garcia, Lloret[1], I. Grinberg[2], E.R.B. McCabe[2].* 1) Dept Pediatrics, Univ California, Los Angeles, Los Angeles, CA; 2) University of California, Los Angeles, Los Angeles, CA.

Background: Multiple intestinal atresia with immunodeficiency (MIA-I) is an exceptionally rare disorder characterized by atretic regions throughout the gut, often from the pylorus down through the colon, with heterogeneous forms of combined immunodeficiency. Histologically, the intestinal atresias demonstrate a sieve-like appearance. Although the pathogenesis of MIA-I is unknown, familial cases suggest an autosomal pattern of inheritance. Purpose: To characterize the disorder of MIA-I and to identify genes potentially involved in its pathogenesis using expression microarray and genomic single nucleotide polymorphism (SNP) microarray platforms. Methods: Clinical history was obtained from medical records. Immunologic evaluation was completed using standard laboratory methods. RNA was isolated from whole blood and primary T cell cultures, and was analyzed using a genomic RNA expression microarray platform. A genome-wide SNP microarray platform was used for genotyping. Genes of interest were identified by gene expression differences of more than three-fold compared to controls. We explored common signaling pathways that were upregulated or downregulated in MIA-I using Ingenuity Pathways Analysis web-based software. We genotyped individuals from three families, each with affected and unaffected members. Results: At our institution, two unrelated patients with MIA-I were found to be panhypogammaglobulinemic and lymphopenic. Both demonstrated diminished lymphocyte proliferation to mitogens. Conclusions: MIA-I is a rare disorder that results in catastrophic loss of gut function and death from severe infections. Care should be taken to evaluate neonates with MIA-I. MIA-I may involve a single gene defect or partial defects in multiple pathways that alter both gastrointestinal and lymphocyte development. Mediators of embryonic patterning may be involved in the pathogenesis of MIA-I and should be further explored.

## 2602/F/Poster Board #118

**Neonatal primary spontaneous pneumothorax in 5 members of a family.** *B. Hall[1,2], M.E. Pierpont[2,3].* 1) Minnesota Perinatal Physicians, St Paul, MN; 2) Children's Hospitals and Clinics of Minnesota, Genetics Division, St. Paul, MN; 3) University of Minnesota, Minneapolis, MN.

Primary spontaneous pneumothorax (PSP) results in partial or complete lung collapse. It occurs more frequently in adolescent or adult males. Additional risk factors for PSP include smoking, increased height, and family history. Neonatal onset of pneumothorax has been reported to have an incidence of 1-2% at term and 6% in preterm infants and is usually associated with underlying complications such as meconium aspiration, hyaline membrane disease, or vigorous resuscitation. Rarely has neonatal pneumothorax been described as familial. We describe a family with 5 affected individuals in 2 generations with neonatal presentation of PSP. Three female siblings developed documented bilateral pneumothoraces, and the first 2 (born in 1969 and 1971) died in the first day of life. The third sister born at 35-36 weeks was treated and survived. Her childhood health was unremarkable and without recurrence. She had 3 sons, 2 of whom developed bilateral pnuemothoraces shortly after birth requiring treatment. There have been no further pnemothoraces in these boys to ages 5 and 7 years. It has long been suggested that an underlying abnormality of the connective tissue may be involved in the etiology of familial PSP. Disorders such as Ehlers Danlos syndrome(EDS), Marfan syndrome, alpha-1- antitrypsin deficiency (AAT) have been considered in the differential diagnosis. More recently, mutations in the FLCN gene have been found in families with apparently isolated PSP as well as in those with a disorder characterized by skin folliculomas and renal cancer, called Birt-Hogge-Dube syndrome. Examination of these boys and their mother does not support a clinical diagnosis of Marfan syndrome nor EDS. Due to a history of early cirrhosis and emphysema in a maternal great aunt, testing for AAT was performed and was negative. No mutation was found by sequencing of the FLCN gene. Array CGH was normal. PSP usually occurs in older individuals. Only 3 reports describing neonatal onset of familial PSP were noted upon review of the literature. Two of the 3 reports involved affected siblings while the 3rd had affected persons in more than one generation. Previous case studies have suggested dominant with reduced penetrance, polygenic, X-linked or recessive inheritance patterns. It is unclear at this time if individuals who experience PSP later in life do so as the result of the same underlying cause as the few reported cases of neonatal PSP.

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## 2603/F/Poster Board #119

**Screening of 1033 patients with mental retardation, developmental delay and/or dysmorphic features by aCGH.** *O.K. Rodningen, B.E. Kristiansen, C. von der Lippe, H.S. Sorte, M. Moller, K.L. Eiklid.* Department of Medical Genetics, Oslo University Hospital, Ulleval, Oslo, Norway.

Microarray-based comparative genomic hybridization (aCGH) is a powerful method for detection of genomic imbalances in individuals with mental retardation, developmental delay and/or dysmorphic features. The method allows for the detection of DNA copy number changes not previously detected by conventional cytogenetic techniques. We present the results of aCGH analysis in 1033 probands, of which the majority were karyotyped (G-banded chromosome analysis at a resolution of 450-550 bands) before aCGH was performed. DNA was extracted from blood, fibroblasts or FFPE (Formalin Fixed and Paraffin Embedded) tissue, and aCGH was performed using 44K, 105K or 244K Human Genome CGH Oligo Microarrays (Agilent Technologies). Microdeletions and/or microduplications were detected in 261/1033 probands (25,3%). An abnormal karyotype had previously been detected in 75 probands, hence aCGH was performed in order to specify the origin of extrachromosomal material, to identify deletions or duplications in translocation breakpoints, or to map genes involved in the aberrations. Of these 75 probands, 13 had no copy number changes detectable by aCGH, indicating a balanced aberration. In the remaining 62 probands, the fine mapping provided new information about the complexity of the rearrangements, and identified additional pathogenic aberrations that would have been missed without aCGH. Of the microdeletions/duplications identified in the probands with a normal karyotype, 54 were similar to, or overlapping regions associated with known syndromes, and 145 were previously undescribed. In most instances, the chromosomal anomaly was judged to be the underlying cause, or probable cause, of the patient's abnormal phenotype. Our data demonstrates the utility of aCGH as a powerful diagnostic tool for detection of genomic imbalances.

## 2604/F/Poster Board #120

**Delineation of a novel lethal autosomal condition characterized by alveolar capillary dysplasia and limb reduction anomalies.** *A. Innes.* Dept Med Gen, Alberta Children's Hosp, Calgary, AB, Canada.

Alveolar capillary dysplasia (ACD) is amongst the common causes of lethal primary pulmonary hypertension (PPHN) in the newborn. It is ultimately unresponsive to inhaled nitric oxide and ECMO. Definitive diagnosis requires lung biopsy, and is characterized by a reduction in number of capillaries, with a decreased blood-air interface. The deficit likely occurs during the pseudoglandular and canalicular stages of lung development. Over 100 cases have been reported. The etiology of most cases is unknown, but many familial cases have been reported. Associated malformations are seen in over 50% of patients, recurrent anomalies involve the cardiovascular, genitourinary and gastrointestinal systems. Mutations in STRA6 have been identified in patients with anophthalmia and malformations including ACD. Until recently, the molecular basis for the majority of children with ACD was unknown. However, microdeletions of chromosome 16q24.1q24.2 or heterozygous mutations in FOXF1 at that locus, have recently been identified in a cohort of patients with ACD and associated congenital anomalies (Stankiewicz et al, 2009). These patients did not have major limb anomalies. One previous study excluded mutations in two candidates: BMPR2 and EMAPII (Sen et al, 2004)in children with ACD. We have encountered 5 children from 3 families born to consanguineous parents (2 different ethnic groups) that presented with limb reduction anomalies and died of PPHN as newborns. In those who had autopsy, ACD was confirmed as the etiology of the PPHN. Previously, a consanguineous family had been reported where 3 children had variable features of ACD and/or limb anomalies(Witter et al, 2001). Review of the literature identified 3 other reports of 4 children with limb reduction anomalies and ACD (Cullinane et al, 1992; Simonton et al, 1993; Steinhorn et al, 1997). Therefore, we are aware of 12 patients with ACD and limb reduction anomalies. Given consanguinity in 4 families, this is likely an autosomal recessive condition. This paper will include a clinical delineation of this syndrome, with a focus on the clinical details of the 4 consanguineous families. As mice deficient in the gene eNOS have a pulmonary phenotype reminiscent of ACD and variable limb malformations (Han, 2006), this was a potential candidate gene. However, linkage was excluded to eNOS in one family we studies. A homozygosity mapping approach will be applied in an attempt to identify the gene for this lethal disorder.

## 2605/F/Poster Board #121

**New dominant osteochondrodysplasia with brachydactyly and short humerus.** *D. Lacombe[1], M.A. Delrue[1], S. Mundlos[2], A. Toutain[3], J.F. Chateil[4].* 1) Dept Medical Genetics, CHU Bordeaux, Université de Bordeaux, Bordeaux, France; 2) Dept. Genetics, Charité Hospital, Berlin, Germany; 3) Dept. Genetics, CHU Tours, France; 4) Dept Radiology, CHU Bordeaux, Université de Bordeaux, France.

We report a three-generation family affected with a chondrodysplasia transmitted as an autosomal dominant trait. The phenotype mainly associates short humerus, incurvation of radius and brachydactyly and is not already reported to the best of our knowledge. Enzo was born at 39 WG by cesarean section, after a pregnancy marked by ultrasound identification of shortness of humeri (< 3° centile). At bith, weight was 3000 g, height was 50 cm, and OFC was 35.5 cm. Examination showed short arms, a radial deviation of hands, and brachydactyly. At age 7 months, height was 68 cm and weight 8.6 kg. Mental development were normal. X-rays noted short humerus, incurvation of radius with hypoplasia of the proximal part of the ulna, short metacarpals except abnormally shaped second one (probable fusion between 1st and 2nd metacarpals), short 2nd, 4th, and 5th middle phalanges, and short metatarsals. Audiogram was normal. This was the first child of a 31-year mother. Her height was 154 cm, weight was 51 kg, and OFC 56 cm. She had short arms, a radial angulation of forearms, brachydactyly of hands and feet with short second phalanges of hands. X-rays showed short and broad humerus, incurvation of radius with relative hypoplasia of the lateral condyle, short metacarpals, short 2nd, 4th, and 5th second phalanges, and short 1st, 4th and 5th metatarsals. Vertebrae were normal. She had a normal hearing. She had two brothers, one affected and one non-affected. Height of the affected brother was 180 cm. He had the same skeletal phenotype including triphalangeal thumbs and associated a mixed deafness. The great-mother also had the same osseous dysplasia with brachydactyly and short second phalanges. Her height was 155 cm and she had a limitation of prono-supination. She had a deaffness since age 15 years. She also had a brother affected with the same chondrodysplasia and a height of 160 cm. This combination of skeletal features does not fit into any identified phenotype. The brachydactyly is closed to the A1 type (Farabee) of brachydactyly due to IHH gene mutrations. A mutation was excluded by sequencing of the entire coding regions in the IHH gene encoding the Indian Hedgehog protein and in the GDF5 gene in Enzo and in his mother. It seems to be a new chondrodyplasia phenotype or part of the A1 brachydactyly spectrum.

## 2606/F/Poster Board #122

**Ryzomelic shortening, cardiac malformation, didelphic uterus, and accessory spleen: a new syndrome.** *Z.D.N.M Lumack[1], D. Brunoni[1], N.L. Sobreira[2], A.B. Perez[1].* 1) Centro de Genética Médica (CGM) - Universidade Federal de São Paulo (UNIFESP)-São Paulo, Brasil; 2) Institute of Genetic Medicine, Johns Hopkins University of Medicine - Baltimore, MD.

Skeletal dysplasias are a heterogeneous group of more than 200 disorders characterized by abnormalities of cartilage and bone growth, resulting in abnormal shape and size of the skeleton and disproportion of the long bones, spine, and head. Their modes of inheritance are heterogeneous and some of them have known molecular bases. Diagnosis of skeletal dysplasia is based on the most severely affected segment of the skeletal; rhizomelic shortening (short proximal segments) is present in patients with achondroplasia, hypochondroplasia, the rhizomelic type of chondrodysplasia punctata, the Jansen type of metaphyseal dysplasia, spondyloepiphyseal dysplasia (SED) congenita, thanatophoric dysplasia, atelosteogenesis, diastrophic dysplasia, and congenital short femur. Certain clinical features may be of value as diagnostic indicators, although they may not be specific or consistent, for example, heart anomalies are associated with chondroectodermal dysplasia, lethal short-limbed skeletal dysplasias, and Majewski syndrome. He we describe a 19 years-old Brazilian girl, born to consanguineous parents, who has disproportionate short stature with rhizomelic shortening, macrocephaly, short neck, hypertelorism, increased intermamillary distance, pectus carinatum, brachydactyly, single flexion crease in both hands, small feet, increased space between first and second toes. Abdomen USG showed accessory spleen, endovaginal USG showed didelphic uterus, and echocardiogram showed interventricular septum dyskinesia, moderate pulmonary valve stenosis, interauricular communication type ostium secundum, hypertrophy of right chambers, mitral insufficiency and mild tricuspid insufficiency. C-banded karyotype: 46,XX. X-ray showed dolicocephaly, bilateral rudimentary cervical ribs, narrowed humeral head and colo, short and vertically narrowed iliac bones, defective fusion of the spinal process of sacral vertebraes, short femur, patella luxation, genu valgo, reduction of proximal tibial epiphyses, and shortness of 1st metatarsus. The manifestations in that patient do not match any of the skeletal dysplasia known to be associated with rhizomelic shortening or with anyone of the others radiographic features described here. We consider that the constellation is unique and apparently represents a previously unrecognized syndrome.

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## 2607/F/Poster Board #123

**Autosomal dominant connective tissue disorder presenting with short stature, clubfeet, sparse hair, hyperextensible joints, hernias and distinctive facial features - a new syndrome?** *D. Myles Reid[1], E. Goh[2], C. Barnett[2], B. Lee[3], A. Hinek[4], D. Chitayat[1,2].* 1) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, ON, Canada; 2) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, ON, Canada; 3) Department of Humanities, York University, Toronto, ON, Canada; 4) Heart Centre - Cardiovascular research, Hospital for Sick Children, Toronto, ON, Canada.

Heritable connective tissue disorders is a family of more than 200 disorders that result from gene mutations causing change in the structure and development of skin, bones, joints, ligaments, teeth, heart, blood vessels, lungs, eyes, ears and hollowed viscera. The most known conditions are Ehlers-Danlos syndromes, Marfan syndrome, Loeys-Dietz syndrome and osteogenesis imperfecta. Most of these conditions are associated with life threatening manifestations including fractures, rupture of the aortic root and arch and other blood vessels, spontaneous pneumothoraces and rupture of hollowed viscera including the intestine, eye globe and uterus. We report a hitherto new autosomal dominant connective tissue disorder. The proband, a 56-year-old female, was born to non-consanguineous parents of English, German and Scottish descent, had short stature, hyperextensible joints, right talipes, thin translucent skin, abnormal fingers and toes, sparse hair, osteopenia and some thrombocytopathy. She had ruptured colonic diverticulum, an epigastric hernia, left abdominal wall incisional hernia and a left inguinal hernia. Her echocardiogram was normal. She had easy bruisability, a history of difficulties in blood clotting and menorrhagia. Family history showed that her mother, her two sisters, a nephew and a niece, born to one of these sisters, have the same clinical manifestations. Immunohistochemistry done on cultured skin fibroblasts obtained from the proband, revealed normal deposition of elastic fibers and lack of fibrillin 1 in the microfibrillar scaffold. Surprisingly the microfibrillar structures demonstrated strong presence of fibulin 1 organized in microfibrills. Since rtPCR analysis demonstrated normal expression of fibrillin 1, we conclude that the main problem here could be due to the lack of normal proteolytic processing of newly synthesized fibrillin 1, which prevents their polymerization and assembly into the microfibrills. Further studies are needed to identify the candidate proteinase responsible for fibrillin 1 processing that may be deficient in this patient. A similar situation where decreased production of fibrillin 1 can be compensated by increased production of fibulin 1 occurs in Marfan syndrome. However, in this case the genetic expression of fibrillin 1 was normal and the clinical manifestations are not consistent with Marfan syndrome.

## 2608/F/Poster Board #124

**Familial tracheomalacia associated with a 5p15.33 microdeletion.** *D. Abuelo[1], N. Shur[1], J. Padbury[2], S.R. Gunn[3].* 1) Dept Pediatrics, Divion of Genetics,Rhode Island Hosp and Hasbro Childrens' Hosp, Providence, RI; 2) Dept Neonatology, Women and Infants' Hosp, Providence, RI; 3) Combimatrix Molecular Diagnostics, Irvine, CA.

Congenital or primary tracheomalacia is a rare condition caused by abnormal cartilage which is insufficient to maintain airway patency during the respiratory cycle. It can be associated with several genetic conditions, including 22q-, CHARGE, Larsen and Kniest syndromes. Although congenital autosomal dominant laryngomalacia has been described (OMIM 150280), congenital tracheomalacia is not listed in OMIM. We report a mother and 3 children with dominantly inherited congenital tracheomalacia associated with a chromosome 5p microdeletion. The mother, now age 27, was born with respiratory distress which was treated with a tracheostomy. She has short stature, learning disabilities and a weak, high-pitched voice. She has had 3 children with 3 different fathers, all of whom also have congenital tracheomalacia. The oldest son, now age 6, was born at 30 weeks gestation and was hospitalized 5 times for tracheomalacia and respiratory distress between the ages of 2 months and 3 years. He continues to have stridor with exertion and respiratory infections. Psychomotor development has been delayed, and he has subtle dysmorphism, consisting of almond shaped palpebral fissures and a prominent nasal root. His 2 ½ year old sister has tracheomalacia, mild dysmorphism and developmental delay. The 9 month-old sister has tracheomalacia, a short neck, mild hypertelorism and relative microcephaly. Oligonucleotide array CGH analysis showed a single copy number decrease of 4.18Mb of the region 5p15.33→ pter in all four affected family members. FISH results confirmed the deletion. There are approximately 30 genes between the breakpoints. Of interest is that this 5p microdeletion is smaller, but has some overlap with the 5p deletion associated with cri-du-chat syndrome. A mouse model has shown that an evolutionary conserved transmembrane protein called TMEM16A is required for normal cartilage formation, however its locus is on chromosome 11. One or more of the deleted genes in our family must also be important for normal tracheal cartilage formation.

## 2609/F/Poster Board #125

**Hand dysmorphology: Dermatoglyphics-A neglected clue to suspect microdeletions.** *Y. Lacassie[1], V. Myrtle[2], S. Sathyamoorthi[3].* 1) Dept Ped/ Div Clin Gen, LSU Hlth Sci Ctr, New Orleans, LA; 2) Volunteer Children's Hospital and Pre-med student at University of Georgia, Athens, GA; 3) Volunteer Children's Hospital and Pre-med student at Tulane University, New Orleans, LA.

With the advent of aCGH techniques, most clinicians, ignoring the usefulness of detailed evaluation, immediately request laboratory testing rather than spending time on PE. Often, the examination of face, hands, and feet provide the most important diagnostic clues. The examination of hands and feet, other than looking for single transverse palmar flexion crease, is frequently omitted. For more than 40 years, the senior author has used the analysis of hands, particularly dermatoglyphics to suspect cryptic chromosomal abnormalities (Lacassie, Y, Stahls, PF. Chromosomal abnormalities predicted by the absence of "d" triradius. AJHG Suppl Vol 61 (#4)-47th Annual Meeting ASHG, 1997, Baltimore). The advent of aCGH is allowing the detection of a large number of microdeletions/ duplications missed with standard high resolution chromosomal techniques. We were interested in finding out if dermatoglyphic abnormalities are also suggestive of microdeletions/duplications. Material and methods. Between June 1, 2007 and June 1, 2009, the senior author had 1217 encounters corresponding to 984 patients. In 157 patients with an evident genetic disorder but unspecific diagnosis (syndromes in identification or SINID) we performed aCGH (15.9%), obtaining 57 (36.3%) with abnormal results. These 157 patients do not include patients with recognizable classic numerical or structural chromosomal disorders. We are currently analyzing the dermatoglyphics findings in both groups, those patients with normal and abnormal microarrays. Although the absence of d (or a and b) triradius is highly predictive of a more substantial chromosomal abnormality, the preliminary results show that there are several minor dermatoglyphic traits that seem to be more frequent in the population with microdeletions and/or duplications. During this presentation, for the benefit of the audience we will review the normal dermatoglyphics traits and the most common abnormalities associated with some recognizable chromosomal syndromes. We will stress those features that suggest the possibility of a microdeletion/duplication. We conclude that the use of some dermatoglyphic findings associated with the antecedents of intrauterine and postnatal growth retardation, delayed developmental milestones and /or mental retardation or autism, dysmorphic features and/ or congenital malformations and neurological manifestations, help to yield a higher percentage of cryptic chromosomal abnormalities.

## 2610/F/Poster Board #126

**Novel copy number alterations from the Korean child with craniofacial microsomia.** *J.O. Lee[1], E.J. Seo[1,2], K.R. Jun[1,2], M. Hong[1], H.W. Yoo[1,3], I.S. Park[1,3].* 1) Genome Research Center for Birth defects and Genetic disorders, Asan Medical Center, Seoul, Korea; 2) Dept. of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea; 3) Dept. of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

Hemifacial microsomia is characterized by facial asymmetry, preauricular facial tags, ear malformations, and hearing loss. Ear malformations can include microtia, anotia, or aural atresia, and hearing loss. We studied copy number alterations (CNAs) from the Korean 3-yr-old male with craniofacial microsomia. He had left small-sized deformed ear, hearing loss, facial asymmetry, microcephaly, and development delay. We detected two CNAs, 1.3 Mb heterozygote deletion on 10p12.31 and 227 kb hemizygous duplication on Xq21.1, by array comparative genomic hybridization using Agilent's Human Genome CGH Microarray Kit 244A. Deleted region on 10p12.31 contains only one gene, PLXDC2 (plexin-domain containing 2) which encodes for the relatively uncharacterized transmembrane protein with an area of nidogen homology and a plexin repeat (PSI domain) in its extracellular region. In the mouse study, Plxdc2 is expressed in the embryonic mouse, with particular emphasis on the developing central nervous system and developing heart. The hemizygous duplication on Xq21.1 caused duplication of transcription factor gene, TBX22, involving the regulation of developmental process. Mutations of this gene causing loss of function have been found in patients with cleft palate with ankyloglossia. We validated copy number changes of PLXDC2 and TBX22 using quantitative real-time PCR. These two genes are not harbored within CNV region from public CNV database and showed normal copy numbers in 126 normal Korean individuals. This study suggests that PLXDC2 deletion and TBX22 duplication appear to be novel CNAs and either of them can be associated with craniofacial microsomia.

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## 2611/F/Poster Board #127

**A Novel Autosomal Recessive Syndrome with Congenital Stargardt-like Macular Dystrophy and Corpus Callosum Agenesis.** *P. Bitoun[1], E. Pipiras[2], J. Gaudelus[3], B. Benzacken[2], A. Delahaye[2].* 1) Gen Med, CHU Paris-Nord, Hopital Jean Verdier, Bondy, France; 2) Histology Embryology and Cytogenetics, CHU Paris Nord, Hopital jean Verdier, Bondy; 3) Pediatrics, CHU Paris Nord, Hopital Jean Verdier, Bondy.

Introduction: Many forms of macular dystrophies have been described including Stargardt disease with ABCA4 mutations , dominant Stargardt-like disease due to ELOVL4 mutations , Best disease with mutations in the VMD2 gene,Carolina macular dystrophy, Bietti's disease with crystalline deposits, and age related macular degeneration linked to ABCA4, CFH, C2 and ARMS2 mutation or predisposing risk alleles. Diffuse Retinal dystrophy associated with corpus callosum agenesis has been described in Aicardi syndrome which often involves developmental delay. Patients and Methods: The authors describe a patient with a novel syndrome with congenital macular dystrophy presenting with congenital nytagmus and low vision associated with corpus callosum agenesis. This 5 year-old girl presenting with low vision was seen for genetic counseling. Parents have given informed consent for participation in genetic research. Results: She was born at term from consanguineous first cousin parents with nystagmus by 6 month and corpus callosum agenesis seen on prenatal ultrasound and normal karyotype on amniocytes . She has a healthy brother and sister. Cranial ultrasound confirmed corpus callosum agenesis. Her distance visual acuity was 20/200 bilaterally and near acuity of Parinaud 4. She had no photophobia nightblindness or dyschromatopsia. Her fundus examination showed bilateral oval lesions of macular atrophy . Angiography confirmed macular atrophy without autofluorescence. Electrophysiology showed bilateral normal flash ERG and flicker response and symetrical pattern VEP showing good response to 60° and 30° but no response to 15° pattern VEP at right and a delayed P100 response at left . These anomalies are compatible with macular bundle dysfunction. OCT examination showed complete macular atrophy with thinning of the retina and interruption of the photoreceptor layer in the macular area . Macular thickness was 102 microns at right(60% normal) and 92 (50% normal) at left ,Macular volume was 4.65 mm3 at R and 4.32 mm3 at L. 15 Hue Desaturated color vision test showed slight dyschromatopsia. She had a normal karyotype, psychomotor development and intelligence.Gene testing was negative for ABCA4 mutations. Both parents had normal vision, ERG, VEP, OCT and angiography. Conclusion: This is the first presentation of a congenital developmental Stargardt-like macular dystrophy syndrome with corpus callosum agenesis and apparently recessive inheritance.

## 2612/F/Poster Board #128

**De novo Triple Segmental Aneuploid of 1p, 1q and 4q in a Girl with Hypertrophic Cardiomyopathy, Muscle Hypotonia and Multiple Congenital Anomalies.** *G. Ma[1,2], Y. Ke[1,3], D. Lee[1], M. Chen[1,4,5,6].* 1) Department of Medical Research, Department of Genomic Medicine and Center for Medical Genetics, Changhua Christian Hospital, Changhua, Taiwan; 2) Institute of Biochemistry and Biotechnology, Chung Shan Medical University, Taichung, Taiwan; 3) Department of Pediatrics, Changhua Christian Hospital, Changhua, Taiwan; 4) Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua, Taiwan; 5) Department of Obstetrics and Gynecology, College of Medicine and Hospital, National Taiwan University, Taipei, Taiwan; 6) Department of Medical Genetics, College of Medicine and Hospital, National Taiwan University, Taipei, Taiwan.

Segmental aneuploids are a significant cause of morbidity and mortality in children, with an estimated incidence of 0.7-1 in 1000 births. Different segment aneuploids often have different clinical effects, depending upon the genes included in the aberrant regions. Children with hypertrophic cardiomyopathy (HCM) combining with muscle hypotonia (MH) are rare and the underlying case remains unclear. We report on a girl with HCM, MH and multiple congenital anomalies, in whom a de novo triple segmental aneuploid involving 1p13-1p21 trisomy, 1q32-1qter trisomy and 4q35-4qter monosomy was demonstrated by molecular cytogenetic analyses (G-banding, FISH, SKY and array-CGH). We examined the breakpoints, sizes and genes included of the segmental aneuploids, and discussed the possible genotype-phenotype correlations of the composite aneuploidy, with an emphasis on the two rare pediatric conditions of HCM and MH. By reviewing the literature we suggest that: (1) the 1q32.2-qter trisomy plays a major role in the appearance of the clinical phenotype and results in distal 1q trisomy syndrome in our patient; (2) increased gene dosages of two sarcomeric genes, TNNT2 and ACTA1, in distal 1q could be responsible for HCM; (3) the 4q35-qter monosomy could contribute to MH; (4) the critical regions for distal 1q trisomy syndrome and Kabuki make-up-like syndrome were located at 1q42 and 1p21-p22, respectively.

## 2613/F/Poster Board #129

**An unusual case of thrombocytopenia explained by a balanced translocation (Xp; 11q) in a female with random X-inactivation.** *N. Shur[1], P. Cotter[2], M. Gorre[2], E. Enriquez[2], G. Harappanhaly[1], D. Abuelo[1].* 1) Rhode Island Hospital 593 Eddy Street Providence, RI 02906; 2) Combimatrix Molecular Diagnostics 310 Goddard Suite 150 Irvine, CA 92618.

A 19-year-old female with a developmental delay and seizures was hospitalized for a six month history of thrombocytopenia . Her medications included phenyotoin, which she had taken for a number of years and valproic acid, which had been added in the last year. Hematology-oncology transfused her with platelets and performed a bone marrow biopsy which ruled out leukemia. Review of her history revealed that she was the 8 1/2 pound product of a full-term uncomplicated pregnancy to a 28 year-old G2 mother. Syndactyly between her right second and third finger was noted, which was repaired at age 3, but otherwise she appeared normal. At 5 months of age, a seizure disorder was diagnosed. She walked at age 2 ½ and began using two word sentences at age 7. An extensive metabolic work-up was done prior to referral at age 8 to genetics clinic. A high-resolution karyotype revealed 46X, t(X; 11) (p11.1; q25). On our P.E, weight and height were normal, and she had a head circumference of 52.5 cm (10-20%). Dysmorphic features included a slightly low anterior hairline, bushy eyebrows, a broad nasal bridge, slight hypertelorism, borderline low-set ears, and a high-arched palate. She had diffuse bruising. Neurological examination revealed mild ataxia. She was able to carry on a simple conversation. We concluded that she had the phenotypic effects of a partial 11q deletion disorder -- caused by partial inactivation of the X with the translocation. 11q deletion syndrome, also known as Jacobson syndrome: characteristics include dysmorphic features; syndactyly; global delays; and Paris-Trousseau syndrome characterized by thrombocytopenia and platelet dysfunction. Seizures have not been widely reported. FISH analysis showed a signal pattern with a translocation disrupting the 11q25 region corresponding to BAC clone Rp11-298F20. X-inactivation studies revealed a random inactivation pattern. An oligoarray returned as normal. In patients with 11q deletions, medications like phenytoin and valproic acid, which can exacerbate platelet dysfunction, should be avoided. After genetics consultation, our patient was switched to a ketogenic diet. Her thrombocytopenia resolved. This case is the first reported of Jacobson caused by a Xp; 11q translocation. Our patient's story demonstrates the importance of reviewing any rare genetic diagnosis in a patient presenting with seemingly mysterious symptoms.

## 2614/F/Poster Board #130

**Junctional epidermolysis bullosa. The identification of rare novel mutation in the LAMA3 gene.** *E. GUZMAN, H. SHUHAIBER, HANS H., K. ANYANE-YEBOA.* COLUMBIA UNIVERSITY MEDICAL CENTRE- NEW YORK PRESBYTERIAN HOSPITAL, DEPARTMENT OF PEDIATRICS, NEW YORK, N.Y.

Junctional epidermolysis bullosa (JEB) represents a genetically heterozygous group of bullous disorders characterized by dermo-epidermal separation resulting from mutations affecting the main dermo-epidermal adhesion factor, laminin-5, its cellular receptor, integrin alpha6B4, or collagen XVII. We report the identification of a new mutation of LAMA3, encoding laminin-5 alpha3 subunit in one infant of a Lebanese family. 8- day- old newborn descending from 1st degree consanguineous marriages, presented a lethal form of EBJ-Herlitz.. She had yellow and peeling blisters noted over hands, sacrum and buttocks also recurrent esophageal blisters leaving her unable to feed and gain weight. Histologic, ultrastructural and immunofluorescence studies were performed in order to ascertain the diagnosis and to direct genetic analysis. Immunohistology of frozen skin samples revealed an extremely reduced immunoreactivity for the alpha3 laminin-5 subunit. Our patient was found was found homozygous for c.4335dupA mutation in exon 32 of LAMA3 gene. The c.4335dupA is a single base duplication(A) causing a frameshift starting with codon Leu1446, changing residue to Isoleucine, then creating a premature Stop codon at position 11 of the new reading frame, denoted Leu1446Ilefs11. This expected to result in nonsense-mediated mRNA decay or in protein truncation. Susequently, testing both parents showed heterozygous carriers of the c.4335dupA Mutation in the LAMA3 gene. Ayoub et al 2005 first described this founder mutation in two Lebanese families. This newly identified mutation results in reduced synthesis of alpha3 chain and truncation of its C-terminal domain, which is crucial for the intermolecular interactions of laminin-5. This new case further underscores the geographical specificity of EBJ mutations linked to founder effects which are amplified by consanguineous marriages in genetically isolated populations who have immigrated to the United States.

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## 2615/F/Poster Board #131

**Absent orbicularis oris and 22q11.2 deletion.** *R. Madankumar[1], L.C. Higgs[2], N. Chandy[2], D. Kronn[2].* 1) Dept. of Reproductive Genetics, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; 2) Dept. of Medical Genetics, Westchester Medical Center, Valhalla, NY.

Background: Deletion of 22q11.2 may present as a variety of syndromes such as DiGeorge(DGS), Velocardiofacial(VCFS)and Conotruncal face anomaly. CATCH22(Cardiac anomaly, Abnormal facies, Thymic hypoplasia, Cleft palate, Hypocalcemia)is used to encompass these syndromes which share a common genetic etiology. Case report: A 15 month old girl was seen for asymmetric smile due to absence of right orbicularis oris muscle. She was born to 32 year old G2P1001, full term by NSVD, birth weight was 6lbs 14oz and apgars were 8 & 9. Pregnancy was unremarkable and she has a healthy 3 year old sister. Since birth she was noted to have asymmetric facial movements, prominent while crying or smiling. Paternal history is notable for Factor V Leiden mutation. During her initial genetic evaluation at 10 months, her milestones were felt to be age appropriate with no facial dysmorphism except for asymmetric smile and a high arched palate. Other systems were normal clinically. Although initially reported normal, a subsequent review of her echo reveals an insignificant atrial septal defect and her hearing evaluation was normal. At 15 months of age she was not walking, her height was at 10th and weight and head circumference were at less than 5th percentile. Her karyotype revealed 46,XX,del(22)(q11.21q11.23) and FISH analysis revealed a deletion within 22-ish del(22)(q11.2q11.2)(-TUPLE1-), suggesting DGS/VCF syndrome. Calcium level, CD3 and CD4 counts were normal. The patient is a heterozygote for factor V Leiden mutation and additional thrombophilia workup was negative. Oligonucleotide micro array analysis, early intervention evaluation and a feeding study were requested on the patient and FISH 22q analysis was sent on the parents. Discussion: Asymmetric smile should alert one to suspect a 22q11.2 deletion. To date, the genes responsible for all features of this contiguous gene syndrome have not been identified. Loss of one particular gene, TBX1, has been found to be responsible for cardiac defects and studies have shown that CRKL gene which maps within the 22q11.2 region was associated with cardiac, parathyroid and craniofacial anomalies. Chromosome and FISH analysis alone will not always help to determine the expected phenotype. More studies on genotype and phenotype correlation in conjunction with microarray techniques may better define the syndrome. Prenatal microarray studies in future may assist in counseling mothers carrying pregnancies detected with 22q11.2 deletions.

## 2616/F/Poster Board #132

**A microduplication of the Rubinstein-Taybi region on 16p13 in a girl with cleft palate and severe mental retardation.** *G. YESIL[1], BWM. VAN BON[2], A. HOISCHEN[2], JA. VELTMAN[2], BBA. DE VRIES[2], B. TUYSUZ[1].* 1) Istanbul University, Cerrahpasa Medical Faculty, Deapartment of Medical Genetics, Turkey; 2) Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

A 2,5 years-old female child was admitted to our unit with cleft palate and multiple congenital anomalies. She was the third child of non-consanguineous healthy parents. On physical examination, weight, height and head circumference were 9600 gr (<3p), 79 cm (<3p) and 47 cm (3-50p), respectively. She had cleft palate, hypertelorism, short palpebral fissures, a flat and broad nasal bridge, left ptosis and epicanthus, right esotrophia, large and anteverted nares, long philtrum, large mouth, hypoplastic, large and low set ears, microretrognathia, sparse and fine hair and eyebrows, clinodactyly, nail hypoplasia on fifth fingers and short hands. Radiological findings showed short distal phalanges on the both hands and feet and coxa valga. Small ASD was revealed on echocardiography. Psychomotor development was delayed as she sat unsupported at 11 months and walked at 32 months. At the age of 6 years, her language skills were very poor as she could say only 10 words. The karyotype revealed 46, XY. A genome wide single nucleotide polymorphism array (SNP array 6.0) was done according to the standard of Affymetrix GeneChip protocol (Affymetrix Inc, Santa Clara, California, USA). Array analysis showed a duplication of 1091 markers of the Rubinstein Taybi region on 16p13.3. The gain had a size of 2.1 Mb (chr16: 2, 645, 930-4, 728, 276 Mb, Hg18). Only three patients have been reported with microduplication of the Rubinstein Taybi region on 16p13.3. The patients had dysmorphic facies, moderate mental retardation, atrial septal defect, vertebral fusion on C5-C6, pes cavus and clawed toes and equinovalgus. The presented patient had overlapping features with previously studies like short palpebral fissure, hypertelorism, broad nasal bridge, malformed and low set ears, sparse hair and ASD. Our patient also showed short stature, cleft palate and severe mental retardation with delayed speech had not been reported previously. These findings were reported in patients who had cytogenetically apparent 16p13 duplication. It seems that the severity of the condition in our patient is strongly related with the large duplication with 2.1 megabases.

## 2617/F/Poster Board #133

***MEF2C* haploinsufficiency is associated with severe mental retardation, seizures, hypotonia, thinning of corpus callosum and delay of white matter myelinisation.** *B. Nowakowska[1], E. Obersztyn[1], K. Szymanska[2,3], D.W. Stockton[4,5], A. Patel[6], M. Bekiesinska-Figatowska[7], E. Bocian[1], K. Szczaluba[1], S.W. Cheung[6], P. Stankiewicz[1,6].* 1) Dept of Medical Genetics, Institute of Mother & Child, Warsaw, Poland; 2) Dept of Clinical and Experimental Neuropathology, Mossakowski Medical Research Center, Polish Academy of Sciences, Warsaw, Poland; 3) Dept of Child Psychiatry, Medical University of Warsaw, Warsaw, Poland; 4) Wayne State University School of Medicine, Division of Genetic and Metabolic Disorders, Detroit, MI; 5) Children's Hospital of Michigan Division of Genetic and Metabolic Disorders, Detroit, MI; 6) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 7) Dept of Diagnostic Imaging, Institute of Mother & Child, Warsaw, Poland.

Myocyte enhancer-binding factor 2C (MEF2C) is as a transcription factor expressed at high level in muscle, heart precursor cells, and brain. In brain, MEF2C has a crucial function in the homeostatic control of activity-dependent synaptogenesis that plays a role in the establishment of functional neuronal circuits during development and memory storage. We present three patients with severe mental retardation, in whom we identified overlapping deletions in 5q14.3 involving *MEF2C* using a clinical oligonucleotide array CGH. In case 1, array CGH (CMA V7.4) revealed an ~ 110 kb deletion encompassing first three exons of *MEF2C* in a 3 year old patient with severe psychomotor retardation, periodic tremor and abnormal motor pattern with mirror movement of the upper limbs observed during infancy, abnormal EEG, epilepsy, absent of speech, autistic behavior, bruxism and mild dysmorphic features. MRI of brain showed mild thinning of corpus callosum and delay of white matter myelinisation in the occipital lobes. Case 2, an ~ 1.8 Mb deletion of *TMEM161B* and *MEF2C* was found in a child with severe developmental delay, hypotonia, and agenesis of corpus callosum. Case 3, an ~ 5.6 Mb deletion containing *EDIL3*, *COX7C*, *RASA1*, *CCNH*, *TMEM161B*, and *MEF2C* was identified in a patient with developmental delay, hypo- / hypertonia, microcephaly, seizures with abnormal EEG, and hirsutism. MRI and PET studies showed abnormal basal ganglia and globus pallidus, and cortical dysplasia. The common clinical features observed in all three patients include severe psychomotor retardation, seizures, hypotonia, abnormal EEG and MRI results with mild thinning of corpus callosum and delay of white matter myelinisation. Cardoso et al (2009) reported three overlapping deletions in 5q14.3q15 distal and adjacent to the common deleted region in our patients, associated with periventricular heterotopia, mental retardation, and epilepsy, and suggested candidate genes, *GPR98*, *CETN3*, *NR2F1* and *MCTP1*. One of these deletion contained *MEF2C* and in two other cases, the proximal breakpoints of the deletions mapped close but distal to *MEF2C*. We suggest that position effect cannot be excluded. *MEF2C* was postulated to play an important role in heart development; however, no heart defect was observed in any of the cases. We propose that haploinsufficiency of *MEF2C* is responsible for the severe neurological phenotype in our patients.

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## 2618/F/Poster Board #134

**Familial 18q22.3→18q23 Deletion.** *D.L. Pickering[1], B.J. Dave[1], J.D. Eudy[2], A. Haskins Olney[1], R. Lutz[1], K. Platt[3], W.G. Sanger[1].* 1) Department of Pediatrics and Human Genetics Laboratory, Munroe Meyer Institute for Genetics and Rehabilitation, University of Nebraska Medical Center, Omaha, NE; 2) Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE; 3) Boystown National Research Hospital, Omaha, NE.

We describe a family with an 18q22.3-q23 interstitial deletion and corresponding clinical features of the 18q22-qter deletion syndrome. The clinical phenotype of the proband and two maternal half-siblings includes short stature, a seizure disorder, developmental delays, hearing loss, and aural atresia with clefting of the soft palate. The deletion was initially identified by BAC aCGH followed by confirmatory FISH studies. Precise definition of the breakpoints was achieved by using oligonucleotide aCGH which redefined the minimum region of deletion as a 4.1 megabase interstitial deletion at 18q22.3-q23, from linear location 70565916bp - 74719434bp (hg18). The previously defined region associated with the cleft palate component in the 18q22.3-qter deletion syndrome has been reported to be 7.2 megabases, our study has helped refine this minimum deleted region. There are nine genes located in the deleted region, and two of them are strong candidates that might account for at least three of the observed anomalies. We assert that a strong candidate gene for two of these anomalies, cleft palate and congenital aural atresia, is teashirt zinc finger-1 (*TSHZ1*), an evolutionarily conserved putative zinc finger transcription factor. Galanin receptor-1 (*GALR1*), a receptor for the neuropeptide galanin, could be considered a candidate for seizures in these patients and may explain the association of epilepsy with 18q deletion syndrome. Oligonucleotide aCGH more accurately defines the breakpoints while identifying the specific genes involved in chromosomal deletions. Particularly in this familial study, the use of oligonucleotide aCGH facilitated precise phenotype/genotype correlations and has helped refine the minimum deleted region associated with 18q deletion syndrome. Our study underscores the importance of using high resolution aCGH in clinical investigations for accurate definition of the genetic anomaly and help gain a better understanding of the functional role of specific genes located within the critical regions.

## 2619/F/Poster Board #135

**Use of chromosome microarray analysis to map an Xq interstitial deletion in a female.** *M.E. Pierpont[1,2], B. Hall[1,3], D. Freese[4].* 1) Children's Hospitals and Clinics of Minnesota, St Paul, MN; 2) University of Minnesota, Minneapolis, MN; 3) Minnesota Perinatal Physicians, United Hospital, St. Paul, MN; 4) Mayo Clinic, Rochester, MN.

Chromosome microarray analysis (CMA) has allowed for better definition of cytogenetic deletions. We report a 4 year old girl who was seen for initial evaluation at age 18 months with developmental delay, hypotonia, macrocephaly, macrosomia, and extensive liver hemorrhages. Facial features include broad forehead, pale irises, epicanthal folds, depressed nasal bridge, protruding ears, and curly hair. Sotos syndrome was initially suspected but FISH analysis and sequencing of NSD1 gene were negative. Cytogenetic analysis at the 600 band level revealed a Xq27.2q28 deletion. A concurrent FISH study showed no evidence of a terminal deletion or rearrangement involving the subtelomere region. Additional studies included FMR1 gene testing which showed a CGG repeat number of 30 on both alleles. Due to our suspicion that the FMR1 gene may be involved in the Xq deletion, copy number analysis was performed for FMR1 which confirmed a whole gene deletion on one allele. At age 40 months, the patient experienced a second life threatening episode of intraparenchymal liver hemorrhages. Due to worsening liver failure and renal failure, the patient was treated with dialysis and eventually liver transplantation. Since that time, the patient has been relatively well. At age 4 years, she continues to be developmentally delayed. She is able to walk and can speak a few words. Recently, CMA was performed which further defined the boundries of the deletion to Xq27.1q28. Major genes that were deleted as a result include F9, FMR1, AFF2 (FRAXE), SOX3, IDS and MTM1. Deletions of FMR1 and AFF2 are known to be associated with cognitive and developmental delay and overgrowth. This patient displays a phenotype consistent with that reported in other females with a deletion of FMR1. What is unusual about her is that she had life threatening episodes of liver dysfunction and intraparenchymal hemorrhages, which have been reported in some patients with myotubular myopathy (MTM). She lacks the classic facial appearance of MTM but exhibits mild hypotonia. CMA analysis has allowed a more complete understanding of the genes involved in this deletion and how they contribute to the patient's phenotype and the potential for associated medical complications.

## 2620/F/Poster Board #136

**A Japanese infant with 1q41q42 deletion detected by using array-CGH.** *H. Yoshihashi[1,2], S. Hayashi[3], N. Furuya[2], K. Kurosawa[2], J. Inazawa[3].* 1) Division of Medical Genetics, Gunma Children's, Medical Center, Shibukawa, Japan; 2) Division of Medical Genetics, Kanagawa Children's, Medical Center, Yokohama, Japan; 3) Department of Molecular Cytogenetics, Medical Research Institute and School of Biomedical Science, Tokyo Medical and Dental University, Tokyo, Japan.

Array-based CGH has enabled the detection of novel genomic disorders using a genotype-first approach. We have screened genomic aberrations in patients with multiple congenital anomalies and mental retardation (MCA/MR) using MCG Whole Genome (WG) Array, which harbors 4523 BACs throughout the human genome (Hayashi S et al. ASHG 2007). Here, we report on a case with a small interstitial deletion of 1q41q42. Case: The propositus was a 3-year-old boy. At 38 weeks of gestation, he was born by Cesarean delivery due to CPD. Birth weight was 2680g (-0.7SD), length45.0cm (-1.9SD), and head circumference 34.0cm (+0.5SD). He had right club foot, and distinctive facial appearance (down-slanting palpebral fissures, thick lips, spaced teeth, long philtrum, upturned nares). Standard karyotype was normal. In infancy, his other complications included developmental delay, strabismus, laryngomalacia, undescended tesitis, and febrile seizures. Cytogenetic study: Decreases in the ratio of BAC clones around 1q41 were detected [arr cgh 1q41q42.11(RP11-211K12→239E10)×1], consistent with a heterozygous deletion. Based on our FISH mapping analysis, we estimated the size of monosomic region of about 6.1Mb< from 1q41 (RP11-224O19) to 1q42.11 (RP11-367O4). This case was found with de novo deletion of 1q41q42, resulting from the parental chromosomal study. Conclusions: Deletions of 1q41q42 have been infrequently reported. Shaffer et al. delineated clinical manifestations and breakpoint locations for the seven subjects with 1q41q42 deletion (Shaffer et al.,2006). Some clinical findings of our patient (facial features, club foot, seizures, mental retardation) shared common manifestations to those of the group with 1q41q42 deletion. The deleted region of our subject included the smallest region of overlap (<1.17 Mb) and contained the DISP1 gene, suggested as a candidate gene causing the features in the previous report. The common clinical features and cytogenetic results found in our case support that deletion of 1q41q42 is a new microdeletion syndrome. In conclusion, aCGH provides a useful strategy to investigate cryptic genomic aberrations responsible for unknown MCA/MR, and/or to establish a new syndrome.

## 2621/F/Poster Board #137

**Proximal interstitial 4q deletion associated with macrocephaly, plagiocephaly, ptosis, intraventricular hemorrhage, hypotonia, and growth and global development delays.** *R. Zambrano[1], E. Lisi[1], S. Morsey[2], D. Batista[2,3], T. Wang[1].* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Kennedy Krieger Institute, Baltimore, MD; 3) Department of Pathology, Johns Hopkins University, Baltimore, MD.

Proximal 4q deletions are rare and not well characterized cytogenetically and phenotypically. The majority of the reported cases were studied using standard karyotyping methods with loosely defined clinical features including macrocephaly; facial dysmorphism; small hands with short fingers and clinodactyly; hypotonia, developmental delays and growth retardation. Delineation of the deletions is important to understand the clinical phenotype, implicate novel genes that underlie the pathogenesis, and differentiate disease-causing genomic imbalances from benign copy number variations. We report clinical and molecular cytogenetic studies of a *de novo* genomic deletion involving chromosome 4q in a 19 month-old male with macrocephaly, plagiocephaly, recurrent intraventricular hemorrhage; ptosis, nasolacrimal duct obstruction; facial dysmorphism; mild hydronephrosis; hypotonia; significantly delayed global development, physical growth, and bone age. BAC-microarray CGH with 4200 clones (BlueGnome) identified a genomic deletion at 4q21.21-21.23 that was confirmed by FISH. The deletion boundaries (79,156,795 to 86,063,181 bps) and size (6.9Mb) were determined using the Illumina 610,000 SNParray platform. A total of 30 annotated genes were identified within the deleted region using KaryoStudio 1.0.3 Build 36.1 (Illumina) and are predicted to be monosomic in this patient. Among these, at least 6 genes are known to express at high levels in the brain, at least 3 genes (*BMP3*, *BMP2K* and *PRKG2*) are known to be involved in long bone growth and maturation; 1 gene, *ANXA3* has been implicated as a novel angiogenic factor; and *FRAS1* encodes an extracellular matrix protein essential in normal dermal adhesion during development, and homozygous mutations in this gene have been implicated in Fraser syndrome 1. Further characterization of genes that are involved in this deletion should help to understand the genetic basis for the constellation of findings in this patient and implicate important genes in normal brain and skeletal development in humans.

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## 2622/F/Poster Board #138

**Unique deletion 1q42: phenotype-genotype correlation in a patient with corpus callosum agenesis and MCA.** *I. Filges[1], B. Roethlisberger[2], N. Boesch[1], P. Weber[3], A.R. Huber[2], K. Heinimann[1], P. Miny[1].* 1) Division of Medical Genetics, University Children's Hospital and Department of Biomedicine, Basel, Switzerland; 2) Center of Laboratory Medicine, Cantonal Hospital, Aarau, Switzerland; 3) Division of Neuropediatrics, University Children's Hospital, Basel, Switzerland.

Interstitial deletions of 1q are rarely reported. Deletions of different extent as well as a variable phenotype have been described. Dysmorphic features, developmental delay and midline defects are common clinical features. Severe phenotype apparently resembles Fryns Syndrome. We report on a female patient who is the 3rd child of healthy, non-consanguineous parents with uneventful family history. Agenesis of the corpus callosum has been diagnosed prenatally by ultrasound and MRI. The child was born at term with growth parameters in the lower normal range. After delivery she presented with dysmorphic features including frontal bossing, midface hypoplasia and very high nasal bridge, hypoplastic nails, bowed lower extremities and bilateral cataract. Bilateral cleft palate was diagnosed, agenesis of the corpus callosum was confirmed and heart malformation was excluded, radiologic skeletal survey was normal. At the age of 5 months she presented with global developmental delay, but did not develop seizures until to date. Standard karyotyping revealed 46,XX. Array-CGH analysis (NimbleGen HG18 WG Tiling 385k CGH v2.0) showed a 5.45Mb interstitial deletion on the long arm of chromosome 1. The centromeric breakpoint is situated at 221.886-221.895 Mb, the telomeric breakpoint is located at 227.323-227.340 Mb. The deleted region comprises at least 53 genes and can be cytogenetically defined as 46,XX,del(1)(q41q42.13). Locus-specific FISH-analysis of both parents did not reveal an imbalance. This unique de novo deletion mainly affects the proximal region of 1q42 and includes only 0.2Mb in the 1q41 region. In contrast, patients decribed in the literature (Shaffer LG et al., 2007; www.decipher.sanger.ac.uk) show a deletion mainly exceeding into the 1q41 region. The deletion of our patient is not including the DISP1 gene which has been considered as a possible candidate for the developmental defects in the phenotype (Shaffer LG et al., 2007). We suggest a more distal region 1q42.11q42.12 being critical especially for cleft palate. To our knowledge only one additional patient with a deletion 1q42 is known with an agenesis of the corpus callosum (Puthuran MJ et al., 2005), but a critical region had been rather proposed to 1q44 (van Bon BWM et al., 2008). Positional effect and gene interaction might contribute to the disease model.

## 2623/F/Poster Board #139

**Atypical Simpson-Golabi-Behmel syndrome phenotype in a patient with duplication of *glypican-4* (GPC-4), and normal *glypican-3* (GPC-3).** *G. Scharer[1], A. Dameron[1], M. Raymond[1], B. Lunt[2], M. Springer[2], K. Swisshelm[2].* 1) Dept. of Pediatrics, Div. of Clinical Genetics & Metabolism, The Children's Hospital/ University of Colorado Denver, Aurora, CO; 2) Colorado Genetics Laboratory, Dept. of Pathology, University of Colorado Denver, Aurora, CO.

Simpson-Golabi-Behmel syndrome (SGBS) is an X-linked disorder associated with cognitive disabilities, pre/postnatal macrosomia, including macrocephaly, and other characteristic physical features. Most cases have been linked to mutations in the *glypican-3* gene (GPC-3) in chromosome region Xq26. Here we report the case of a 7 year old male with phenotypical features very similar to SGBS, with hypotonia and marked cognitive disabilities (absent speech), but with low normal growth and microcephaly. Routine genetic/metabolic work-up was negative, except for a subtle copy number variation (CNV), a one BAC clone duplication at 132.29 Mb on Xq26, detected by whole-genome array based comparative genetic hybridization (aCGH, Blue Genome CytoChip v2.01 BAC array). Interestingly, the duplication affected a known gene, *glypican-4*, which is located in close proximity to *glypican-3* (132.50-132.95 Mb). Clinical testing for deletions/duplications (MLPA) in GPC-3 did not show an extension of the duplication into the reading frame of that gene. Concurrent parental DNA studies did reveal maternal inheritance of the Xq26 duplication. While the mother did not have physical features consistent with SGBS, she did have a history of seizures and mild learning disabilities; and at least 2 maternal male relatives were reported with hypotonia, cognitive disabilities and motor developmental delays. While we can only hypothesize that the identified CNV on Xq26 is the actual cause for our patient's clinical presentation, the phenotypical overlap with SGBS and the involvement of a gene (GPC-4) in the glypican family of cell surface heparin sulfate proteoglycans, which is tightly clustered with GPC-3, make a dose-dependent effect more likely. There is currently no evidence that both genes interact in a counter-regulatory fashion, but the contribution of GPC-4 should be considered in the variability of the SGBS phenotype.

## 2624/F/Poster Board #140

**A 7 Mb partial trisomy 13q34 links postaxial polydactyly to an uncharacterized gene PCID2.** *Y. Ning[1], J. Meekins[1], M. Butler[1], M. Skinner[2], R. Shippy[3], C. Greene[2].* 1) Dept Pathology, Univ Maryland, Baltimore, MD; 2) Dept Pediatrics, Univ Maryland, Baltimore, MD; 3) Affymetrix, Santa Clara, CA.

Postaxial polydactyly is a frequent congenital malformation characterized by fifth digit duplication in hands and/or feet. It can be inherited as an isolated trait or occur as a part of a syndrome. Mutations in the GLI3 gene on 7p have been well-documented to result in postaxial polydactyly in a number of families. Another candidate region was indicated by the observation of postaxial polydactyly in approximately 75% of trisomy 13 cases. Several case reports described that this phenotype was associated with partial trisomy 13q22-qter. Here we report our recent finding of a newborn boy with hypertelorism, wide flat nasal bridge, cleft lip and palate, as well as postaxial polydactyly on both hands. Karyotype analysis showed a terminal deletion of 4p15.2, consistent with a clinical diagnosis of Wolf-Hirschhorn syndrome. The presentation of postaxial polydactyly, which is unusual for the Wolf-Hirschhorn syndrome, prompted fluorescence in-situ hybridization (FISH) investigation to rule out a cryptic translocation. A 13q telomeric probe revealed an unbalanced 4;13 translocation, with monosomy for 4p15.2 and trisomy for 13q34. The mother's chromosomes were normal and the father was not available. In order to determine the size of the unbalanced translocation and establish a genotype-phenotype correlation, we used SNP-oligo microarray approach and identified a terminal 7 Mb of trisomy 13 in our patient. A database search led to the identification of 36 genes in this region. Thirty genes appear to have known functions, and 6 genes are currently uncharacterized. One of these 6 genes is PCID2, PCI domain containing 2. The PCI domain is a highly conserved domain, predicted to be involved in protein-protein interaction. Significantly, PCID2 was found, through large scale "interactome mapping" using a yeast two-hybrid system, to be interacting with SHFM1, encoding split hand/foot malformation (ectrodactyly) type 1. These findings suggest that PCID2 is a candidate gene involving the postaxial polydactyly phenotype. Further characterization of this gene will advance our understanding of the molecular basis of trisomy 13-associated postaxial polydactyly.

## 2625/F/Poster Board #141

**Dissecting the 13q14 microdeletion syndrome to define the critical region for mental retardation.** *M. Amenduni[1], F.T. Papa[1], R.D. Clark[5], M. Bruttini[1], V. Disciglio[1], M.A. Mencarelli[1], M.C. Epistolato[2], P. Toti[2], A. Marozza[1], F. Mari[1], T. Hadjistilianou[3], S. De Francesco[3], A. Acquaviva[4], E. Katzaki[1], M. Mucciolo[1], F. Ariani[1], A. Renieri[1].* 1) Molec Biol, Medical Genetics, Siena, Siena, Italy; 2) Department of Human Pathology and Oncology, University of Siena, Siena, Italy; 3) Retinoblastoma Referral Center, Department of Ophtalmology, University of Siena, Siena, Italy; 4) Department of Pediatrics, Obstetrics and Reproductive Medicine, Italian retinoblastoma registry, University of Siena, Siena, Italy; 5) Divisions of Medical Genetics and Ophthalmology, Retinoblastoma Program, Childrens Hospital Los Angeles, Los Angeles, CA, USA.

Retinoblastoma (RB) is the most common intraocular cancer of early childhood. Tumor development is caused by inactivation of both alleles of RB1 gene, located in 13q14.2. In most cases RB1 is inactivated by point mutations, but also complete gene deletions and gross-sized molecular deletions have been observed. When the deletion involves part of the RB1 surrounding genome, it causes a contiguous gene deletion syndrome characterized by retinoblastoma, mental retardation and dysmorphic facial features. In a previous work, using array-CGH, we defined two critical region for mental retardation (MR) and dismorphic features (Caselli et al.,2007). These regions contained 50 genes and 4 of them appeared to be good candidates for the neurological phenotype: NUFIP1, HTR2A, and the protocaderin genes PCDH8 and PCDH17. Recently we collected five additional patients, three with isolated retinoblastoma and an RB1 large deletion and two presenting RB together with MR and dismorphic features. In these patients, we performed array-CGH (Agilent 44K and 105K) to better define the critical region for MR. In two patients with isolated RB, the deletions included only the RB1 gene, while in the third patient we identified a larger rearrangement of about 8 Mb. We compared the deleted region with the 11 Mb and 34 Mb rearrangements, detected in the two patients presenting also MR. The comparison of these overlapping deletions, allowed us to restrict the critical regions previously identified. The centromeric sub-region now contains 7 genes and NUFIP1 and HTR2A are excluded as candidates genes. The telomeric sub-region now contains 28 including PCDH8 and PCDH17. These results contributed to narrow the critical regions for MR and dysmorphic features and to dissect the syndrome, pointing the attention on the possible role of protocaderin in the generation of the neurological phenotype. These proteins are, indeed, integral membrane proteins which are thought to function in signalling pathways and in cell adhesion in a central nervous system specific manner.

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## 2626/F/Poster Board #142

**Genotypic-Phenotypic Association Study of Chromosome 8p inverted duplication deletion syndrome.** *J. Youngblom[1], R. Davis[2], J. Gregg[2], G. Fisch[3], E. Stark[1].* 1) Dept Biol Sci, California State Univ, Turlock, CA; 2) M.I.N.D. Institute, University of California, Davis, Sacramento, CA; 3) Colleges of Dentistry and Nursing, New York University, New York, New York.

Individuals diagnosed with chromosome 8p inverted duplication deletion manifest a wide range of clinical features and cognitive impairment. To date, a systematic genotypic -phenotypic association study has not been conducted on individuals with this cytogenetic diagnosis. The purpose of this study is to employ array CGH technology to more precisely define the cytogenetic breakpoints and regions of copy number variation found in different individuals with this cytogenetic condition, and compare these results with their phenotypic features. Three families were included in the study, and each family has only one affected child. Blood samples were obtained for array CGH analysis from the affected children and each of the parents for two of the families. The array results were compared with the clinical presentation and cognitive assessment of each patient. The latter included IQ tests, VABS for behavior assessment, and CARS for autism. Comparison of the microarray results among the patients showed regions of consistency where chromosomal deletions and duplications occurred. For example, 2 of the 3 patients have terminal deletions with the breakpoints near the boundary between 8p22 and 8p23.1. However the existence of this deletion did not correlate with the severity of the clinical outcome, i.e. both of the patients with the deletion were less severely affected overall than the patient without the deletion. Additionally, 2 (one most severe and the other least affected) of the 3 patients have a small deletion near the 8p11.22 and 8p11.23 boundary. The duplicated segments show greater variability among the patients. A common core duplicated region was found in all 3 patients that includes all of 8p22 and extends midway into 8p21.3. One of the patients showed an interesting triple stepwise increase in duplication of adjacent segments within this exact region, with no other duplicated regions involved. The other 2 patients have duplications that extend from this core region, albeit in opposite directions, and both are more severely affected physically and cognitively. All the parents showed normal microarray results, except one parent showed a duplication in a region on chromosome 5q of unknown significance. This study will assist in providing a more refined prognosis for patients given this cytogenetic diagnosis, and may help to identify critical genes responsible for the specific physical and cognitive features associated with this condition.

## 2627/F/Poster Board #143

**Clinical and genetic characterization in a patient with complete deletion of TSC2.** *D.R. Amrom[1, 2], F. Andermann[2, 3,4,7], J. Hall[2,5], B. Zifkin[4], E. Andermann[1,2,6].* 1) Neurogenetics Unit, Montreal Neurological Hospital and Institute, Montreal, Quebec, Canada; 2) Department of Neurology & Neurosurgery, McGill University; 3) Epilepsy Service, Montreal Neurological Hospital and Institute; 4) Seizure Clinic, Montreal Neurological Hospital and Institute; 5) Department of Neurosurgery, Montreal Neurological Hospital and Institute; 6) Department of Human Genetics, McGill University; 7) Department of Pediatrics, McGill University.

Purpose Tuberous sclerosis complex (TSC) is caused by a wide spectrum of mutations within the TSC1 and TSC2 genes, mostly point mutations and small indels, while large deletion/duplication mutations are much less frequent and mostly identified in the TSC2 gene. We report the clinical and molecular data of a patient with TSC due to a single copy loss of TSC2. Methods We performed a study of the TSC phenotype and genotype in a patient with a clinical diagnosis of TSC. TSC1 and TSC2 DNA sequencing, as well as TSC2 DNA deletion test were performed, followed by an oligonucleotide microarray using 1543 loci. Results A 28-year-old man had focal seizure onset at 1 year of age well controlled by phenobarbital. TSC was diagnosed at age 5 years. He attended regular school until age 17 years, but had learning difficulties. An intraventricular tumor was found and remained stable for several years. At age 22 years, he developed cognitive deterioration and ataxia, repeat CT scan showed an increased volume of the intraventricular lesion and hydrocephalus. A right frontal subependymal giant cell astrocytoma (SEGA) was resected and seizures recurred. At the age of 26, CT scan and sonography of the abdomen showed angiomyolipomas of the liver, and multiple small angiolipomas as well as several cysts of both kidneys. Two years later, seizures increased and an MRI showed a residual right intraventricular SEGA with an increased cystic component and hydrocephalus, as well as multiple cortical and subcortical tubers. He had endoscopic fenestration of the intraventricular cyst, resection of the glial scar and right residual intraventricular SEGA. He developed left hemiparesis due to an epidural hematoma which was subsequently drained. DNA studies revealed deletion of all exons (1-41) of the TSC2 gene. Microarray analysis detected a single copy loss of 25 oligonucleotide probes from the short arm of chromosome 16 at 16p13.3. This was estimated to be approximately 194.3 Kb in size; it overlapped the TSC2 locus and extended in the 5' direction, containing at least 10 other genes. There was no evidence for TSC in available family members. Quantitative dosage analysis of genomic DNA in the mother and brother did not show any TSC2 deletion nor abnormal DNA sequence variants; the father was not available for genetic testing. Conclusions To our knowledge, the variant detected here has not been previously reported. It appears to be associated with a severe TSC phenotype.

## 2628/F/Poster Board #144

**Marinesco-Sjögren syndrome (MSS): Genotype-Phenotype Correlation and Heterogeniety.** *A. Parsian[1], S. El-sayed[2], E. El-Sobky[2], F. Kok[3].* 1) Div Neuroscience & Behavior, NIAAA/NIH, Bethesda, MD; 2) Department of Pediatrics, University of Ain Shams, Cairo, Egypt; 3) Department of Pediatrics, University of Sao Paulo, Sao Paulo, Brazil.

Marinesco-Sjögren syndrome (MSS [MIM 248800]) is an autosomal recessive multisystem disorder characterized by congenital cataracts, cerebellar ataxia, progressive muscular weakness (chronic myopathy), growth and mental retardation. Peripheral neuropathy, acute rhabdomyolysis, hypergonadotrophic hypogonadism and skeletal deformities have been described occasionally. We sequenced BAP/SIL1gene using genomic DNA from members of an Egyptian pedigree and found a homozygous mutation at position 1312 (C to T) in exon 10 that creates a stop codon (Q438X) in all four affected members. However, two of the older affected pedigree members did not present any sign of ataxia. Sequencing the same gene using genomic DNA from a proband from a Brazilian family revealed a G deletion in exon 2 of the gene that also creates a stop codon. Some of the symptoms of this proband include severe developmental delay, muscular dystrophy and severe apendicular and axial ataxia. A detailed comparison of the phenotypes of the five affecteds in these two pedigrees and related genotypes will be presented. This study clearly shows the effect of the position of the mutations and the severity of the phenotypes.

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## 2629/F/Poster Board #145

**Concordance in Genotype and Discordance in Phenotype found in Monozygotic Twins with Allagile Syndrome.** *H. SHUHAIBER, E. GUZMAN, K. ANYANE-YEBOA.* PEDIATRICS, COLUMBIA UNIVERSITY MEDICAL CENTRE, NEW YORK, N.Y.

Alagille syndrome(AGS) is an autosomal dominant disorder affecting multiple organ systems. The phenotype in Alagille syndrome is highly variable both within families. We report monozygotic twins with Alagille syndrome concordant for a mutation in Jagged1 but discordant for clinical phenotype. The twins' monozygosity was confirmed by molecular testing. This heterozygous C>T was identified in exon 23 resulting in replacement of an arginine codon with a stop codon from CGA to TGA at amino acid position 900. The mutation is denoted as c.2698 C> T at the cDNA level or p.Arg900Stop (R900X) at protein level. Twin A had cholestasis with ductopenia on liver biopsy. Whereas, twin B had paucity of bile ducts with mild centrilobular cholestasis. Both twins had a normal eye exams. Twin A had a Tetrology of Fallot whereas Twin B had a normal heart. The twins described here, demonstrated to be monozygous by molecular testing, share an identical de novo mutation in JAG1 yet display a conspicuous discordance in clinical phenotype.Krantz- 1998; was the first to describe the variability in phenotypic manifestations in AGS among family members sharing the same mutation. In particular, cardiac defects, which are more common in monozygotic twins, usually affect only one of the twin pair. Several genetic hypotheses have been proposed to account for discordance in phenotype in monozygotic twins; among these are somatic mosaicism, differences in deletion size when chromosome deletions are present, and modifying genetic loci. The relationship between genotype and phenotype in AGS remains unclear. Both girls are clearly significantly affected by their mutation, but the pattern of the disease significantly differs by their organ involvement. Twin A being liver and heart, whereas twin B being purely liver . However, it remains difficult to conclude whether the phenotypic discordance results from factors arising from the twinning process, or from the same mechanism that generates the variability seen in family members sharing the same mutation, or in fact a combination of both mechanisms. It is possible that the genotype predisposes individuals to disease and the twinning process adds to the severity of the effect. Currently, testing is underway for the parents to see if one of them carries this mutation since both of them are completely asymptomatic. Further studies are necessary to elucidate the factors that modify the effects of JAG1 mutations on phenotype.

## 2630/F/Poster Board #146

**Increased Bone Mineral Density and Body Mass Index in Families of Osteogenesis Imperfecta: a New Type of OI?** *C. Li.* Dept Pediatrics, McMaster Children's Hosp, Hamilton, ON, Canada.

Osteogenesis Imperfecta (OI) type I is the mildest type of OI and typically manifest with decreased bone density and increased risks for fractures. The patient usually is of thin built, of borderline short stature and with blue sclera. They may also experience dental problems, hearing problems, easy bruising and joint laxity. I report two pedigrees with a milder form of OI confirmed by molecular analysis. Interestingly affected individuals have higher bone density and body mass index. Joint laxity appears to be more pronounced in these affected individuals as well. The first pedigree is of Iraqi origin with at least 37 individuals with a history of multiple fractures. Four of them, age 10, 10 (twins), 13 and 52, were assessed by the author. In addition to blue sclera and fractures, novel features on clinical examination include facial features that are not reminiscent of OI. All of them were overweight. Severe joint laxity was noted in one of them. Bone mineral density studies revealed generalized increased bone density in all four of the affected, up to +3.5 standard deviations from age-match means, most pronounced in the spine. Molecular analysis revealed a mutation c.3359A>C; p.Asp1120Ala in one allele of COL1A2 in the carboxy terminal proteolytic conversion site of procollagen to collagen, in these four affected individuals . Another family of European Caucasian background was assessed by the author. The proband is a 2-year old boy referred for assessment of behavioral issues. He was noted to have blue sclera, joint laxity and dental crowding but no history of fracture. His father, a half-sister by the same father, paternal grandmother and two paternal grandaunts were reported to have blue sclera, joint laxity/ dislocation and dental crowding as well. The father is overweight, of a stocky built. Molecular analysis revealed a novel mutation in the COL1A2 gene, c.380G>A, p. Gly127Asp in the triple helix in the proband and his father. The proband was noted to have osteosclerostic changes on X rays. In summary, I report two pedigrees of multiple individuals with OI confirmed by molecular analysis. Although they exhibit some features commonly associated with OI type 1, affected and presumed (by history, not assessed by author) affected members of both families have increased bone density and are overweight, two features very distinctly contrary to the typical findings of OI and suggest a new type.

## 2631/F/Poster Board #147

**Spondyloperipheral dysplasia - Clinical and radiographic delineation of a novel COL2A1 missense mutation in the C-Propeptide.** *C.T. Thiel[1], H.-G. Doerr[2], H. Sticht[3], A. Rauch[1,4].* 1) Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Germany; 2) Children's and Adolescents' Hospital, University of Erlangen-Nuremberg, Erlangen, Germany; 3) Institute of Biochemistry, Division of Bioinformatics, University of Erlangen-Nuremberg, Erlangen, Germany; 4) Institute of Medical Genetics, University of Zurich, Schwerzenbach-Zurich, Switzerland.

Mutations in the type II collagen gene (COL2A1) result in a broad spectrum of skeletal dysplasias. Most of these are located in the coding region for the helical part of the $\alpha$1(II)-chain and lead to alterations of the triple helix formation or to nonsense-mediated decay (NMD) as observed in Stickler syndrome. In contrast, truncating mutations coding for the C-propeptide have been recently identified as the underlying cause of spondyloperipheral dysplasia [SPD, MIM 271700]. We present an 11 year-old female with characteristic features of SPD-like short-trunk dwarfism and brachydactyly E-like changes. Growth retardation was first noted at 25th week of gestation and she was born at 36th weeks of gestation with a birth weight of 1990 g (-1,7 SDS) and length of 41 cm (-2,9 SDS). Psychomotor development was normal. Her HC followed the 50th to 97th c. whereas her growth remained below the 3rd c. with a proposed adult height of 145 cm. Growth hormone treatment was futile. There have been no signs of hearing impairment or eye problems as reported in other cases of SPD. X-ray evaluation at 11 years of age confirmed a spondyloepiphyseal dysplasia with an accelerated bone age, delayed ossification of the pubic and ischial bones and degenerative changes of the femoral head, horizontal acetabulae, epiphyseal changes and shortening of the metarcapals and the distal phalanges, mild metaphyseal widening at the knee joint and a platyspondyly with fish-mouth vertebrae. Mutational analysis of the COL2A1 gene revealed the de-novo missense mutation c.4250T>C (p.F1417C) within the C-propeptide coding region. All known mutations in patients with SPD result in a premature stop codon within the C-propetide disrupting disulfide bonds formed by cysteine residues or trimer association specific recognition sequences. Therefore, it has been proposed that the C-propeptide plays a crucial role in the association of the procollagen chains in helix formation, which might be disturbed by truncating mutations. As our patient presented with mild clinical and characteristic radiographic features of SPD caused by the first reported missense mutation forming a new cysteine residue, this results support the hypothesis of a conformational change of the C-propeptide by alteration of the disulfide bonds composition. This is of special relevance as accumulation of free procollagen II chains with a calcification-promoting effect of the C-propeptide has been proposed as the underlying cause of SPD.

## 2632/F/Poster Board #148

**Noonan syndrome and related disorders: genotype & phenotype correlations.** *B. Lee[1,2,3], C. Cheon[4], J. Kim[2], G. Kim[2,3], J. Park[3], H. Woo[1,2,3].* 1) Pediatrics, Asan Medical Center, Seoul, Korea; 2) Genome Research Center for Birth Defects and Genetic Disorders, Asan Medical Center, Seoul, Korea; 3) Medical Genetics Clinic and Laboratory, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea; 4) Department of Pediatrics, Children and Adolescent Hospital, Pusan National University, Gyeongsang-nam-do, Korea.

Noonan syndrome (NS) and related disorders (NSLD) are caused by dysregulation or gain-of-function mutation in a member of RAS-MAPK signaling pathway. With more causative genes identified, their genotypic and phenotypic heterogeneities emerge as complex and overlapped. In this study, we analyzed genotype-phenotype correlations with 71 Korean cases with NS or NSLD. Clinical diagnosis were NS in 59 (83%) and NSLD in 12 (17%) cases; 5 with Cardiofasciocutaneous syndrome (CFC), 5 with Costello syndrome (CS) and 2 with LEOPARD syndrome (LS). Male predominance (76%) was noted in NS, but short stature and delayed development were more common in NSLD. Mutations were identified in 34 cases with NS (57.6%): PTPN11 in 19, SOS1 in 11, RAF1 in 3 and KRAS in 1 case. Five cases with CFC carry mutations in BRAF and the rest in MEK1. All the 5 cases with CS and 2 cases with LS harbor mutations in HRAS and PTPN11, respectively. No mutation was found in MEK2. Three cases with RAF1 mutation have hypertrophic cardiomyopathy. Patent ductus arteriosus was more common in cases with PTPN11 mutation than SOS1. Developmental delay was more common in cases with mutation in BRAF or HRAS, with CFC or CS, than cases with PTPN11. There was no correlation in pulmonary stenosis, chest deformity, abnormal genitalia, lymphedema, hearing difficulty, or bleeding tendency. In conclusion, although genotype-phenotype overlap exists between NS and NSLD, searching for common genotypes according to each phenotype can be recommended. Developmental delay is an important sign for the differential diagnosis of NS and NSLD. Further study is needed to identify new mutations in NS with unknown genotypes.

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## 2633/F/Poster Board #149

**Axenfeld-Rieger anomaly and Axenfeld-Rieger syndrome; clinical and genomic analyses on three patients with chromosomal defects at 6p25.**
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Axenfeld-Rieger anomalies, a group of genetically and phenotypically heterogeneous disorders leading to aberrant development of the anterior chamber of eyes, frequently accompany a variety of major and minor anomalies of systemic organs. So far mutations in two genes, FOXC1 and PITX2, which code transcription factors, are known to cause Axenfeld-Rieger anomalies. We encountered three unrelated Japanese patients with Axenfeld-Rieger anomalies accompanied by a variety of anomalies of systemic organs. GTG-banded chromosome analysis showed terminal deletions of a short arm of chromosome 6 in two patients and inv(6)(p25q14) in the other. FISH and DNA array analyses revealed that the former two patients had 5.0-5.7 Mb and 6.6 Mb 6p terminal deletions, respectively, and FOXC1 is apparently deleted in both. In the other patient, the inversion break point at 6p25 is in or very close to FOXC1 locus since BAC clones encompassing FOXC1 gave split signals while clones RP11-157J24 and RP11-265E5, distally and proximally closest neighbors to FOXC1, gave single signals at the original and inverted position on the inv(6), respectively. We characterized the spectrum of extra-ocular findings in these patients and compared them with those in the literature in accordance with several common genetic causes; mutations of FOXC1, 6p terminal deletion including FOXC1, and PITX2 mutations. Patients with PITX2 mutations seemed to have umbilical and dental anomalies more frequently than those with FOXC1 mutations. On the contrary, patients with FOXC1 mutations tend to have hypertelorism and defects in heart and central nervous system much more than those with PITX2 mutations. Patients with 6p terminal deletion usually have mental defects and major systemic anomalies as results of haploinsufficiency of multiple genes. Recognition of the difference in the spectrum of extraocular findings in patients with Axenfeld-Rieger anamalies may lead better clinical management.

## 2634/F/Poster Board #150

**Co-segregation of autosomal recessive polycystic disease and Bardet-Biedl syndrome.**
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Autosomal recessive polycystic disease (ARPKD) is associated with enlarged kidneys and biliary dysgenesis. The disease phenotype caused by mutation in *PKHD1* is highly variable, ranging from neonatal death to later presentation with minimal kidney disease. A different kidney phenotype as well as discordance for postaxial polydactyly in two affected patients from a family prompt us to report the present observation. The first fetus presented at 24 weeks of pregnancy with massively enlarged kidneys and oligohydramnios leading to abortion at 26 weeks. Kidney and liver specimens showed radially oriented collecting tubule cysts and hepatic fibrosis. This fetus carry two distinct missense mutations in *PKDH1* gene inherited from both parents. Whereas the first fetus had no associated clinical features except fibrosis of the liver, the second fetus presented at 20 weeks with postaxial polydactyly and hyperechogenic kidneys without corticomedullary differentiation. Diagnosis of Bardet-Biedl (BBS) phenotype was established at birth on the combination of clinical findings and compound heterozygosity for at least the common *BBS10* mutation and the maternal *PKD1* mutation. Further molecular testing is in process (collaboration with N Katsanis). We show for the first time that (1) co-segregation of mutations at *PKDH1* and *BBS10* genes may cause BBS phenotype,(2) discordance for clinical presentation in affected siblings with primary cilium defect may reflect a multiple mutations loading.

## 2635/F/Poster Board #151

**Exon-focused microarray analysis of candidate genes in Autism.**
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We hypothesize that de novo and recent mutations cause a major fraction of autism cases. These mutations would not be detected by genome-wide linkage studies. Much progress in understanding the etiology of autism is derived from the use of array technologies to detect genomic deletions and duplications followed by sequencing of candidate genes within these genomic regions. Objectives: The goal of this work is to discover novel genomic deletions and duplications causing autism with a particular focus on determination of exon copy number to identify small deletions and duplications that would implicate a single protein coding gene. Genes suspected of causing autism via haploinsufficiency would then be sequenced in other autism individuals. Methods: Customized Agilent arrays were designed to cover all the exons of tens to hundreds of autism candidate genes. Genes were selected based on literature support that they might cause autism (73 genes), on neurological or synaptic function (253 genes), or on an implied role in epigenetic regulation (294 genes). A one-million oligonucleotide array to test copy number for nearly all exons in the genome was also developed. The study group included 98 patients from South Carolina Autism Project, 64 local patients, and 22 autistic individuals from AGRE collection. Results: These experiments identified several very small and apparently benign copy number variants (CNVs). Multiple lymphoblast cell lines were trisomic for various chromosomes. These were interpreted to represent cell culture artifacts. We found 5 cases with pathogenic alterations. Three of these abnormalities were deletions: a 15q13.3 deletion encompassing the CHRNA7 gene, a paternally inherited 2-Mb deletion causing haploinsufficiency of the BDNF and LIN7C genes, and a 6p23 deletion. In addition, there were two duplications involving X-linked genes and the 15q11-q13 imprinted domain. The X-linked duplication was detected in males and included a maternally inherited 615-kb duplication encompassing the OCRL1 gene. Conclusions: These data provide additional support for the importance of copy number variants in the etiology of autism and suggest that exon-focused microarrays may be an effective method for detecting these chromosomal imbalances and identifying specific causative genes. The utilization of arrays that cover all exons in the genome is predicted to increase the detection of these chromosomal imbalances.

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## 2636/F/Poster Board #152

**X-inactivation studies in a familial case of Simpson-Golabi-Behmel Syndrome.** *S. Yano[1], A. Bagheri[1], K. Moseley[1], Y. Watanabe[2], B. Baskin[3], P.N. Ray[3], A. Nishimura[4], N. Matsumoto[4].* 1) Pediatrics/Gen Div, 1G24, LAC+USC Med Ctr, USC, Los Angeles, CA; 2) Department of Pediatrics and Child Health, Kurume University, Kurume, Japan; 3) Division of Molecular Genetics, Department of Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, Canada; 4) Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan.

Introduction: Simpson-Golabi-Behmel syndrome (SGBS) is an overgrowth/multiple congenital anomalies syndrome with X-linked inheritance and a wide clinical spectrum. Most cases of SGBS attributed to mutations in the glypican 3-gene (GPC3). Typical clinical features include pre/post natal overgrowth, macrocephaly, characteristic facies with prominent eyes and macroglossia, congenital heart defects, kidney anomalies, and skeletal anomalies, including vertebral fusion, pectus excavatum rib anomalies, and polydactyly. Developmental delay is often associated. Childhood cancer is found in about 10 %. Obligate carrier females with GPC3 mutations are usually not affected although they can have a milder phenotype. We describe three affected siblings and their mother in a family carrying c.256C>T (p.Arg86X) mutation in the GPC3 gene. All three siblings showed typical symptoms of SGBS. The mother, however, was asymptomatic. We conducted X-inactivation studies in the carrier females. Case Report: Patient 1 is a 3-year-old Jordanian male, prenatally diagnosed with hydronephrosis, hydroureters, and polyhydramnios. All of his body measurements are larger than 97%ile. He has clinical findings consistent with SGBS. His developmental milestones are delayed. Patient 2 is a 2-year-old younger brother of patient 1. He was born as one of the twins at 34 weeks (BW 3800g), was also prenatally diagnosed with hydronephrosis, hydroureters, and macrocephaly. His development has been delayed. Patient 3 is a female, twin sister of patient 2 (BW 2196g, at 50%ile). She had a congenital diaphragmatic hernia and a large PDA. Her development at age 5 months revealed fine motor delay. By age 8 months she showed signs of overgrowth. Their mother is an asymptomatic carrier of the gene mutation. Molecular Studies: X-chromosome inactivation studies were performed on the androgen receptor (AR) gene. The results indicated the paternal X-chromosome was preferentially inactivated (83 % inactivated) in patient 3. X-inactivation studies revealed uninformative results in the asymptomatic carrier female (the mother) due to homozygous alleles of the AR gene. Discussion: There were no reports of molecular studies in carrier females of GPC3 mutations. X chromosome inactivation studies were conducted to evaluate relationship of skewed X inactivation and SBGS female phenotype. The studies showed significantly skewed X-inactivation in the female with SBGS phenotype.

## 2637/F/Poster Board #153

**Mild phenotype of the dominant negative mutation in the tyrosine kinase domain of *KIT* may suggest a second modifier gene for piebaldism.** *N. Oiso[1], T. Suzuki[2], K. Fukai[3], T. Motokawa[4], K. Yokoyama[4], Y. Hozumi[2], A. Kawada[1].* 1) Dept Dermatology, Kinki Univ Sch Med, Osaka-Sayama, Japan; 2) Dept Dermatology, Yamagata Univ Sch Med, Yamagata, Japan; 3) Dept Dermatology, Osaka City Univ Grad Sch Med, Osaka, Japan; 4) Cutaneous Drug Research Dept, POLA Chemical Industries, Inc, Yokohama, Japan.

Piebaldism is a congenital disorder characterized by white forelock and localized leukoderma. In some piebald patients, café-au-lait spots are present. Human piebaldism is caused by a mutation of the *KIT* gene encoding the transmembrane receptor tyrosine kinase, c-kit. The pathogenesis is assumed that a defect is present in the migration and differentiation of melanoblasts from the neural crest. In piebaldism, genotype-phenotype relationship in *KIT* is commonly present. We would like to present a unique family with piebaldism. The mutation detected was a missense mutation, supposedly causing severe phenotype, however the phenotype of the proband, the brother, and the fathere was mild, very mild and unpenetrant, respectively. A 4-year-old girl had leukoderma on the forehead and three of four extremities, and café-au-lait spots on the normally pigmented skin. The proband's 7-year-old brother had patchy leukoderma on the left wrist and café-au-lait spots. The proband's 35-year-old father did not have poliosis or leukoderma. However, the father had café-au-lait spots. Sequence analysis revealed a missense mutation p.F584L in the tyrosine-kinase domain of the *KIT* gene in the proband, the brother and the father. It is believed that a missense mutation in the intracellular tyrosine kinase domain have a dominant-negative effect, reducing *KIT* function by ~75%, resulting in a characteristically severe phenotype. The phenotype in the affected members in this study was expected to be severe. The mild to unpenetrant phenotype in this family may suggest an existence of a modifier gene for the phenotype of piebadism. Recently, we reported a very severe Japanese piebald patient with auburn hair color caused by a novel loss-of-function missense mutation p.P832L in *KIT* and homozygous inactive variants p.I120T in *MC1R* (in press, *Br J Dermatol*). This indicated the *MC1R* gene as a modifier gene for piebaldism. We hypothesized that activating variants of the *MC1R* gene might be present in the family, resulting in the milder phenotype of the leukoderma. However, the sequence result of the *MC1R* gene showed no variants altering the function of MC1R. Identification of modifier gene which upregulates or compensates the function of c-kit may offer a novel therapeutic strategy in piebaldism. The family may give a new insight into the function and regulation of SCF/c-kit signaling in melanogenesis.

## 2638/F/Poster Board #154

**Co-occurring diagnoses among FMR1 premutation allele carriers.** *J. Hunter, J. Rohr, S. Sherman.* Dept Human Genetics, Emory Univ, Atlanta, GA.

Following the discovery of two disorders associated with premutation alleles of the fragile X mental retardation gene (FMR1), primary ovarian insufficiency (FXPOI) and a tremor/ataxia syndrome (FXTAS), numerous studies have examined other potential co-morbid conditions, including neuropsychological deficits. Here, the frequency of self-reported diagnoses obtained through medical history interviews from FMR1 premutation carriers and non-carriers ages 18 to 50 were analyzed. Study subjects included 537 women, 334 of whom carry the premutation, and 151 men, 37 of whom carry the premutation. Men with the premutation did not report any medical conditions at higher rates compared with non-carriers, controlling for age, ethnicity/race, and household income. Women with the premutation reported mental health disorders (i.e., ADHD, anxiety and depression) more often than non-carriers. However, after adjusting for covariates, these increased rates were not statistically significant. Additional follow-up analyses examined the consequence of ovarian dysfunction as a cause of co-occurring conditions. Women with an indication of ovarian insufficiency (i.e., irregular cycles) reported more thyroid problems, depression/anxiety, and gastrointestinal problems. Since only women, not men, reported these conditions more often, the relationship between FXPOI and hormone irregularities in women should be explored for a potential link with the increase in the reported medical conditions.

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## 2639/F/Poster Board #155

**PTPN11 genotype and phenotype correlation of Noonan syndrome.** *Y. Ke[1,2], D. Lee[1], G. Ma[1], M. Chen[1,3,4].* 1) Center for Medical Genetics, and Department of Medical Research, Changhua Christian Hospital, Changhua, Taiwan; 2) Department of Pediatrics, Changhua Christian Hospital, Changhua, Taiwan; 3) Department of Obstetrics and Gynecology, and Department of Medical Genetics, College of Medicine and Hospital, National Taiwan University, Taipei, Taiwan; 4) Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua, Taiwan.

Noonan syndrome (NS) is a developmental disorder caused mainly by mutations in PTPN11 gene. Here, we used the NS scoring system of van der Burgt et al (1994) [Am J Med Genet 53:187-191] for diagnostic criteria of NS. Besides, we performed PTPN11 mutation analyses in six NS patients and one prenatal chylothorax case. Five missense PTPN11 mutations were identified in five cases: three mutations were in exon 3, one in exon 8 and the other one in exon 12. Two patients carried the 922A>G (Asn308Asp) has classic NS symptoms with mild mental retardation. Two cases carried the 182A>G (Asp61Gly) , one has classic NS symptoms and the other is a prenatal case with chylothorax. One patient with 1403C>T (Thr468Met) has classic NS facial symptoms with dominant hypertrophic cardiomyopathy; while the diagnosis of this patient was changed to LEOPARD syndrome thereafter since the mutant site is known to be the common mutant site of LEOPARD syndrome and the patient developed café-au-lait spots at the age of 5 year-old. The other one patient with the mutation 218C>T (Thr73Ile) was suffered from juvenile myelomonocytic leukemia (JMML) with the initial presentation of pancytopenia suspect of severe sepsis. Our results, together with recent reports, suggest that: (1) all the neuro-cardio-facial-cutaneous syndromes share a common genetic and pathophysiologic basis; (2) dysregulation of the RAS-MAPKinase pathway is caused by germline mutations in genes involved in this pathway; (3) more genes causing related syndromes and other disease will be discovered in the near future since a substantial number of genes involved in the pathway are not yet associated with known syndromes or diseases.

## 2640/F/Poster Board #156

**Is PiSS Alpha-1 Antitrypsin Deficiency Associated with Disease?** *D. McGee[1], L. Schwarz[1], R. McClure[1], L. Peterka[1], F. Rouhani[2], M. Brantly[2], C. Strange[1].* 1) Division of Pulmonary and Critical Care Medicine, Medical University of South Carolina, Charleston, SC; 2) Division of Pulmonary and Critical Care Medicine, University of Florida Health Science Center, Gainesville, FL.

BACKGROUND: Alpha-1 antitrypsin deficiency (AAT) is an inherited condition that predisposes to lung and/or liver disease. The most common allele found at the SERPINA1 locus is the M allele that codes for normal amounts of AAT in a codominant manner. The two most common deficiency alleles are Z and S. Currently, there is little data available regarding clinical risks associated with the PiSS genotype. METHODS: Participants were recruited through the Alpha Coded Testing study and the Alpha-1 Foundation Research Registry. Nineteen study participants (PiSS) and 29 age, sex, and state matched control participants (PiMM) were telephone interviewed by a certified genetic counselor using a standardized questionnaire. Demographic features, cigarette smoking, vocation, medication history, and clinical diagnoses were subjected to comparison. Statistical analysis was performed. Finally, a comprehensive literature review was performed by two investigators. RESULTS: 12/19 (63.16%) study participants reported the presence of lung and/or liver disease compared to 12/29 (41.38%) control participants. There was no difference in the frequency of psychiatric diagnoses (anxiety, depression, bipolar disorder) in study participants (47.4%) vs. controls (48.3%) or fetal loss (14.7% vs. 13.9%) in the biological mother of the participants. There was a higher frequency of medication allergies in the study population (42.11% vs. 6.90%). CONCLUSIONS: The PiSS genotype was associated with a high incidence of obstructive lung disease in the study population. Selective bias intrinsic in testing for AAT deficiency and the rarity of the PiSS genotype will make future study of this association dependent on population-based tests.

## 2641/F/Poster Board #157

**Holoprosencephaly mutations in the Dutch population.** *A.D.C. Paulussen[1], C. Schrander Stumpel[1,2], J. Herbergs[1], C. de Die Smulders[1], S. Stegmann[1], Y. Arens[1], M. Vreeburg[1], G. Tan-Sindhunata[6], M. Collee[3], A. van Haeringen[4], A. Hoogeboom[5], K. Lichtenbelt[3], A. Lachmeijer[6], W. Kerstjens Frederikse[7], M.L. Kwee[6], J.A. Maat Kievit[5], G. Mancini[5], M. Simon[5], I. Stolte Dijkstra[7], H.J. Smeets[1,2].* 1) Dept Clinical Genetics, Maastricht University Medical Center, Maastricht, Netherlands; 2) Research Institute Growth and Development (GROW), University Maastricht, Maastricht, The Netherlands; 3) Department of Clinical Genetics, University Medical Center, Utrecht, The Netherlands; 4) Department of Human and Clinical Genetics, Leiden University Medical Center, The Netherlands; 5) Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands; 6) Department of Clinical Genetics, VU University Medical Center, Amsterdam, The Netherlands; 7) Department of Genetics, University Medical Center Groningen, Groningen, The Netherlands.

Holoprosencephaly (HPE) is a severe malformation of the brain that involves abnormal formation and septation of the developing central nervous system. The prevalence is 1:250 during early embryogenesis, but the live born prevalence is only 1:16,000. The etiology of HPE is extremely heterogeneous and can include both a teratogenic and/or genetic basis. We studied four genes known to be involved in HPE, namely SHH, ZIC2, SIX3 and TGIF by sequence and MLPA analysis. A series of in total 80 sporadic and familial HPE cases with a variable clinical spectrum has been analysed. We detected 19 pathogenic mutations (24%) in total, of which 3 in SHH, 7 in ZIC2 and 9 in SIX3. Only one mutation (Alanine-tract expansion in ZIC2) was reported previously and detected twice in this population, all others were novel. Two mutations were complete gene deletions (one SIX3, one ZIC2 deletion) of which the deletion sizes were further characterized using the 250K Nsp I Affymetrix SNP array. The ZIC2 deletion was too small to be detected with this array, the SIX3 deletion also comprised the SIX2 gene and was approximately 1,37 Mb in size. The familial mutations displayed considerable heterogeneity in clinical expression, which makes it difficult to establish genotype-phenotype correlations and indicates that although the mutation detected is essential, it is not the only factor involved. Additional environmental factors and modifier genes will play a role as well. HPE development is probably a multihit process, which implicates more genes, illustrating the importance of further identification of new genes or risk-factors.

## 2642/F/Poster Board #158

**A t(5q;17q) in a patient with acampomelic campomelic dysplasia.** *H. Vernon[1], N. Sobreira[1], E. Lisi[1], E. Wohler[2], J. Hoover-Fong[1], G. Thomas[2], D. Valle[1], A. Hamosh[1].* 1) Inst Gen Med, Johns Hopkins Hosp, Baltimore, MD; 2) Kennedy Krieger Institute, Baltimore, MD.

Campomelic dysplasia (CD), MIM #114290, is an osteochondrodysplasia caused by point mutations in *SOX9* on 17q24.3 or by chromosomal aberrations with breakpoints primarily upstream *SOX9* (Smith et al., 2009). About 10% of the cases do not exhibit campomelia, acampomelic CD (ACD). ACD is more frequent in patients who survive neonatal period and is associated with translocations and breakpoints upstream *SOX9*. A diagnostically important aspect of CD/ACD is male-to-female sex reversal that occurs in about two-thirds of affected 46,XY males. The published CD/ACD translocation breakpoints upstream of *SOX9* fall into 2 clusters: a proximal cluster 50-375 kb and a distal cluster 789 kb to 932 kb upstream of *SOX9*. In a single CD case with a complex but balanced translocation, the 17q breakpoint is exceptional and maps ~ 1.3 Mb downstream of *SOX9*. Here we described an 18 month old boy with ACD. He has a flat round face, midface hypoplasia, flat supraorbital ridges, hypertelorism, small palpebral fissures, flat nasal bridge, small nose, low-set ears, cleft palate, micrognathia, short neck with redundant posterior skin folds, hydrocephalus, and tracheobronchomalacia. He also had pyloric stenosis, a feature not described in CD/ACD. X-rays showed short thorax with kyphoscoliosis, 11 pairs of ribs, no scapulae bilaterally, short but unbowed long bones, and normal skin. His karyotype was interpreted as being apparently balanced, 46,XY,t(5;17)(q23.2;q24). SNP array analysis (Illumina 550) revealed a 100 kb region on 5q23.2 (123,742,816 to 123,843,769 bp) in the area of the cytogenetic breakpoint containing 26 genotyped SNPs with reduced intensity and apparent loss of heterozygosity, indicating a hemizygous deletion. This region lacks annotated genes (UCSC). Chromosome 17q was normal by this assay. Interestingly, *ITGB4*, 3.5 Mb downstream of *SOX9*, has been implicated in epidermolysis bullosa lethalis with pyloric atresia (MIM #226730). This observation together with the occurrence of pyloric stenosis in our patient suggests that his chromosomal rearrangement may also affect *ITGB4* expression. Further analysis of the chromosome 17q breakpoint in our patient may disclose new distant regulatory elements affecting *SOX9* and/or *ITGB4*.

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## 2643/F/Poster Board #159

**An atypical case of pseudoxanthoma elasticum with abdominal cutis laxa: evidence for a clinical disease spectrum.** *O. Vanakker[1], B. Leroy[1,2], L. Schurgers[3], I. Pasquali-Ronchetti[4], D. Guerra[4], P. Coucke[1], A. De Paepe[1].* 1) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 2) Department of Ophthalmology, Ghent University Hospital, Ghent, Belgium; 3) VitaK & Cardiovascular Research Institute, Department of Biochemistry, University of Maastricht, Maastricht,The Netherlands; 4) Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy.

Introduction. Pseudoxanthoma elasticum (PXE) is characterized by papular lesions in flexural areas of the skin, a retinopathy and cardiovascular complications, resulting from calcification and fragmentation of the elastic fibre core. Recently, a PXE-like syndrome (PXE-L) was identified, featuring generalized cutis laxa, associated with a mild retinopathy and a clotting deficiency. Contrary to the ABCC6 mutations in PXE, PXE-L is caused by mutations in the GGCX gene, encoding a $\gamma$-carboxylase responsible for activation of vitamin K (VK)-dependent calcification inhibitors such as matrix gla protein (MGP) and osteocalcin (OC). We present a patient with a clinical, histological and biochemical overlap phenotype between PXE and PXE-L. Methods. Molecular analysis of ABCC6 and GGCX was done via direct sequencing. Biochemical measurements of mineralization inhibitors and VK were done in serum using sandwich and competitive ELISA. Immunohistochemical (IHC) stains on lesional skin biopsies from the neck and abdomen were done using conformation-specific antibodies against carboxylated (c) and uncarboxylated (uc) MGP and OC. Results. The proband presented with clinical features typical for PXE - a yellowish reticular pattern in the anterior neck and severe retinopathy - though his phenotype was dominated by a marked cutis laxa of the abdomen, as in PXE-L. Ultrastructural evaluation of the skin revealed calcium deposits in the periphery of elastic fibres, typical for PXE-L. IHC stains revealed marked staining for ucMGP and ucOC, seen in PXE and PXE-L, but its distribution was not confined to the middermis - as in PXE - but rather affected the whole dermis as in PXE-L. Measurement of circulating levels of c- and ucMGP and OC revealed an elevated ucOC/cOC ratio, as in PXE-L (normal in PXE), while ucMGP/cMGP ratios were normal, as in PXE (elevated in PXE-L). Molecular analysis revealed the patient to be compound heterozygous for 2 known ABCC6 mutations, while the GGCX gene harboured a reported gain-of-function polymorphism. Circulating VK levels were however severely decreased, possibly neutralising the effect of the latter. Conclusion. The findings in this patient, reminiscent of both PXE and PXE-L, suggest that PXE-L may represent a spectrum of ectopic calcification disorders who are clinically and pathogenetically related to PXE. In addition, the low VK serum levels may suggest that a deficient VK status has a role in the pathogenesis of these disorders.

## 2644/F/Poster Board #160

**The p.L145F Mutation in the LRP5 Gene Responsible for Familial Exudative Vitreoretinopathy was Identified in a Patient with Osteoporosis-Pseudoglioma Syndrome.** *Y. Watanabe[1], S. Yano[2], S. Narumi[3], T. Hasegawa[3], M. Yoshino[1], T. Matsuishi[1].* 1) Dept Pediatrics, Kurume Univ, Kurume, Japan; 2) Dept of Genetics/Pediatrics, Univ of Southern California, CA, USA; 3) Dept of Pediatrics, Keio Univ School of Medicine, Tokyo, Japan.

**Background:** Osteoporosis-pseudoglioma syndrome (OPS; MIM 259770) is an autosomal recessive disorder characterized by severe osteoporosis, bone fragility, hyperextensible joints, and early infantile onset blindness. Mutations in the low-density lipoprotein receptor-related protein 5 gene (*LRP5*) are thought to be responsible for this rare genetic condition. Besides OPS, mutations in *LRP5* are also identified in patients with autosomal dominant and recessive forms of familial exudative vitreoretinopathy (FEVR), and autosomal dominant Osteopetrosis/Hyperostosis/Osteosclerosis. There have been more than 65 mutations reported in *LRP 5* and at least 57 missense/nonsense mutations have been identified. Genotype/phenotype correlation has been established at some extent in the LRP5 gene mutations (Minrong et al. 2005). **Case Report:** A 23-year-old Japanese male was diagnosed with OPS at age 10 years with the typical clinical findings. Mutation analysis of *LRP5* showed compound heterozygous mutations with p.T552M/p.L145F. The mother was asymptomatic at age 32 years when her son was diagnosed. She was also tested for *LRP5* mutations and her bone mineral density was evaluated. The mother was found to be heterozygous for p.L145F and her bone mineral density was found to be significantly reduced. Ophthalmologic examination on the mother was unremarkable. The father of the patient was unavailable for the studies. **Discussion:** The p.T552M mutation has not been reported in any clinical phenotypes associated with *LRP5* mutations. Qin et al. (2005) reported a familial case of autosomal dominant type FEVR due to p.L145F: three children with typical FEVR and the mother who exhibited mild retinal findings and reduced bone mineral density. In our case, the mother of the proband carried p.L145F although she did not develop FEVR. It is unclear why this particular mutation showed detrimental effects in the eye development in one family and did not in another. Our familial case may indicate weak genotype/phenotype correlation in *LRP5* mutations and may suggest involvement of other epigenetic factors particularly in the eye development. **References:** Minrong et al.: Clinical and molecular findings in Osteoporosis-Pseudoglioma syndrome. Am J Hum Genet: 77:741-753, 2005 Qin et al.: Complexity of the genotype-phenotype correlation in familial exudative vitreoretinopathy with mutations in the LRP5 and /or FZD4 genes. Human Mutation: 26;104-112, 2005.

## 2645/F/Poster Board #161

**Evidence suggesting digenic inheritance of Waardenburg syndrome type II with ocular albinism.** *T.L. McGregor[1], E. Spector[2], P.W. Chiang[2].* 1) Pediatrics, Vanderbilt School of Medicine, Nashville, TN; 2) Pediatrics, UC Denver School of Medicine, Aurora, CO.

Waardenburg syndrome (WS) is a series of auditory-pigmentation disorders inherited in an autosomal dominant manner. In most patients, WS2 results from mutations in the *MITF* gene (WS2 type A). *MITF* encodes a basic helix-loop-helix transcription factor that activates transcription of tyrosinase and other melanocyte proteins. The clinical presentation of WS is highly variable, and we believe that Tietz syndrome and WS2 with OA are likely two variations of WS2 due to the presence of modifiers. One family with molecular diagnosis of WS2 co-segregating with ocular albinism (OA) has previously been reported. A digenic mutation mechanism including a *MITF* mutation and the *OCA1/TYR*$^{R402Q}$ hypomorphic allele was proposed to be the cause of OA in this family. Here, we present a second WS2 family with OA and provide evidence suggesting the *OCA1/TYR*$^{R402Q}$ allele could not cause OA in this family. A child who presented with OA and a familial history of WS2 is presented. No other family members with WS2 exhibited the OA phenotype. The patient and selected members of his family underwent molecular analysis of multiple genes involved in the pigmentation pathway. We hypothesize the presence of a novel *OCA3/TYRP1* mutation together with the *MITF* del R217 mutation account for the OA phenotype in this family. Since *MITF* is a transcription factor for pigmentation genes, a mutation in *MITF* plus a heterozygous mutation in *OCA3/TYRP1* together provide an adverse effect crossing a quantitative threshold; therefore, WS2 with OA occurs. We have previously hypothesized that the clinical spectrum and mutation mechanism of OCA depend on the pigmentation threshold of an affected individual. This unique family has provided further evidence supporting this hypothesis.

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## 2646/F/Poster Board #162

**The 12q14 microdeletion syndrome, 4 further cases.** *R. Regan[1], S.A. Lynch[2], C. Costigan[3], N. Foulds[4], A. Collins[4], A.C. Thuresson[5], F.H. Sharkey[6], D.R. Fitzpatrick[6].* 1) School of Medicine and Medical Science, University College Dublin, Dublin 4, Ireland; 2) National Centre for Medical Genetics, Our Lady's Children's Hospital , Crumlin, Dublin 12, Ireland; 3) Department of Endocrinology & Diabetes, Our Lady's Children's Hospital, Crumlin, Dublin, Ireland; 4) Wessex Clinical Genetics Service, Southampton University Hospitals Trust, Princess Anne Hospital, Southampton, UK; 5) Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden; 6) Medical Genetics Section, MRC Human Genetics Unit, Western General Hospital, Edinburgh, UK.

The 12q14 microdeletion syndrome is characterised by low birth weight, failure to thrive, short stature and developmental delay. Osteopoikilosis has been described in some cases. We report 4 children with chromosomal deletions, detected by microarray analysis, that extend between 12q13 to 12q15. Cases 1 and 2 have severe growth retardation with a diagnosis of query Silver-Russell syndrome, one with deletion of 8.4Mb spanning 12q13.3 to 12q14.3. This child weighed 2 kg at birth. The second child has a 11.2 Mb deletion of 12(q13.2;q15) and also has short stature and weighed 1.9 kg at 37 weeks. She is currently requiring PEG feeding. A third case has normal growth but his abnormality, a 6.2Mb deletion of 12(q13.3;q14.2), ends proximally at 12q14.2 suggesting that the major growth gene lies between 12q14.2 to 12q14.3. Two of the children (case 1 & 3) had similar facial dysmorphism with long eyelashes and a cupids bow. There was no evidence of osteopoikoilosis in any of our cases. A previous report by Menten and colleagues (2007) described three patients with a deletion of 12q14 who presented with growth retardation & osteopoikilosis. There was a 3.44 Mb common deleted region which contains 13 RefSeq genes, including LEMD3, the causal gene for osteopoikilosis. Mari et al. (2009) recently described a case with primordial dwarfism and a smaller deletion of the 12q14.4 region. The child, a boy, had severe pre and postnatal growth failure and mild developmental delay. There was a history of poor feeding and failure to thrive, similar to the phenotype of primordial dwarfism or severe Silver-Russell syndrome (SRS). Buysse et al (2009) described two children, one of whom had an intragenic deletion of HMGA2 and short stature providing evidence that HMGA2 is the likely growth gene. HMGA2 lies at 12q14.3 which would explain the normal growth in our Case 3 whose deletion ended proximal to this region. Loss of function of Hmga2 in the mouse results in the pygmy phenotype that combines pre and postnatal growth failure, with resistance to the adipogenic effect of overfeeding. We conclude that the 12q14 microdeletion syndrome is a recurrent deletion syndrome and our cases, particularly Case 3 provide further evidence that HMGA2 is the causative gene for growth retardation.

## 2647/F/Poster Board #163

**A boy with atypical phenotype of NF1 with a type 1 microdeletion.** *M. Poyhonen[1,2], H. Kehrer-Sawatzki[3], T. Lonnqvist[4], P. Alhopuro[2], P. Hoglund[5], L. Valanne[6], L. Messiaen[7], S. Kivirikko[2].* 1) Dept Med Gen, Univ Helsinki, Helsinki, Finland; 2) Dept Clin Gen, Univ Hospital, Helsinki, Finland; 3) Inst Hum Gen, Univ Ulm, Ulm, Germany; 4) Hosp Children and Adolescent, Helsinki, Finland; 5) Rinnekoti Foundation, Espoo, Finland; 6) Dept Radiology, Univ Hospital, Helsinki, Finland; 7) Dept Gen, Univ Alabama Birmingham, AL, US.

Patient was born as a first child of healthy, non-consanguineous Caucasian parents at week 38+5. During pregnancy hydronephrosis was seen at 19 weeks of gestation. Birth weight was 3890 g, length 47 cm and head circumference 35 cm. As a newborn dysmorphic facial features, anterior anus and an anocutaneal fistula was seen. The chromosomes (400 bands) were normal. He walked independently at the age of 2 years 4 months, but did not speak. His height was 87.5 cm (slightly below his target height), body mass index (BMI) 16.2 and head circumference 53 cm. His facial features were distinct with high anterior hairline, thick eyebrows, epicanthal folds and hypertelorism. The nasal tip was broad and he had anteverted nostrils. The filtrum was long and the upper lip was thin. The ears were thickened and the ear lobules were uplifted. He had one classical cafe au lait spot. In addition he has had severe feeding problems because of an intestinal malrotation. His MRI revealed high signal intensity lesions of posterior fossa, thalamus and cortical area. The spinal cord was thickened with increased signal at Th8 - Th10. A de novo interstitial deletion of 1.3 Mb in 17q11.2 was detected by commercially available high-resolution oligonucleotide CGH array. Using MLPA P122-C1 NF1 area (MRC-Holland), we found a ~ 1.4Mb type 1 microdeletion, including the *SUZ12P* gene (~363 Kb centromeric of NF1 exon 1) and the LRRC37B probe (661Kb telomeric of the last *NF1* exon). Nearest flanking non-deleted genes probed on the assay are CPD and ZNF207. Some dysmorphic features, intestinal malrotation and some of the brain abnormalities have not been reported previously to be associated with NF1 microdeletion patients. Further work is needed to fine-map the deletion breakpoints.

## 2648/F/Poster Board #164

**Molecular and Clinical Characterization of Bardet-Biedl syndrome in the Saudi Population.** *L. Abu Safieh[1], M. Aldahmesh A[1], H. Shamseddin[1], M. Hashem[1], R. Shaheen[1], H. Alkuraya[2], S. Hazzaa[3], A. Al-Rajhi[2], FS. Alkuraya[1, 4,5].* 1) GENETICS MBC #03, KING FAISAL SPECIALIST HOSPITAL & RESEARCH CENTER, RIYADH, RIYADH, Saudi Arabia; 2) Vitreoretinal Division, King Khaled Eye Specialist Hospital, RIYADH, Saudi Arabia; 3) Department of Surgery, King Faisal Specialist Hospital and Research Center, RIYADH, Saudi Arabia; 4) Department of Pediatrics, King Khalid University Hospital and College of Medicine, King Saud University, RIYADH, Saudi Arabia; 5) Department of Anatomy and Cell Biology, College of Medicine, Alfaisal University, RIYADH, Saudi Arabia.

Bardet-Biedl syndrome (BBS) is a rare autosomal recessive disorder characterized by chronic renal failure,polydactyly,mental retardation,retinal dystrophy,obesity and genitourinary abnormalities.To date 14 genes have been reported to be associated with the disease.Our aim of this study is to genetically and phenotypically characterize BBS in Saudi Arabia not only to make the results available for counseling but also to draw patterns of genotype/ phenotype correlation as well as to discover novel genes which could potentially improve our understanding of the development and function of the organs involved and the retina in particular.We have so far recruited a total of 37 patients representing 16 unrelated families.We applied homozygosity mapping and candidate gene screening approach to identify the disease causing gene(s).Mutations have been identified in 7 families, linkage is already established for 3 families and work is still ongoing for the remaining 6 families. All identified mutations are novel and involve one of three previously reported BBS genes, BBS1, BBS3 and BBS4.While work is still ongoing, several themes seem to emerge from our study:1)Intronic mutations, some were quite deep within the introns, which we have demonstrated their detrimental effect on RNA splicing, are more prevalent than missense mutations in our cohort.In one instance, homozygosity allowed us to reanalyze a gene that was reported by a DNA diagnostic lab to be negative for BBS1 mutations.2)While it has been shown before that BBS3-related BBS tends to be on the milder end of the spectrum, we were able to enroll a family that represents the extreme of this observation where RP was the only abnormality observed in all three affected individuals despite extensive phentoyping.3-)Data on the facial profile of patients with BBS is scarce.However, our analysis suggests that careful dysmorphic exam can suggest the gene involved and we are in the process of calculating the predictive value of the facial profile in prioritizing BBS genes for mutation analysis.In conclusion homozygosity mapping is an extremely powerful tool in the setting of molecular diagnosis of the genetically heterogeneous BBS.When possible, cDNA should be the starting material for mutation analysis among Saudi BBS patients.We confirm earlier reports of the milder phenotype associated with BBS3 mutations.We also suggest that some genotype/phenotype correlation also exists at the level of BBS-related dysmorphia.

## 2649/F/Poster Board #165

**The association of genetic factors in retinopathy of prematurity.** *M. Hiraoka[1], H. Takahashi[1], H. Orimo[2], M. Hiraoka[3], T. Ogata[4], N. Azuma[3].* 1) Ophthalmology, Nippon Medical School, Tokyo, Japan; 2) Biochemistry, Nippon Medical School, Tokyo, Japan; 3) Ophthalmology, National Center for Child Health and Development, Tokyo, Japan; 4) Endocrinology and Metabolism, National Research Institute for Child Health and Development, Tokyo, Japan.

Purpose: Retinopathy of prematurity (ROP) is a major cause of childhood blindness in developed countries. It is known to occur along environmental factors such as preterm gestational age and early oxygen exposure, but even same condition with controlled environment can lead to diversity in ROP phenotype. These facts lead that other internal factors, especially genetic variants may involve in the development of ROP. Familial exudative vitreoretinopathy (FEVR) is hereditary vitreoretinal disorder that has similar clinical manifestations to ROP. In X-linked recessive FEVR, mutations were found in Norrie disease (ND) gene. Mutations of frizzled 4 (FZD4) gene were seen in autosomal dominant FEVR. Low density-lipoprotein receptor-related protein 5 (LRP5) gene was reported to have variants in both autosomal dominant and recessive pattern of FEVR. To evaluate the genetic involvement in ROP, we have screened advanced ROP for those three candidate genes of FEVR. Methods used: Seventeen advanced ROP subjects were enrolled in this study. All cases have received surgical treatment in their eyes. Genomic DNA from each patient underwent PCR and direct sequencing of ND, FZD4, and LRP5 genes respectively. As for ND and FZD4 genes, coding region and 5' untranslated regions (UTR) were examined. Entire coding regions of twenty-three exons were examined for LRP5 gene. Summary of results: One case showed heterozygous mutation in 5' untranslated regions (UTR) of ND gene. Two cases had heterozygous mutations in coding region of LRP5 gene. No case showed mutaion in FZD4 gene. These findings suggest that genetic changes contribute to the development of ROP.

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## 2650/F/Poster Board #166

**46,XY,del(1)(q23q25),t(4;11)(q31.3;q21) possibly associated with significant short stature.** *A. Nishimura[1], Y. Hiraki[2], N. Matsumoto[1].* 1) Dept Human Gen, Yokohama City Univ Med, Yokohama, Japan; 2) Hiroshima Municipal Center for Child Health and Development, Hiroshima, Japan.

We report a case of 46,XY,del(1)(q23q25),t(4;11)(q31.3;q21) associated with significant short stature. The case at 3 4/12 years of age presented with growth retardation [height 65 cm (-8.5 SD), weight 5,890 g (-4.5SD)], developmental delay, prominent forehead, strabismus, high arched palate, cryptorchidism, bilateral simian lines, and bilateral inguinal hernia. Growth hormone defect and antithrombin III deficiency were also noted. We analyzed the genomic structure by high-resolution SNP array 6.0 (Affymetrix). We could detect del(1)(q24.3q31.2). No other obvious chromosomal abnormalities were found. Long PCR successfully amplified the fragment spanning the 1q24.3-q31.2 deletion breakpoints, which later determined at the nucleotide level. The deletion size was 19,497 Kb. Furthermore, we analyzed the t(4;11)(q31.3;q21) by FISH. All the chromosomal abnormalities occurred as *de novo*. The antithrombin III deficiency is supposed to be caused by the *ATIII* (*SERPINC1*) deletion (1q25.1). The possible association between these chromosomal abnormalities and clinical features including short stature will be discussed.

## 2651/F/Poster Board #167

**COL1A1 gene mutation analysis and phenotype picture of Czech osteogenesis imperfecta patients.** *I. Mazura[1,2], I. Marik[3], D. Zemkova[3,4], M. Kuklik[3], O. Hudakova[3], F. Nutsu-Mazurova[3], M. Sedova[1,2], S. Mazurova[5], J. Zvarova[1,2].* 1) Dept. of Biomed.Informatics, Institute of Computer Science, Prague, Czech Republic; 2) Institute of Computer Science, Academy of Science CR, Prague, Czech Republic; 3) Ambulant Centre for Defects of Locomotor Apparatus, Prague, Czech Republic; 4) Department of Pediatrics, University Hospital Motol, Prague, Czech Republic; 5) 1st Medical Faculty, Charles University Prague, Prague, Czech Republic.

**Introduction.** Osteogenesis imperfecta (OI) is inherited disorder, the most common inborn disease of fibrous connective tissue characterized by increased fragility of skeleton. First four types (I.-IV.) are characterized by COL1A1 and COL1A2 gene mutations. Nucleotide structure of two COL1A1 gene regions (helical domain) plays important role in interaction between collagen monomer and fibronectin, cartilage oligomeric matrix protein, different integrins and metalloproteinases. **Aim of study.** In this study the COL1A1 gene mutations were compared with clinical, anthropological, biochemical and radiological results. **Patients and methods.** Group of 55 patients (27 M/ 28 F) aged from 1.5 to 56 years were classified into particular types of the OI according to both Sillence (1979) and the new Glorieaux (2003) classification. All the collected data of patients were gathered into the complex database and statistically evaluated trying to find some relations among genetic, anthropometric, clinical and radiological parameters. **Results.** The most important factor which affected the severity of disease was the type of OI, determined by the severity of collagen affection. We analysed two COL1A1 regions rich in lethal mutations (regions 691-823 and 910-964) and we summarized list of mutations of Czech OI patient group according to Marini's study (2007). The most important phenotype parameters distinguishing the types are body height and length of lower extremities. Platyspondyly, deformity of ribs and dentinogenesis imperfecta are parameters explaining the variability in body height among patients within the same type of OI. **Conclusion.** Dividing of OI types according to the new clinical classification and detail molecular genetic analysis of binding sites of COL1A1 gene give more consistent pictures of the OI types. The type of OI determines the severity and progress of the disease. One of the most important parameter is body height. This work was partially supported by the grant no. 1M06014 of Ministry of Education, Youth and Sport, Czech Republic.

## 2652/F/Poster Board #168

**Potocki-Lupski SyndromeA Microduplication Syndrome Associated with Oral Pharyngeal Dysphasia and Failure to Thrive.** *C.R. Soler-Alfonso[1], K.J. Motil[2], P. Robbins-Furman[1], J.E. Friedman[2], A. Eifert[2], J.R. Lupski[1], K. Fraley[2], L. Potocki[1].* 1) Molecular and Human Genetics , Baylor College of Medicine, Houston, TX; 2) Children's Nutritional Research Center, Department of Pediatrics, Texas Children's Hospital. Houston, TX.

Objective: Failure to thrive is a recognized feature in children with Potocki-Lupski Syndrome (PTLS) [duplication 17p11.2]. This study describes the growth characteristics of 19 subjects with PTLS from birth through adolescence in conjunction with relevant physical features and objective data from swallow function studies. We hypothesized that failure to thrive observed in PTLS is in part due to oral and motor dysphasia and hypotonia. Study design: We studied 19 individuals with PTLS who were evaluated through an Institutional Review Board (IRB)-approved clinical protocol at Texas Children's Hospital. Height and weight measurements were obtained by standardized methods and from review of medical records. These measurements were converted to weight-for-age, length-for-age and weight-for-length Z-scores. A swallow function study performed with a speech pathologist present was performed in order to assess phases of swallowing. Otolaryngologic examination delineated anatomical contributing to swallowing difficulty. Results: While only 10% of PTLS newborns fell into the small for gestational age range, as a group, children with PTLS have birth weights and lengths that are, on average, one standard deviation (SD) below the mean. Although there is a trend towards more normal growth in early childhood, these children follow their own growth curve over time and remain one SD below the mean for weight-for-age and weight-for-length. Poor feeding during neonatal period and infancy was observed in 89% (17/19), infantile hypotonia was reported in 84% (16/19), 68% (13/19) had a history of failure to thrive, and 26% (5/19) required tube feedings. Swallow function studies indicate that all subjects have at least one abnormality observable, and the most common finding is gastroesophageal reflux (GERD) seen in 53% (10/19). Conclusions: Our results indicated that as a group, newborns with PTLS weigh less than the general population and do not catch up to the normal curve in childhood. Factors such as hypotonia and oral pharyngeal dysphasia likely contribute to the lack of catch-up growth; however, our data, in conjunction with data from animal models of PTLS strongly suggest that intrinsic factors have a major impact on growth in this chromosomal microduplication syndrome. This study supports that a diagnosis of PTLS should be considered in the differential diagnosis of failure to thrive, hypotonia, and/or oral pharyngeal dysphasia.

## 2653/F/Poster Board #169

**Losartan for Ehlers Danlos IV.** *P.J. Benke.* Pediatric Genetics, Joe Dimaggio Children's Hosp, Hollywood, FL.

A 4 year old boy was brought to Florida from the Dominican Republic for diagnosis and treatment. His small size, easy bruisability, hyperextensible joints, thin skin, thin hair, poor subcutaneous tissue with highly visible veins, and thin knee scars suggested Ehlers Danlos Syndrome IV (ED IV). This was confirmed by demonstration of c.3554 G>A mutation in Exon 49 of the Col3A1 gene. The parents were informed that there was no known therapy and that anticipated surgical treatment of abdominal crises and anticipated arterial aneurysms in the second and third decades of life was extremely difficult. Because recent studies on a different collagen disorder, Marfan Syndrome, suggested that Losartan, which blocks Angiotensin II receptors and cytokine signals, was effective in treating aortic root dilation, Losartan was suggested as an off-label use of an approved drug. A dose of 25 mg/day was started and was well tolerated. The dose was increased to 50 mg/day in 1 month, and 75 mg/day in 6 months. After 3 months, his skin became thicker (from 0.5 to 0.9 cm), his hair thicker, he demonstrated less bruising, and his abdominal veins were not as visible. Continued improvement over a year was noted, and no side effects or hypotension were observed. The appearance of the veins over his wrists and feet did not change, and his growth rate did not improve. Losartan may not be the answer to all the vascular defects found in EDIV and may not help other patients. However, abdominal vein appearance and skin thickness and skin bruisability in this patient appeared to improve with Losartan therapy.

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## 2654/F/Poster Board #170

**Terminal osseous dysplasia with pigmentary defects: follow-up of the initial reported family, refinement of the critical region, and exclusion of some candidate genes.** *C. Bacino[1], P. Piccolo[1], D. Del Gaudio[1], I. Van den Veyver[1,2], N. Brunetti-Pierri[1].* 1) Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Ob-Gyn, Baylor College of Medicine, Houston, TX.

Terminal osseous dysplasia with pigmentary defects (TODPD) is an X-linked syndrome with distal limb anomalies and pigmentary skin defects. We have previously described this syndrome in several females from a large, four-generation pedigree. This condition appears to be male lethal. The clinical presentation consists of multiple limb anomalies including brachydactyly, syndactyly, camptodactyly, digital elongation, and distal digital fibromatosis. Other clinical features include hypertelorism, multiple frenula, and characteristic punched-out pigmentary abnormalities over the face, temporal regions, and scalp. The phenotype is highly variable thus suggesting that X-inactivation plays an important role in the expression of the disease. We have recently performed a detailed skeletal evaluation in 4 of the affected individuals. The skeletal surveys showed significant findings including: osteopenia, methapyseal involvement, striation of the distal long bones, vertebral flattening, carpal coalition reminiscent of osteolysis, and brachydactyly among some of the features. These findings suggest that the bone involvement is generalized and not restricted to the distal limbs as previously indicated. Previous molecular studies by our group showed linkage to Xq27.3-Xq28. Using a 6,056 SNP array we have further refined the critical region within the Xq28 region. We have tested a few candidate genes in the region including *BGN* (encoding biglycan), *FLNA* (encoding filamin A), and *FAM58A* which has been recently found to be involved in STAR syndrome that shares some phenotypic similarities with TODPD. However, no pathogenic mutations in any of these genes were found. The identification of the gene responsible for this rare condition will shed light on the molecular pathways leading to the various congenital anomalies of TODPD, in particular the rather unique skeletal phenotype. The identification of the responsible gene will also allow a more accurate genetic counseling to the affected families.

## 2655/F/Poster Board #171

**Incidence of Caffey disease in a tertiary care center: Increasing frequency or increasing awareness?** *A. Guerin[1], E. Goh[1], L. Dupuis[1], E. Cory[2], M. Shouldice[2], J. Maguire[3], A.W. Howard[4], R. Mendoza-Londono[1].* 1) Division of Clinical and Metabolic Genetics and; 2) SCAN Program and; 3) Division of Pediatric Medicine and; 4) Division of Orthopaedics; The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada.

Caffey disease (CD-MIM 114000) is a rare genetic condition characterized by infantile episodes of massive subperiosteal new bone formation involving the diaphyses of long bones, mandible and clavicle, sparing the epiphyses and metaphyses. CD is transmitted in an autosomal dominant manner with incomplete penetrance; the age of onset is typically before 5 months of age and resolves spontaneously around 2 years. Gensure et al (2005) identified a common mutation in the *COL1A1* gene (R836C) in affected individuals. Professional awareness remains an important tool to distinguish CD from other possible etiologies of hyperostosis such as vitamin A intoxication, congenital syphilis, osteomyelitis, and non-accidental injury. We present the clinical and radiological features in four cases evaluated at the Hospital for Sick Children for possible CD between November 2005 and March 2009. Only one case had an identified positive family history of the disease. None of the families were related, and all of the patients were from different ethnic backgrounds. Male to female ratio was 1:1. The first presenting symptom in all cases was swelling and pain of the tibia with radiological findings of periosteal reaction and hyperostosis in the absence of trauma. The age of onset ranged from birth to 10 weeks. In all four cases the children presented to peripheral centres, and were then referred to orthopedics or the suspected child abuse and neglect (SCAN) clinics. All had radiological findings consistent with CD. Molecular analysis identified the common R836C mutation in *COL1A1* in 2 cases. Results for the remaining two are pending. CD is a rare genetic condition with approximately 100 reported cases, many identified before molecular testing was available. Penetrance for the phenotype is incomplete and only 79% of mutation carriers will manifest symptoms. It remains unclear as to why in our referral base four such cases would present in such a short period of time. Our findings suggest that CD may be more common that previously described. Factors that may explain this include better access to healthcare and increased parental and physician awareness of potential genetic diagnoses in the differential diagnosis for child abuse and neglect. There is also the possibility of a yet unidentified environmental trigger. Systematic evaluation and follow-up of these patients may allow for better understanding of CD and its presentation and improved counseling for families.

## 2656/F/Poster Board #172

**Achondrogenesis Type IA (Houston-Harris) Case Report.** *P. Paez[1], J. Acosta[2], I. Zarante[2], M. Olaya[3], C. Carvajal[2].* 1) Dept Cundinamarca, Universidad el Bosque, Bogota, Colombia; 2) Instituto de Genética Humana, Pontificia Universidad Javeriana, Bogotá Colombia; 3) Departamento de Patologia, Pontificia Universidad Javeriana, Bogotá Colombia.

Introduction: It is considerated as the most severe forms of chondrodysplasia in humans. Apperars to be autosomal recessive, but the mutant gene is unknown. It is characterized radiographically by deficient ossification in lumbar vertebrae and absent ossification in the sacral, pubic and ischial bones and clinically by stillbirth or early death. Also they present severe micromelia, a disproportionately large cranium due to marked edema of soft tissues. Case Report: We describe the case of 22 years old Colombian (South America) woman, who said that during her third pregnancy initiated controls at twenty third week with the first prenatal ultrasound examination revealed severe shortened limbs anomaly because made prenatal detail ultrasound showed enlargement of the lateral ventricles, narrow thorax, severe pulmonary hypoplasia, a poorly ossification of vertebral bodies, severe micromelia, extremely shortened limbs and multiple fractures of long bones and ribs, club foot varo and clou hands. All of these sonograms findings gave us a presumptive prenatal diagnosis. At 26th week is induced labor and she gave stillbirth to 900 gram and 39 centimeter female with evidence of the disproportionately large head and micromelia. Then, the pathology report was compatible with Achondrogenesis type IA (OMIM: 200600) and the radiology report was severe poorly ossification of vertebral bodies and sacrum, hypoplasia and fractures ribs, and shortened limbs. It may be differentiated from other skeletal dysplasias.

## 2657/F/Poster Board #173

**Dramatic vascular phenotype in an Indian cohort of FBLN4 mutant patients.** *A. De Paepe[1], F. Malfait[1], M. Renard[1], M. Kappanayil[2], K. Kumar[2], V. Sunitha[2], S. Nampoothiri[2], B. Loeys[1].* 1) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 2) Departments of Pediatric Genetics and Pediatric Cardiology, Amrita Institute of Medical Sciences and Research Center, Kochi, India.

Autosomal recessive cutis laxa, type 1 (AR-CL I) is a genetically heterogeneous group of disorders with variable systemic involvement. Both mutations in FBLN-4 and FBLN-5 (fibulin-4 and 5) have been identified. FBLN4 mutations were found in AR-CL I patients with aortic/arterial aneurysm, tortuosity and stenosis. We report 8 unrelated patients (4M/4F) from the same ethno-religious background of North-Kerala, India. Consanguinity is documented in 4/8 families. All patients presented with cardio-respiratory problems during infancy. Echocardiography and CT-scan revealed marked dilatation and tortuosity of the complete aorta, neck vessels, and dilatation or stenosis of pulmonary arteries. Other shared phenotypic features included generalized hyperextensible skin, umbilical or inguinal hernia, generalized hypotonia with delayed gross motor development and mild facial dysmorphism, including micrognathia, hypertelorism, long face and high palate. Seven out of eight patients died during infancy (15 days-17 months), mostly due to cardiorespiratory failure. Direct sequencing of FBLN4 revealed a homozygous single base substitution (c.608A>C) in 7 out of 8 probands. This mutation results into an p.Asp203Ala substitution which affects the highly conserved DINE consensus sequence of the fourth cbEGF domain. In the only surviving 8th proband (3 years old), we found compound heterozygosity for this mutation with a c.679C>T, causing a p.Arg227Cys in the same cbEGF domain. The individuals reported here share several characteristics with two previously reported patients with an FBLN4 mutation, including (mild) cutis laxa, tortuous systemic vessels, aneurysm of the aorta and pulmonary arteries and cardiorespiratory problems. Most patients suffer from severe disease resulting in perinatal or infantile death. The mutations reported in this cohort of 8 patients have not been reported before but affect similarly highly conserved residues in the cbEGF domains of fibulin-4. The identification of a single mutation in several individuals from the same ethno-religious background strongly suggests a founder effect. Whereas the FBLN4-associated phenotype is predominated by the vascular abnormalities, mutations in FBLN5 are associated with severe cutis laxa and emphysema, compromising life expectancy. Study of a fbln4 mouse model and human aortic tissues have revealed a dysregulation of the TGFbeta signaling pathway, confirming an key role for this pathway in aneurysm formation.

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## 2658/F/Poster Board #174

**Psychiatric disorders in neurofibromatosis-1: Mayo Clinic experience.**
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Background and Aim: Neurofibromatosis type 1 (NF-1) is the most common autosomal dominant disorder in the general population. The association between NF-1 and psychiatric disorders is not well understood. The aim of this case-control study is to investigate the occurrence and spectrum of psychiatric disorders in subjects with NF-1 and evaluate the association between patient characteristics and psychiatric comorbidities. Methods: Medical records were screened to identify the subjects having diagnostic codes for both NF-1 and psychiatric disorders between 1994 and 2007. An age and sex matched control group with a diagnosis of NF-1 and no psychiatric disorders was formed. Medical records of cases and controls were reviewed manually for case ascertainment and data on demographics and clinical characteristics were collected. A Wilcoxon signed rank test was used to compare case-control pairs for continuous variables and McNemar's test was used to compare pairs for categorical variables. Results: Case group comprised 112 NF-1 subjects (50 [44.6%] female) with psychiatric disorders. The median age of the cases at the time of initial evaluation at the institution was higher than the controls (18 vs 16, respectively, $p<0.001$). The most common psychiatric diagnoses in the case group were attention deficit hyperactivity disorder (44, 39.3%), depression (41, 36.6%) and anxiety (23, 20.5%). In the paired analysis, absence of subdermal neurofibromas was significantly associated with psychiatric disorders in the NF-1 population (14.3% of cases had subdermal neurofibromas compared to 32.1% of the controls, $p=0.0007$). Disruptive behavior disorders were significantly associated with presence of café au lait spots ($p=0.001$) and Lisch nodules ($p=0.01$). Mood disorders were significantly associated with presence of cutaneous ($p=0.008$) and spinal neurofibromas ($p=0.04$). Anxiety disorders were significantly associated with presence of cutaneous neurofibromas ($p=0.008$). Conclusions: The most common psychiatric disorders observed in NF-1 population were attention deficit hyperactivity disorder, depression and anxiety. Defining a possible association between clinical characteristics and the development of psychiatric disorders in NF-1 patients may help in identifying subjects at risk.

## 2659/F/Poster Board #175

**Clinical Features of Taiwanese Patients with Osteogenesis Imperfecta.**
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Background and Purpose: Osteogenesis imperfecta (OI) (MIM# 166200, 166210, 259420, and 166220) is a congenital disorder characterized by increased bone fragility and low bone mass. Despite its prevalence, information regarding the clinical features of this genetic disorder is lacking in Taiwan. Thus, this study aimed at characterizing the clinical features of OI patients in Taiwan so that a practical correlation could be established for distinguishing different clinical subtypes of OI. Methods: Review of medical records identified 48 patients with OI (33 females and 15 males; age range, 2 months to 53 years) during the study period from January 1996 through June 2008. Diagnosis and classification, using the classification system of Sillence et al, were based on clinical and radiological characteristics. We also analyzed the data of these patients among different subtypes, including clinical presentations, physical examinations and bone mineral density findings. Results: There were 33 females and 15 males with age ranging from 2 months to 53 years. Among these patients, 19 were classified as type I, 10 as type III, and 19 as type IV. Height SDS were $-0.76\pm0.91$ in type I, $-9.54\pm4.36$ in type III, and $-3.02\pm2.70$ in type IV. BMD SDS were $-3.16\pm2.00$ in type I, $-5.35\pm2.13$ in type III, and $-4.19\pm1.77$ in type IV. The clinical feature of blue sclera was present in 89% patients of type I, 80% of type III, and 58% of type IV. Eighty percent of type III patients had DI, in contrast to 5% in type I and 42% in type IV. Annual fracture rate was $0.8\pm0.7$ in type I, $4.4\pm0.6$ in type III, and $2.0\pm0.7$ in type IV. In type I patients, 84% had a family history of OI, compared to 0% in type III, and 37% in type IV. Among three subtypes, there existed statistically significant differences ($p<0.05$) in the following clinical features: height SDS, weight SDS, BMD SDS, DI, bone deformity, scoliosis, walking ability, annual fracture rate, and family history. However, no significant differences were found in blue sclera ($p=0.075$) and hearing loss ($p=0.832$). Conclusion: Nine of the 11 clinical features examined, including height SDS, weight SDS, BMD SDS, dentinogenesis imperfecta, bone deformity, scoliosis, walking ability, fracture rate, and family history, were significantly different among three types of OI patients, which may be of help to evaluate patients and to establish their prognosis.

## 2660/F/Poster Board #176

**MULTIDISCIPLINARY STUDY OF PEDIATRIC PATIENTS WITH DUCHENNE MUSCULAR DYSTROPHY. 28 CASE REPORTS AT THE HOSPITAL PARA EL NINO POBLANO.**
*R.G. Ruiz[1], J.R.M. Aparicio[2,8], A. Ortiz[3], M.A. Cubillo[4], F.E. Romero[5], C.M. Gutierrez[6], H.M.L. Hurtado[7], E. Huitzil[8], P.F. Sierra[9], S.M. Chatelain[10].* 1) Pediatric Rheumatology; 2) Medical Genetics; 3) Ortopedics; 4) Rehabilitacion; 5) Nutrition; 6) Pediatrics; 7) Cytogenetics, Hosp para el nino Poblano, Puebla, Mexico; 8) Estomatology, Benemerita Universidad Autonoma de Puebla, Mexico; 9) Medical Genetics, Hospital de la Mujer SSA, Puebla, Mexico; 10) Biotechnology, Universidad Autonoma Metropolitana, Mexico DF.

INTRODUCTION. Duchenne muscular dystrophy (DMD) is an inherited disorder characterized by rapidly progressive muscle weakness beginning in the lower part of the body including pelvis later affecting all the body DMD is the most common form of muscular dystrophy and hypertrophy. It usually affects male patients, but in rare cases it can also be observed in female patients. It is considered an X-linked recessive inherited disease. A milder form of this disease is known as Becker Muscular dystrophy (BMD). In BMD, most of the symptoms are similar to Duchenne, but the onset is diferent. MATERIAL AND METHODS. A group of 28 pediatric patients were diagnosed as Duchenne muscular dystrophy by enzimatic tests (CPK, DHL and Aldolasa), muscular biopsy and the dystrophine proteine was also investiated. Tne patient was also studied by its clinical syntoms includings Gowers sign. All of them were treated with deflazacort and L-carnitine, their different clinical evolution among them was then analyzed. Weigth, size, arterial measure and glucose every three months as control was also performed. CONCLUSIONS. DMD is named after the French neurologist Guillaume Benjamin Amand Duchenne (1806-1875), who first described the disease in the 1860s. One third of the cases are known to be caused by development of spontaneous mutations in the dystrophin gene. Boys with DMD develop weak muscles because the muscle fibers become distrophic and hipertrphic, loosing their natural function, due to mutations in the dystrophin gene, which is located at the sexual chromosome X, which encodes a cell membrane protein in myocytes. L-carnitine and deflazacort dose concentration was used acording to clinical evolution, if muscular strenght decreased, the dose of deflazacort was used at 0.3mg/kg. This study shows that L-carnitine and deflazacort have an important impact on health, improoving their muscle function and providing a better quality of life. Few side effects were observed during the treatment among the patients REFERENCES. | 1. Cardamone M, Darras BT, Ryan MM Inherited myopathies and muscular dystrophies. Semin Neurol. 2008 Apr;28(2): 250-9. 2. Dellavalle, A. et al. Pericytes of human skeletal muscle are myogenic precursors distinct from satellite cells. Nature Cell Biology 9 255-267 (2007).

## 2661/F/Poster Board #177

**High incidence of progressive postnatal cerebellar enlargement in Costello syndrome.**
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Costello syndrome (CS) is a Ras-MAPK pathway disorder resulting from heterozygous germline *HRAS* mutations. Physical manifestations affect all organ systems and cognitive impairment is typical. Structural CNS anomalies have not been systematically assessed. We hypothesized that an identifiable phenotype of structural CNS abnormalities contributes significantly to morbidity. **Methods:** CNS imaging studies on 24 pts with *HRAS* mutations (20 p.G12S; 2 p.G12A; 1 each p.G13C, p.T58I) were reviewed and correlated to clinical data. **Results:** Features included relative macrocephaly resulting in a prominent forehead (22/24), ventriculomegaly (11/24), and crowding of the posterior fossa due to a large cerebellum (filling the lowest portion of the posterior fossa in 20/24; narrowing the extra-axial space around the cerebellum in 16/24; and frank Chiari 1 malformation in 7/24). Progression of the herniation was apparent in 9/11 with serial studies. Cerebellar herniation resulted in compression of the medulla or high cervical cord in 9/24. Posterior fossa crowding led to syrinx formation in 5/16. Surgeries included shunt placement in 4, posterior fossa decompression in 6, tethered cord release in 2, and 3rd ventriculostomy in 1. **Conclusion:** While macrocephaly and prominent forehead are well-recognized in CS, the high frequency of cerebellar enlargement relative to the posterior fossa (83%) was unexpected. We speculate that, similar to the process suspected in macrocephaly-capillary malformation syndrome, abnormally rapid postnatal growth of the cerebellum leads to narrowed extra-axial spaces, reduced CSF resorption, and increased intracranial pressure leading to ventriculomegaly, progressive postnatal tonsillar herniation and syrinx formation. CNS abnormalities contribute significantly to morbidity with 8/24 (33%) individuals requiring a shunt, ventriculostomy or posterior fossa decompression. Symptoms of tonsillar herniation are similar to the typical presentation of infants with CS, including poor feeding, respiratory distress with mixed apnea, ocular palsy and constant arching. Older children complain of headache, which may present as severe irritability in infants. Individuals with a syrinx were more likely to have progressive scoliosis. While we don't have sufficient data to make specific treatment recommendations, baseline MRI studies should be considered, and upward crossing of OFC percentiles or new neurologic symptoms should prompt further studies.

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## 2662/F/Poster Board #178

**CUTIS LAXA AUTOSOMAL RECESSIVE ASSOCIATED TO CONTRACTURE.** *F. Suarez, J. Acosta.* Inst de Gen Humana, Univ Javeriana, Bogota, Colombia.

INTRODUCTION Among the genetic disorders of connective tissue is the cutis laxa, defined as a skin disorder characterized by “loose” skin (redundant) and compromise of various internal organs as a result of the shortage of elastic fibers, it can be local or generalized. Its inheritance can be autosomal dominant, autosomal recessive or X linked recessive CASE REPORT We describe a male patient, first pregnancy, mother 22 years old, father 28 years old, no relatives. Preterm delivery, weight 2700 grams, length 48 cms, congenital hypotonia and pulmonary hypertension. Currently, patient with low weight, low height, microcephaly, plagiocephaly, punctiform anterior fontanel, bulging front and widow’s peak- Marcus Gunn phenomenon with tears, bilateral inguinal hernia, diastasis rectus. Bilateral rhizomélic shortening and contractures in the hands, with marked limitation fingers extension, clubfoot. Redundant loose skin on the neck and extremities. Generalized hypotonia, poor sucking, Moro’s reflex incomplete. The pacient also presents hip dislocation and dysplasia, metaphisiary craniosynostosis, widening, long bones, agenesis of the corpus callosum, colpocefalia. Skin biopsy with scattered irregular papillary dermis, elastic fibers and decreased granular appearance. CONCLUSION In this case the authors believe that the patient has an autosomal recessive Cutis Laxa Type II whose main features are delayed growth and development, bulging front, skeletal dysplasia, including hip dysplasia, pulmonary/vascular compromise, hernias and diverticula in mucosas. It is remarkable that in this case the classic joint hiperlaxitud is not present and, contrary, the limb contractures are evident, as well as malformations in the central nervous system; These finding makes us think about a new association of Cutis Laxa so far not described in the medical literature indexed.

## 2663/F/Poster Board #179

**Late Manifestations of Tricho-Rhino-Pharangeal syndrome: Expanded Skeletal Phenotype in Adulthood.** *K. Izumi[1], M. Takagi[2], AS. Parikh[1], A. Hahn[1], SN. Miskovsky[3], G. Nishimura[4], C. Torii[2], K. Kosaki[2], T. Hasegawa[2], DE. Neilson[5].* 1) Dept Gen, Case Western Reserve Univ, Cleveland, OH; 2) Dept Pediatrics, Keio Univ, Tokyo, Japan; 3) Dept Orthopaedic Surgery, University Hospitals Case Medical Center, Cleveland, OH; 4) Dept Radiology, Tokyo Metoropolitan Kiyose Children’s Hospital, Tokyo, Japan; 5) Division of Human Genetics, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH.

Tricho-Rhino-Phalangeal syndrome (TRPS) is characterized by sparse or fine hair, bulbous and pear-shaped nose, and digital abnormalities. This syndrome is caused by mutations or deletions of TRPS1 gene. Although the longevity of the patients with TRPS is believed to be unchanged from the general population, late onset symptoms and adult phenotypes related to TRPS have not been well studied. In a 31-year-old male with chronic joint pain, hypotrichosis, and morphologic findings suggestive of TRPS, radiologic evaluation revealed ostepenia (lowest T scores of -2.3 evaluated by dual-energy x-ray absorptiometry) and severe osteoarthritis contributing to the multiple joint pains. Direct sequencing of the entire coding regions of TRPS1 revealed a heterozygous c.2755C >G transition leading to a missense mutation of a highly conserved amino acid, p.Leu919Val, in exon 6 of TRPS1. Given the functional role of TRPS1 in chondrocyte proliferation and apoptosis in growth plate, and the anatomic location of TRPS1 expression, TRPS1 represents a candidate gene for bone homeostasis regulation. Osteopenia might be an under-recognized phenotype in adulthood due to the altered function of TRPS1. We propose that bone mineral density assessment using dual-energy x-ray absorptiometry scan should be included in the routine evaluation of TRPS patients in adulthood.

## 2664/F/Poster Board #180

**Cross-Sectional and Longitudinal Assessment of Aortic Dilation in Ehlers-Danlos Syndrome.** *C.L. Atzinger, R.A. Meyer, P. Khoury, Z. Gao, B.T. Tinkle.* Cincinnati Children’s Hospital Medical Center, Cincinnati, OH.

The Ehlers-Danlos syndromes (EDS) are a group of inherited connective tissue disorders characterized by joint hypermobility and dermal findings. Previous studies have found aortic dilation in patients with the hypermobile and classic types of EDS (Wenstrup et al., 2002; McDonnell et al., 2006). However, the small sample size and cross-sectional nature of these studies did not allow for comment on the progression and clinical implications of these findings. Clinical experience suggests that progression of aortic dilation is rare and aortic root measurement tends to normalize with age. However, there has been no longitudinal study looking at the natural history and clinical significance of the aortic root findings in these patients. The purpose of this study was to assess aortic root dilation in a larger population of patients with hypermobile and classic EDS as well as provide longitudinal data regarding aortic growth to help define management. All patients with EDS seen in the Ehlers-Danlos Syndrome clinic at Cincinnati Children’s Hospital routinely receive echocardiograms as part of clinical care. A retrospective chart review of patients seen from 1995 to present was conducted. Of the 214 patients with a diagnosis of hypermobile or classic EDS who had an echocardiogram during this time, 22 (10.3%) had a dilated aorta on their first echo. Of the 98 patients who had at least 2 echocardiograms during this time, 26 had echocardiograms both in early childhood (before age 14) as well as in late childhood or adulthood (after age 14). In early childhood, 23% (6/26) of patients had aortic root dilation while after age 14, only 4% (1/26) showed aortic dilation. This change was statistically significant (p=0.0253). No patient with a normal aortic root in childhood developed dilation in later childhood or adulthood. These results suggest that while patients with hypermobile or classic EDS have an increased risk for aortic dilation, this dilation tends to remain stable or normalize. The lack of progression suggests that intervention may be unnecessary and that adults with normal aortic measurements may not need continued screening by echocardiogram.

## 2665/F/Poster Board #181

**Lowry-Wood Syndrome: Is this a rare or under-recognised condition?** *D. Josifova[1], K. Becker[2], M. Bober[3].* 1) Clinical Genetics, Guy’s Hospital, London, United Kingdom; 2) North Wales Clinical Genetics Service, Glan Clwyd Hospital, Rhyl, and Institute of Medical Genetics, University Hospital of Wales, Cardiff, UK; 3) Division of Genetics-AIDHC 1600 Rockland Road Wilmington, DE 19803-3607.

Lowry-Wood Syndrome (LWS) is a rare, multi-system disorder presenting with prenatal onset, non-progressive microcephaly, multiple epiphyseal dysplasia, short stature and developmental delay. In approximately half of the reported cases a degree of visual impairment was also observed. The ocular phenotype included congenital nystagmus, optic hypoplasia and retinitis pigmentosa which developed in the late teens or early twenties. Although this may reflect the variability of the condition, it is possible that the visual impairment becomes more prevalent with age. All the patients had learning difficulties within the moderate range. The natural history of LWS is poorly understood and long term prognosis difficult to predict. Orthopedic complications such as joint dislocations, early arthritis and limited joint mobility are likely to occur at a young age. Progressive visual deterioration affects at least a proportion of patients. There is no evidence that the life span may be reduced however, co-morbidity secondary to the reduced mobility and/or repeat surgery may have impact on the quality of life. The aetiology of LWS is unknown. Autosomal recessive inheritance has been considered however with few exceptions, all the reported cases have been singleton suggesting that a new dominant mutation is a plausible explanation. Chromosome analysis and CGH array testing have been normal. We present four new cases of LWS: identical twin sisters and two unrelated patients, and review the literature.

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## 2666/F/Poster Board #182

**Case Report: A New born with Camphomelyc Dysplasia.** *J. Bernal, A. Palacios, G. Gordillo, I. Zarante.* Inst Gen Humana, Univ Javeriana, Bogota, Colombia.

The Campomelic Dysplasia is a chondrodysplasia with a estimated prevalence of 1/100.000 to 1.6/100.000 births and a autosomal dominant inheritance. It's characterized by early death (50% of the cases are either born dead or dies within the firsth 24 hours) secondary to respiratory distress as a result of small thoracic cage, narrow larynx, hypoplastic trachea and possibly, CNS-bases hypotonia. It's a heterogeneous disorder with bent, short bones, extreme hypotonia at birth, characteristic cranio-facial features like: frequent macrodolicocephaly, wide fontanel, short, narrow and upslanted palpebral fissures, ocupal hypertelorism, flat nasal bridge, long philtrum, micrognathia, cleft palate; congenital bowel lower limbs with pretibial skin dimples, short limbs, short neck, narrow thorax, talipes equinovarus, fewer ribs. The external genitalia range from unambiguous female external genitalia to hypospadias with a bifid scrotum and an enlarged clitoris in genotypic XY males. We described a new born product of a second pregnancy, with prenatal US that report Ambiguous genitalia and presumed Osteogenesis Imperfecta type II. Delivery was by cesarean section because of podalic presentation. To the physical examination the first day of life we found abnormal face with high forehead, flat, small face, hypertelorism, depressed nasal root, micrognathia, cleft palate, low-set ears; he has an s mall thoracic cage and secondary respiratory distress; the limbs are short with cutaneous dimpling over bowed tibias and bilateral talipes equinovarus. In the genitalia level the new born has a Transposition penis-scrotum. During the hospitalization they order an echocardiogram which turns out to be normal, an thorax X-Ray which shows 10 pair of ribs. We see him again at 30 days of life, the physical examinations shows low weight, short height which accentuate the phenotype. The parents brings and abdominal ultrasound scan with normal report; the karyotype reports 46,XX in all studied cells. All the findings in this case have been previously reported in the literature. We report this case because of the genitalia findings; usually the patients present a declare pattern of ambiguous genitalia, specially on genotypic males, but our case present finds compatible with a masculine phenotype.

## 2667/F/Poster Board #183

**Three women with Hallerman-Streiff syndrome.** *H. Numabe[1,2], K. Muto[3], Z. Yamagata[4].* 1) Department of Medical Ethics, Kyoto University Graduate School of Medicine, Kyoto, Japan; 2) Department of Clinical Genetics, Kyoto University Hospital, Kyoto, Japan; 3) Department of Public Policy, Human Genome Center, The University of Tokyo, Tokyo, Japan; 4) Department of Health Sciences, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan.

Hallermann-Streiff syndrome (HSS) was characterized by early-onset cataract, microphthalmia, small pinched nose, and hypotrichosis. We present three adult female cases. Two of them have children. Case 1: A 37-year-old woman was born to a 22-year-old mother and a 26-year-old father at 40 weeks of gestation. Birth weight was 2750 g, height 49 cm, and head circumference 31.5 cm. At the age of 2 years, she was diagnosed as HSS because of cataract, growth retardation, and dysmorphic phenotype. She is married, but has no child. She is suffering from mild spinal canal stenosis. Case 2: A 38-year-old woman was born to a 24-year-old mother and a 31-year-old father at 41 weeks of gestation. Birth weight was 3100 g, height 50 cm, and head circumference 31 cm. She was diagnosed as HSS because of cataract, growth retardation, and dysmorphic features. She is suffering from obstructive sleep apnea and glaucoma. At the age of 26 years, she delivered a boy by Caesarean section at 33 weeks of gestation. His birth weight was 1760 g, height 38 cm, and head circumference 30 cm. He is now 12-year-old, not affected and healthy. Case 3: A 41-year-old woman was born to an 18-year-old mother at 41 weeks of gestation. Birth weight was 3050 g, height 49 cm, head circumference 32 cm. At the age of 2 years, she was diagnosed as HSS after a cataract operation. She had been suffering from glaucoma, and trabeculectomies were done 3 times. At the age of 21 years, she was delivered a boy by Caesarean section at 39 weeks of gestation. His birth weight was 2830 g, height 47 cm, head circumference 32 cm. At the age of 25 years, she delivered a girl by Caesarean section at 38 weeks of gestation. Her birth weight was 2600 g, height 48 cm, head circumference 31.5 cm. They are now 20-year-old and 16-year-old respectively, not affected and healthy. All the women have congenital cataract, growth retardation, hypotrichosis, characteristic facial feature, and normal intelligence. Secondary glaucoma associated with microphthalmia and cataract operation is the major complication. We thank the members of "Yui-yui"; a Japanese Hallarmann-Streiff syndrome self-support group for their kind information.

## 2668/F/Poster Board #184

**VACTERL Association In A Male Fetus With an Inherited *NIPBL* Gene Duplication.** *A.F. Wagner[1], P.L. Wilson[1], M. Williams Jr.[1], A.G. Wlodaver[2], J. Lee[2], S. Li[2], F.G. Ramji[3], A.A. Elimian[1].* 1) OB/GYN, Univ Oklahoma HSC, OKC, OK; 2) Pediatrics, Univ Oklahoma HSC, OKC, OK; 3) Radiology, Univ Oklahoma HSC, OKC, OK.

We present a 20yo Hispanic female $G_1P_0$ who was referred to our center at 32-5/7wks for multicystic dysplastic kidneys and anhydramnios. An initial ultrasound confirmed these findings as well as a muscular ventricular septal defect and a two vessel umbilical cord. A complete anatomical assessment could not be performed due to the anhydramnios. She was counseled to the grim prognosis for the fetus due to the development of pulmonary hypoplasia. She spontaneously delivered a 2765g male fetus in breech presentation with APGARs of $1^1 1^5 1^{10}$. An intubation attempt was made, but was unsuccessful due to a tracheal band. He passed away at 26min. Cord blood was sent for a karyotype and microarray analysis.

A gross genetic evaluation revealed deformational Potter facies and clubbed feet consistent with anhydramnios. The anus was patent. The right upper extremity had an angulated and shortened forearm with an absent thumb. The left upper extremity was normal.

A skeletal survey was performed which revealed absent right radius and thumb, small thorax with hypoplastic lungs, block vertebrae from L3-5 with a right L4 hemivertebra. There was gas in the stomach indicating a tracheo-esophageal communication, an unusual lucency in the chest, and a dilated esophagus which point to a type 2 tracheoesophageal fistula.

The karyotype came back with a 46,XY karyotype. Whole genome oligonucleotide microarray analysis found a ~312 kb duplication of the *NIPBL* gene. Parental studies found the same duplication in the father. Paternal genetic evaluation is pending.

The constellation of abnormalities in the fetus is consistent with the VACTERL (**v**ertebral, **a**nal, **c**ardiovascular, **t**racheo-**e**sophageal, **r**enal, and **l**imb defects) association. The condition results from a primary polytopic developmental field defect that results from an early prenatal insult to the developing embryo. Previous reports of the *NIPBL* duplication have not shown features consistent with VACTERL except for one case with an absent thumb. The father's probable normal phenotype most probably indicates that the duplication is only a copy number variant and not a cause for the complex anomalies. We recommend that further study of VACTERL cases would benefit from looking for the duplication.

## 2669/F/Poster Board #185

**CFC Syndrome with BRAF mutation at exon 15 in a patient with marked cutaneous symptoms.** *E. Nishi[1], S. Mizuno[1], T. Niihori[2], Y. Aoki[2], Y. Matsubara[2].* 1) Dept Pediatrics,Central Hosp, Aichi Human Service Ctr, Kasugai, Aichi, Japan; 2) Dept Med Genet, Tohoku Univ Sch Med,Sendai,Japan.

Cardio - facio - cutaneous (CFC) syndrome is characterized by cardiac abnormalities , distinctive craniofacial appearance and cutaneous abnormalities. Recent studies have revealed that mutations in BRAF, MAP2K1, MAP2K1/2 and KRAS cause CFC syndrome. Somatic mutations in BRAF have been identified at a high frequency in numerous cancers including melanoma. We here report a detailed clinical description of 19-year-old female with severe mental retardation, intractable epilepsy and significant cutaneous symptoms showing BRAF mutation at exon 15. The patient was a 19-year-old female. She was born to healthy unrelated parents at 38 weeks gestation. Her pregnancy and delivery were not remarkable. Her birth weight was 3494g (97th centile). She began to show failure to thrive and developmental delay during the neonatal period. She also had growth retardation, severe mental retardation, intractable epilepsy, distinctive facial appearance and significant cutaneous symptom during infancy but no cardiac anomaly. She had been unable to walk and confined to bed. Standard chromosome analysis showed normal karyotype. She was initially diagnosed as having Costello syndrome. She was noted to have a number of pigmented naevi, eczema and apparently-circumscribed, protruded proliferative lesion like pemphigus vegetans. Histological examination of affected tissue showed the formation of abscesses with neutrophilic inflammation, but with no malignancy. Mutation analysis for screening the RAS/MAPK-related disorders in her leukocytes disclosed a heterogeneous missense mutation in BRAF within the coding sequence of exon 15. Consequently, she was diagnosed as having CFC syndrome. Her neurological symptoms and cutaneous symptoms were very severe. It shows that BRAF mutation causes various phenotype of neurological and cutaneous manifestations. Somatic BRAF mutations are often found in benign skin lesions and a majority of benign naevi. In our case, the activation of MAPK signaling by the BRAF mutation might be one of the causative factors of the cutaneous lesions. To clarify the genotype-phenotype correlation in tissue lesions of RAS/MAPK disorder syndromes, we have to accumulate a detailed phenotypic description of the various cutaneous symptoms of the syndrome as well as molecular findings.

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## 2670/F/Poster Board #186

**Non chromaffin paragangliomas (glomus jugulare tumors) and hearing loss. Mother and daughter in a Mexican family.** *L. Hernandez-Gomez[1], S.G. Juarez Garcia[2], E. Hernandez Gomez[3], D.O. Gomez Torres[4].* 1) Servicio de Audiologia,(turno vespertino) Instituto Nacional de Rehabilitacion, Mexico. D. F; 2) Servicio de Neuropsicología Infantil (Turno Vespertino), Instituto Nacional de Rehabilitación, Mexico. D. F; 3) Facultad de Estudios Superiores, Iztacala, Universidad Nacional Autónoma de México, Biología; 4) Investigación. Instituto Nacional de Rehabilitación. México. D. F.

Non chromaffin paragangliomas may be isolated or familial. In the head and neck region involve the tympanic, and yugular glomera, (23%), glomus vagale (6%), carotid glomus (84%). Jugulo-tympanic paraganglioma is the most commonly recognized neoplasia involving the middle ear, arising from chemopressure receptors. Auditory findings: pulsative tinnitus occurs in over 50%-70%, otalgia (7-18%), aural fullness (4-18%), and otorrea (3-8%). Hearing loss can be conductive, mixed, or sesorinueral. dizziness and cranial nerve palsies. This tumor usually occurs in middle aged women and its presentation may be familiar, sporadic, bilateral or multicentric. The form of presentation and symptomology do not differ between isolated and familial cases. Clinical presentation of jugulotympanic paraganglioma, when it isn't associated with evidence of hormone secretion. In event of localized disease, paragangliomas often can be treated by surgical excision, but some cases need radiation therapy. Gene map locus 11q23. familial paragangliomas-1 (PGL1) is caused by mutation in the SDHD gene. We We report a Mexican family with 2 affected members with glomus jugulare tumor in 2 generations, in which mother and her daughter are affected. CASE 1: female patient 89-year-old, probando' s mother, hearing loss and glomus jugulare tumor, onset at 60-year-old.Physical exploration feminine with auditory conduct hearing loss, voice and language with alterations. Audiometric test showed sensorineural bilateral middle hearing loss. Speech audiometric test showed sensorineural deficit. Timpanogram: curves A of Jerger bilateral, right saw patron. Stapedial reflex: absent bilateral. Vestibular response to caloric is markedly reduced. CASE 2: female 64 year old, pulsated tinnitus at ear right, constant, being but perceivable in environment silent. Physical exploration feminine with auditory conduct normal, voice and language without alterations. Audiometric test showed normal audition. Speech audiometric test showed normal. Timpanogram: curves A of Jerger bilateral. Stapedial reflex: present bilateral right saw patron. Vestibular response to caloric is normal. Computed tomography of ears. The presence of increase in the caliber of the right jugular vein is identified, being an important reinforcing with the resistance means administration, demonstrating itself the presence of increase in the diameter.

## 2671/F/Poster Board #187

**Pallister-Hall syndrome; report a new case.** *S. Juarez-Garcia[1], L. Hernandez Gomez[2], D.O. Gomez Torres[3], E. Hernandez Gomez[4], V. Valadez Jimenez[5].* 1) Dept Neropsicologia (turno vespertino), Instituto Nacional de Rehabilitacion, Mexico, D.F; 2) Dept Audiologia (turno vespertino), Instituto Nacional de Rehabilitacion, Mexico, D.F; 3) Investigación. Instituto Nacional de Rehabilitacion, Mexico, D.F; 4) Facultad de Estudios Profesionales. Iztacala, Universidad Nacional Autonoma de Mexico. Biologia; 5) Dept Foniatria (turno vespertino), Instituto Nacional de Rehabilitacion, Mexico, D.F.

Pallister-Hall syndrome is caused by mutations in the GLI3 gene, Pallister-Hall syndrome is a pleiotropic autosomal dominant disorder comprising hypothalamic hamartoma, pituitary dysfunction, central polydactyly, and visceral malformations. Clinical features: imperforate anus, laryngeal cleft, abnormal lung lobation, renal agenesis or dysplasia, short 4th metacarpals, nail dysplasia, multiple buccal frenula, hypoadrenalism, microphallus, congenital heart defect, hydronephrosis and hydroureter with absent left kidney and intrauterine growth retardation. All cases were sporadic and chromosomes were apparently normal. The parents were nonconsanguineous. No environmental exposure was common to all cases. Pallister-Hall syndrome is due to an autosomal dominant gene, that most cases are sporadic and the result of new mutation, and that the gene manifests variable expressivity. We present a new case: female Mexican child 4 year moths-old is the first chil of young non consanguineous parentes obteind pretermino. She presented holoprosencephaly, microphthalmia left, cleft lip and palate, bent larynx, agenesis of nasal septum, imperforate anus, hypotelorism, ectopia left kidney, complex urogenital malformations, hidrocolps. with double vagina, uterus bicornis, persistence of arteries conduct That present language disabilities , characterized by being found to level at level of sentences, well directed and structured with articulatory distortions. EEG normal. Test audiometric shower normal audition ABR response in 30dB. Normal intelligence. Karyotype normal.

## 2672/F/Poster Board #188

**De novo chromosomal deletion and duplication in a child with developmental delay and hypotonia.** *E.C. Tan[1], E.C.P. Lim[1], B. Cham[2], I.S.L. Ng[2].* 1) KK Research Center, KK Women's & Children's Hospital, Singapore; 2) Genetics Service, KK Women's & Children's Hospital, Singapore.

The three-year old boy was born full term following an uncomplicated pregnancy to non-consanguineous parents of mixed ethnicity. The pregnancy was reportedly uncomplicated and he was born at term by spontaneous vaginal delivery. Birth weight was 4090g, Apgar scores were 3 at 1 minute and 6 at 5 minutes. During the last stage of labor, fetal heart decelerations were noted on cardiotocography. He was limp at birth, with bradycardia and no spontaneous respiration. Intubation was required for a few minutes. Urine organic acids and amino acids analyses were normal. He was referred to genetics clinic for evaluation because of cleft palate and hypotonia with gross motor and fine motor delay. Cleft palate had been repaired and his facial appearance was normal with no marked dysmorphism. On physical examination, physical growth parameters were found to be within normal limits. Muscle tone was generally decreased, but reflexes were normal. An ejection systolic murmur in the left sternal edge was heard. Brain MRI was reported to be normal but echocardiogram revealed moderate atrial septic defect. He also has sensitive airways and recurrent otitis media requiring grommet tube insertion. His karyotype was reported to be 46, XY, add(11)(?q24). Multiplex ligase-dependent probe amplification analysis using a subtelomeric probe panel revealed gain in 3p26 and loss in 11q25. Quantitative RT-PCR confirmed the gain at Cell Adhesion Molecule L1-like gene (CHL1) at 3p and loss at the Non-SMC condensing II Complex Subunit D3 gene (NCAPD3/KIAA0056) on 11q. Array-CGH with NimbleGen's 384K Human Genome CGH using the MAUI Hybridization System confirmed the copy number changes in the two regions with the loss on 11q estimated to be from q24.2 to qter (approximately 5.8 Mb) and the gain on chromosome 3 from q24.2 to qter (approximately 24.5 Mb). This appears to be a de novo event as chromosome analyses on his parents showed that the father was normal 46,XY and the mother 46,XX. He also has an older sister and a younger sister who are both phenotypically normal. The chromosomal abnormalities involving chromosomes 3 and 11 in this patient suggest that there are genes in these two regions associated with the multiple phenotypic abnormalities. We will further characterize the exact regions duplicated/ deleted and the breakpoints to identify the potential gene loci contributing to the clinical features.

## 2673/F/Poster Board #189

**Single Gene Causes of Isolated Developmental Disability: 7q31 deletion of FOXP2 in a non-dysmorphic, well-grown male with verbal dyspraxia.** *W. Al-Hertani[1,2], M.E. McCready[1,2], J. McGowan-Jordan[1,2], G.E. Graham[1,2].* 1) Genetics, CHEO, Ottawa, ON, Canada; 2) Pediatrics, CHEO, Ottawa, ON, Canada.

Up to 3% of individuals in the general population have some degree of developmental disability (DD), but well-grown, normocephalic and non-dysmorphic individuals with unexplained DD are not typically referred for a Genetics assessment due to the assumption that they are unlikely to have a single gene or chromosomal condition. Our clinic is somewhat unusual, as we often receive referrals from psychiatrists for adult patients with unexplained DD irrespective of the presence of other features. Here we report on a 27-year-old male whose very specific pattern of DD led us to suspect the involvement of FOXP2. An assessment of our patient by a developmental pediatrician at 39 months of age revealed that while some aspects of his development were as high as a 4-year-old level, his receptive language skills were at the level of a 30-36 month old, and his expressive language skills were at the level of a 12-18 month old. Psychometric assessment at 20 years of age showed his verbal skills in the mild intellectual disability range, while non-verbal skills were within the normal range (7th-27th centile). Our patient's past medical history included mild strabismus, astigmatism, major depressive episodes (resolved), generalized anxiety disorder and sleep difficulties that are well controlled by medications. He is otherwise healthy and there is no family history of developmental or speech delay. Physical examination demonstrated a non-dysmorphic, normocephalic male. Chromosome studies showed a normal 46,XY karyotype at a 500-550 band resolution. The role of FOXP2 in language development was first elucidated in 1990 by Hurst et al. Based on recently published reports (Lennon et. al. 2006, Feuk et al. 2006 and Zeesman et. al. 2005) we hypothesized that our patient may have a FOXP2 mutation, but mutation screening of this gene was not available on a clinical service basis and we were unsuccessful in finding an interested research laboratory. We instead ordered a SNP microarray test (Genome-Wide Human SNP Array 6.0, Affymetrix, Inc.), which fortuitously demonstrated a 1.89Mb deletion at 7q31.1-q31.2 (chr7:113,310,091-115,099,094) that included the entire FOXP2 gene. We are investigating the parents for this deletion, although it is most likely de novo in our patient. This case demonstrates that the Genetics clinic can play a role in determining the etiology of unexplained and apparently isolated DD.

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## 2674/F/Poster Board #190

**Chromosome 21q21.1 single clone loss identified in a family with spectrum of developmental delays, learning difficulties, and behavioral problems.** *E. Prijoles[1], M. Pavan[2], P. Newkirk[1], M. Sutcliffe[1,3].* 1) Deptartment of Pediatrics/Division of Medical Genetics, University of South Florida, Tampa, FL; 2) Department of Pediatrics, Division of Child Development, University of South Florida, Tampa, FL; 3) Department of Pathology, All Children's Hospital, Saint Petersburg, FL.

Array comparative genomic hybridization (aCGH) has helped identify novel genomic disorders in patients with developmental delay, cognitive impairment, and mental retardation. The technology has also enabled us to detect copy number variants (CNVs) whose contributions to genetic variation and clinical significance are not yet well understood. We report a family with a single clone chromosome 21q21.1 loss, confirmed by fluorescence in-situ hybridization (FISH), and a spectrum of developmental delays, learning difficulties, and behavioral problems. The proband presented at 21 months of age for language delay, sleep problems and tantrums. Parents reported head-banging behavior and unusual movements. They also questioned the possibility of autistic spectrum disorder. On exam, he was noted to be healthy-appearing without obvious dysmorphic features. EEG and brain MRI were unremarkable. High resolution chromosome analysis and Fragile X DNA testing were normal. Array CGH revealed a single clone loss in copy number within band 21q21.1 with nearest adjacent proximal clone located 0.92 Mb and distal clone 1.33 Mb away. Parents and two siblings of the proband were subsequently tested. Both the mother and 6 year-old sister were confirmed with the loss by FISH. The mother had behavior and learning problems in childhood and has epilepsy, ADHD, and bipolar disorder. She continues to have problems with reading and writing. The sister has behavior problems and learning difficulties, requiring additional assistance in school. She is being treated for ADHD and is being monitored for signs of bipolar disorder. The younger brother had no imbalance confirmed by FISH and clinically presented with mild developmental delay. The proband's father also had no imbalance and has normal intelligence. Additional maternal family history is significant for bipolar disorder, epilepsy, and mental retardation in an uncle and learning problems and mental illness in grandparents. A paternal half brother of the proband is reported to have autism. The chromosomal 21q21.1 imbalance appears to be segregating in those family members with more severe developmental disorder. However, further evaluation of additional family members and delineation of the region is now indicated to investigate the possible clinical significance of this observation.

## 2675/F/Poster Board #191

**Concomitant presence of Cyclopia and sirenomelia in a stillbirth infant before the epidemic focus of sirenomelia in cali - Colombia.** *H. Pachajoa[1], C. Isaza[2].* 1) PhD Student, Genetica, Universidad del Valle, Cali, Colombia; 2) MD, Genetica, Universidad del Valle, Cali, Colombia.

Introduction Sirenomelia and cyclopia are congenital malformations due by early blastogenic defects, with similar epidemiologic patterns, and possible common etiopathogenicity. The sirenomelia or mermaid syndrome is a congenital structural anomaly characterized by an abnormal development of the caudal region of the body with different degrees of fusion of the lower extremities and bears resemblance to the mermaid of Greek mythology. Cyclopia is the most severe form of holoprosencephaly, is characterized by the presence of one central eye product of the fusion of the two eyes and the alobar holoprosencephaly. The prevalence of this two pathologies has been estimated close to 1 in 100 000 births, including stillbirths. Case report We describe the findings in a stillbirth infant with sirenomelia, cyclopia and cephalocele occipital. Born of non consanguineous parents, mother of 16 years old and father of 23 years old by the time of birth; the mother manifested to have used misoprostol at the beginning of pregnancy (first 4 weeks) as a method to abort the fetus. Discussion Recently, a cluster of sirenomelia in the city of Cali was published. This city is located in the Colombian pacific coast. In a maternity follow up by ECLAMC (Latin-American Collaborative Study of Congenital Malformations), an epidemic focus of sirenomelia was reported during a 55-day period between 12.09.04, and 02.02.05. During that period of time 1,064 children were born and four cases presented this rare disease, which was considered unusual and suggesting of the presence of a cluster. The evidence of an infant with both sirenomelia and cyclopia suggest an epidemiologic pattern, and possible common etiopathogenicity in the city of Cali.

## 2676/F/Poster Board #192

**Congenital defects and genetic diseases in prehispanic communities of the colombian and ecuadorian pacific coast, (300 a.c.-600 d.c.).** *C.A. Rodriguez[1], C. Isaza[2], H. Pachajoa[3].* 1) Museo Arqueologico, Universidad del Valle, Cali , Colombia; 2) MD, Dept Morfología, Universidad del Valle, Cali, Colombia; 3) MD, PhD estudent, Dept Morfología, Universidad del Valle, Cali, Colombia.

Introduction: The Tumaco-Tolita culture inhabited the geo-historical region of the colombo-ecuadorian pacific coast during the years 300 A.C.-600 A.D. This culture was characterized for capturing with impressive realism the different pathologies that suffered their population, as well as evidence of genetic illnesses, congenital malformations, and the processes of aging and death. Objetive: To describe the phenotypical evidences of congenital defects and genetic diseases in the ceramic of the Tumaco- Tolita culture of the main museums of Pre-Columbian ceramics of Colombia.. Methods:, A medical geneticist, a doctor with training in medical genetics and an archaeologist examined the collection of the Tumaco-Tolita culture of the main museums of Pre-Columbian ceramics of Colombia. The cases that displayed evidences of congenital defects and genetic diseases were documented. Results: : Out of the 5000 examined pieces, there were found ceramics with clear or suggestive evidence of congenital defects and genetic diseases like Down syndrome, Crouzon syndrome, Achondroplasy, mucopolysaccharidosis (hurler syndrome and Morquio syndrome), sirenomelia and cleft lip. Conclusions: The Tumaco-Tolita culture represented the congenital defects and genetic diseases in their ceramics, and it constituted one of the first registries of these conditions in the Pre-Columbian American cultures and the world, in addition it was found clear evidence that some conditions generated status within the society.

## 2677/F/Poster Board #193

**Provision of Medical Genetic Services in Thailand - Siriraj Experience.** *P. Wasant.* Dept Ped/Div Med Gen, Siriraj Hosp/Mahidol Univ, Bangkok, Thailand.

Genetic Services at the Department of Pediatrics, Siriraj Hospital Faculty of Medicine was established since 1960s' , providing genetic counseling and cytogenetic laboratory. Since 1987 , the Clinical Genetic Services has expanded to include other areas with a mandate of "providing for comprehensive genetic services with a primary role in genetic diagnosis , counseling and prevention" 1. Genetic counseling program. 1.1 Genetics Clinic received referrals from Bangkok and provincial hospitals all over the country ; mainly systemic genetic disorders , dysmorphology , suspected chromosomal disorders , sex differentiation disorders and some cases of prenatal diagnosis. 1.2 Birth Defects Clinic (established in 1991) providing services for infants/ children with cleft lip/palate , neural tube defects, arthrogryposis, congenital hip dislocation etc. 1.3 Genetic Skeletal Dysplasia Clinic (1992) providing services for infants/children with achondroplasia , osteogenesis imperfecta , pyknodysostosis , metabolic bone diseases e.g. mucopolysaccharidosis etc 1.4 Down Syndrome Clinic (1993) serving more than 500-600 families , providing genetic counseling , medical check up , referral to early stimulation program and assisting these children toward integration. 2. Genetic Screening program. e.g. newborn screening for genetic metabolic disorders (congenital hypothyroidism and phenylketonuria) is being planned for routine screen in year 2000 ; including prenatal genetic counseling and maternal serum screening for Down syndrome and Neural tube defect for pregnant women on voluntary basis. 3. Genetics laboratory. Initially only conventional cytogenetic analysis was performed, subsequently biochemical and molecular genetics laboratory is being established. Currently numerous genetic metabolic / inborn errors of metabolism e.g. amino acid , carbohydrate , urea cycle, organic acid, fatty acid oxidation, peroxisomal and lysosomal storage disorders are being identified. The Genetic Metabolic Center was established since June 2000 and has served infants / children with IEM all over the country. 4. Non-governmental organization. Down Syndrome Parents' Support Group at Siriraj Hospital was founded in 1993 , providing for more holistic and personal services to individuals and their families.

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## 2678/F/Poster Board #194

**Providing Clinical Utility: from large-scale bioinformatics to specific audiences.** *D. Maglott[1], A. Astashyn[1], A. Brooks[3], E. Buford[2], F. Cunningham[2], R. Dalgleish[3], P. Flicek[2], L. Forman-Neall[1], J. Lee[1], S. McDaniels[1], W. McLaren[2], L. Phan[1], R. Tully[1].* 1) Natlional Center for Biotechnology Information (NCBI), NIH/NLM, Bethesda, MD; 2) European Bioinformatics Institute (EBI), Hinxton Cambridge, CB10 1SD, UK; 3) University of Leicester, Leicester, UK.

Ever-increasing throughput in generating sequence data has created a wealth of information for investigators to map, analyze, align, compare, view and model. One challenge, therefore, is to identify well-supported content relative to specific regions of the genome. Unfiltered data about genes, proteins, or sequence variants known to be associated with specific diseases may be too much to process for major classes of potential customers of these data. Genetic counselors, physicians, diagnostic testing laboratories, patients and others seeking to support, understand, manage and improve clinical treatment need rapid, reliable methods to retrieve data of interest. Over the past several years, the NCBI has been integrating resources and working directly with the clinical user base to develop and implement tools, displays, and databases that can better meet their praxis needs. We provide an overview of NCBI's strategies to improve access to and representation of clinically relevant data. Included are 1) recent modifications to Online Mendelian Inheritance in Man (OMIM), GeneReviews, and GeneTests, 2) establishing reference gene-specific sequence standards via RefSeq, RefSeqGene and Locus Reference Genomic (LRG), 3) integrating information about variants of clinical interest from multiple sources, and (4) new tools to interrogate multiple databases at the same time.

The LRG project is of particular interest, as it is the product of an international collaboration among EBI, the GEN2PEN consortium, and NCBI to build upon the RefSeqGene project and provide a set of stable gene-specific, genomic standard sequences for reporting variation. The LRG sequences are stable (not versioned), and can represent an idealized allele. Each LRG will also include fixed locations of exons and coding regions for one or more transcripts of the gene. In addition to the static data, the LRG will provide infrastructure for displaying up-to-date information, such as chromosome coordinates, related databases, alternate exon and protein numbering systems. When an LRG is established, the corresponding RefSeqGene sequence will be identical in sequence and exon annotation, and the RefSeqGene will report the LRG accession. This presentation will provide practical examples of these resources (http://www.ncbi.nlm.nih.gov; http://www.lrg-sequence.org).

## 2679/F/Poster Board #195

**MISDIAGNOSIS OF PRADER-WILLI/ANGELMAN SYNDROME DUE TO TECHNICAL LABORATORY ERRORS.** *M. Pervaiz, B. Coffee, L. Bean, M. Hedge, N. Hjelm, S. Askree, M. Adam.* Department of Human Genetics, Emory University School of Medicine, Decatur, GA.

The diagnosis of many genetic conditions relies on the combination of clinical suspicion followed by confirmatory genetic testing. We report two cases in which technical laboratory errors led to the misdiagnosis of either Angelman syndrome or Prader-Willi syndrome. Patient 1 is an 11 year old male with mild to moderate mental retardation. Very little was known regarding his early medical history, as he was in foster care. At the time of presentation to our clinic, he was speaking in complete sentences, had recently been toilet trained, and had gained a fair amount of weight over a short period of time. His foster mother reported a history of hyperphagia and hyperactivity.We recommended testing for PWS. DNA based methylation studies were done.The methodology involves methylation based PCR techniques which can analyze the DNA methylation status of any groups of CpG sites in a CpG island. Sodium bisulfite treatment is the first step in the conversion of unmethylated cytosine to uracil under conditions in which methylated cytosines remain unchanged. The duration of bisulphite treatment can lead to 90% degradation of the DNA. In this case, the maternal allele was overdegraded, leading to maternal allele dropout, and a preliminary positive result for Angelman Syndrome. As his clinical history did not suggest this diagnosis, a confirmatory southern blot test was done and was negative. Our second patient is a 27 month old female who was referred to us with an established diagnosis of PWS by methylation studies. She did not have classic clinical features of this condition, such as hypotonia, feeding difficulties, failure to thrive, or short stature. Therefore a repeat test by methylation sensitive PCR was done in our lab, and again was positive for PWS. Because of our experience with Patient 1, a second methodology was used the confirm this positive result and was negative for PWS. Further analysis demonstrated that the patient DNA contained a SNP within the primer sequence used for the PCR, leading to decreased annealing of the paternal allele, resulting in paternal allele dropout. The same PCR primers for PWS are used by almost every lab in the United States (Kubota et al, 2007), which is the reason that 2 separate clinical laboratories both erroneously reported a positive result for this patient. These 2 cases reinforce the importance of clinical judgment in the diagnosis of patients. Sometimes a positive test result should be confirmed by a second method.

## 2680/F/Poster Board #196

**Frequency of congenital malformations in newborn infants in the Civil Hospital of Tepic. México.** *LE. Wong-Ley[1,2], J. González-Zapata[1], M. Pérez-Nuño[2].* 1) Dept Gen, SSN, Tepic, Mexico; 2) Dept Gen, UAN, Tepic, Mexico.

Summary. Congenital malformations constitute an emerging problem in developing countries due to the fact that other causes are being controlled. México occupies the first place in Latin America and the Caribbean. The objective of this work was to determine the incidence of congenital malformation in the maternity area at the Civil Hospital of Tepic and to analyze its main characteristics. A retrospective study of the newborn's clinical records was realized from January 1, 2007 until December 31, 2008. Objetive. To know the frequency of congenital malformations (CM) in alive new born of the Civil Hospital of Tepic, Mexico and to identify possible risk factors. Material and methods. During 2 years, from January 1st, 2007 to December 31, 2008, they were captured those alive newborn with external congenital malformations, grouping for apparatuses and systems, comparing our results with that reported in the literature. Results. Of total of 9,166 alive births; 4,716 (51,4%) were female, 4,446 (48,5%) male and 2 (0,1%) with the ambiguous genitals. 274 cases were captured of recently born with external congenital defects corresponding to 3%, which determines an incidence of 29,9 cases by each 1.000 new born. It prevailed in the feminine sex (52.7%). Of term they were 67% and preterms 33%. The most frequent malformations were related to the digestive system, heart disease, skeletal malformations and central nervous system and less frequent defects of the genitourinary. In half of the pregnancies prenatal ultrasonography was useful for the diagnosis. The frequency found was upper than that reported by other studies in our country. The most important risk factors were, mothers older than 35 years, multiparity and some consanguinity degree between parents. Conclusions. The congenital malformations constitutes a significant problem of health in our country, some differences were observed in the prevalence of congenital malformations. The CM are an important cause of morbimortality in newborns and we must learn to identify the risk factors that influence its presentation. Key words. Congenital malformations, alive newborn .

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## 2681/F/Poster Board #197

**Detecting genetic associations with rare variants.** *G. Bhatia[1], V. Bansal[2], O. Harismendy[2], E. Topol[2], K. Frazer[2], V. Bafna[1].* 1) Computer Science Department, UC San Diego, La Jolla, CA; 2) Scripps Research Institute, La Jolla, CA.

Genome-wide association (GWA) studies, which search for association between single, common genetic markers and a disease phenotype, have shown varying degrees of success. Recent studies suggest that multiple rare variants at different loci may act in concert to influence etiology of common diseases. However, current GWA studies depend upon the Common Disease Common Variant hypothesis and are not powered to account for disease-causing rare variants (CDRV).

We consider a simple CDRV model where a subset of rare variants at a locus is independently deleterious. These rare variants have modest penetrance (their presence increases disease likelihood significantly), but each explains only a small fraction of the diseased individuals. For this model, we describe an algorithm that efficiently computes a subset of rare-variants that best associate with the disease. We test our approach using extensive simulations. Neutral rare-variants are simulated according to models proposed by Fu, and causal variants are assumed to be under purifying selection are simulated according to Wright's equation. Our method is robust in detecting associations with modest odds ratios in relatively small cohort studies. It significantly outperforms previously proposed collapsing strategies, and is robust to other CDRV models.

Our simulations suggest that associated regions with an odds ratio of 1.5 can be identified with a case control study of 640 cases and 640 controls, with unadjusted p-values of $10^{-16}$. Associated regions with odds ratios of 2.2 can be identified with about 200 case/200 control individuals, while still maintaining genome wide significance. These results suggest that genome wide association using an analysis of rare variants is feasible.

The method was applied to a cohort of 289 individuals, 143 with high BMI and 146 with relatively low BMI. Two genes (187 kb) were sequenced in this cohort using next-generation sequencing. We identified two subsets of co-located variants that showed significant association with BMI (out of a total of 1088 rare variants). These subsets associated showed association with un-adjusted chi-square P-values of 3.6 x 10-4 and 1.4 x 10-4, and permutation based P-values of 0.004 and 8.04 x 10-4. In comparison, the most significant association using single marker tests had a chi-square P-value of 0.002.

## 2682/F/Poster Board #198

**Informatic Sample Handling Processes: A High Throughput Genotyping Facility's Workflow for Sample Information Tracking.** *B. Craig, C. Ongaco, M. Zilka, M. Barnhart, D. Leary, K. Hetrick, B. Marosy, M. Adams-Carr, O. Osimokun, Y. Sun, F. Kistenev, J. Romm, J. Goldstein, E. Pugh, L. Watkins, C. Boehm, K. Doheny.* Center for Inherited Disease Research, Johns Hopkins University, Baltimore, MD.

The Center for Inherited Disease Research was established at Johns Hopkins University in 1996. CIDR provides high quality genotyping services and statistical genetics consultation to investigators working to discover genes that contribute to common disease. The handling of large numbers of samples requires a mechanism for tracking detailed information throughout experimental processes. Progress can be recorded and results retrieved in a manner appropriate to the individual lab's custom procedures. CIDR processes approximately 100,000 samples each year, tracking this information through unique combinations of database types, Web, Java and Perl applications and integration with robotics for sample movements. Use of MySQL®, Oracle® and MS SQL® databases have allowed CIDR to create new and adapt legacy schemas to store changing information about samples for use in downstream processes and release to investigators. Creation of Java and Perl applications to integrate with databases provides the flexibility to implement and adapt custom validation frameworks. These methods are put into operation through unique sample map construction, verification of pedigree and other investigator supplied information, confirmation of sample receipt, recording history of sample movements, queuing samples into production workflows, tracking of problems and recording auxiliary information and quality control (QC) metrics. Development of custom laboratory information management systems (LIMS) for CIDR's Affymetrix® 6.0, Illumina® Infinium and BeadXpress services using JavaScript, Java and the Google Web Toolkit allow for rapid changes to workflows and improved troubleshooting methods based on input from CIDR personnel or vendors. The use of liquid and tube handling robotics has allowed CIDR to streamline sample processing by re-arraying samples with investigator-supplied plating criteria to better mix sample phenotype information prior to processing. Samples can be removed, re-genotyped or replaced based on problems identified throughout CIDR's workflow, thereby optimizing processing time and reagent use. Automated processes perform genotype calling and QC analysis which are linked to the sample for reporting to CIDR personnel and investigators. The use of these resources, as well as the experience to adapt or engineer new ones as needed, permit CIDR to efficiently receive and process large sample studies at present and allow for continued growth into the future.

## 2683/F/Poster Board #199

**Hypertrophic cardiomyopathy multigene analysis by next generation sequencing.** *S. Dames[1], J. Durtschi[1], J. Stephens[1], K. Geiersbach[1], K.V. Voelkerding[1,2].* 1) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, Utah; 2) University of Utah Department of Pathology, Salt Lake City, Utah.

**Introduction.** Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disorder of sarcomere structure/function. At least 16 genes with over 450 mutations have been implicated in HCM. Next generation sequencing (NGS) offers a new approach for complex multi-gene analysis. The goal of the current study was to compare the performance of the Illumina Genome Analyzer and Roche 454 GS FLX platforms for targeted resequencing of genes implicated in HCM. **Methods.** A normal human genomic DNA sample was used for analysis. HCM genes were enriched by long range PCR and equimolar pooled. The pooled amplicons were divided and single end libraries for Illumina and GS FLX sequencing were prepared and sequencing conducted following manufacturer's protocols. Analysis of sequencing data was performed with platform-specific software and DNAstar SeqMan alignment tools using HGB 36.3 reference sequences. All NGS identified HCM exon sequence variations were analyzed by Sanger sequencing for verification. **Results.** Illumina and GS FLX sequencing resulted in coverage averages of 798 and 198 with read lengths of 36 and 235 (ave) bases, respectively. Forty-two GS FLX and 45 Illumina exon variants were identified using >30-fold coverage and >20% read percentage criteria. Thirty-two variants were concordant with 31/32 confirmed by Sanger sequencing. Of non-concordant variants, all were wild type compared to the reference sequence, with half under 30% read percentage. Analysis indicates the remaining variants resulted from short-read misalignments due to high homology between sequenced genes and homopolymer sequencing errors. Over 20 Kb of Sanger sequencing data did not reveal any sequence variants unreported by the NGS technologies. **Discussion.** The current study represents an initial phase in the development of a multi-gene analysis for HCM with the future goal of implementation for diagnostics. The data demonstrate the feasibility of multi-gene enrichment by LR-PCR coupled with NGS for targeted resequencing. The high degree of platform concordance suggests that either Illumina or Roche 454 technologies would be suitable for this application. Each platform has unique features (e.g., read lengths and sequencing chemistries) that need to be considered with respect to data analysis and evaluation/confirmation of variants. Ongoing efforts include the use of pair end Illumina sequencing and evaluation of additional algorithms for variant identification.

## 2684/F/Poster Board #200

**Classifying genomic data using combination of bagging and rank aggregation.** *S. DATTA, V. PIHUR, S. DATTA.* University of Louisville, LOUISVILLE, KY.

Machine learning methods such as clustering and classification are essential tools for analyzing genomic data. However, no single classifier can reliably classify all data sets. Similarly, for a given data set, the results may vary greatly depending on the classifier used and the user's level of sophistication. This is particularly true for high dimensional data sets arising out of microarray and proteomic studies as illustrated by the results of a recent classification competition of grouping breast cancer patients from SELDI spectra held at Leiden University. In this work, we propose to use a collection of classifiers to make the final analysis relatively stable. Also care is taken so that the analysis is adaptive - that is the ultimate classification is close to that obtained using a hypothetically "optimal" classifier for a given data set. The optimization part is carried out using multiple performance measures and a rank-based aggregation. This is different from the traditional "ensemble classifier" whose general goal is to improve the accuracy by averaging individual models constructed using "weak" classifiers. We illustrate the usefulness of our method using a gene expression data set on Acute Lymphoblastic Leukemia and a proteomic data set on prostrate cancer.

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## 2685/F/Poster Board #233

**A statistical framework for differential network analysis (DNA) from microarray data using partial least squares.** *S. DATTA, R. GILL, S. DATTA.* UNIV OF LOUISVILLE, LOUISVILLE, KY.

It has been long well known that genes do not act alone; rather groups of genes act in consort during a biological process. Consequently, the expression levels of genes are dependent on each other. Experimental techniques to detect such interacting pairs of genes have been in place for quite some time. With the advent of microarray technology, newer computational techniques to detect such interaction or association between gene expressions are being proposed which lead to an association network. While most microarray analyses look for genes that are differentially expressed, it is of potentially greater significance to identify how entire association network structures change between two or more biological settings, say normal versus diseased cell types. We provide a recipe for conducting a differential analysis of networks constructed from microarray data under two experimental settings. At the core of our approach lies a connectivity score that represents the strength of genetic association or interaction between two genes. In this paper we use a score that is based on a partial least squares analysis of expression data that was introduced in our previous work. In this presentation, we use these scores to propose formal statistical tests for each of following queries: (i) whether the overall modular structures of the two networks are different, ii) whether the connectivity of a particular set of “interesting genes” has changed between the two networks, and (iii) whether the connectivity of a given single gene has changed between the two networks. We carried out our method on two types of simulated data: Gaussian networks and networks based on differential equations. We show that, for appropriate choices of the tuning parameters, our method works well on simulated data. We also analyze a real data set involving normal versus heavy mice and identify an interesting set of genes that may play key roles in obesity.

## 2686/F/Poster Board #234

**Identification of disease-associated copy number variation using classification techniques.** *J.Y. Hehir-Kwa[1], N. Wieskamp[1], C. Webber[2], C. Gilissen[1], R. Pfundt[1], C.P. Ponting[2], J.A. Veltman[1].* 1) Radboud University Nijmegen Medical Centre, Department of Human Genetics, Nijmegen; 2) MRC Functional Genomics Unit, University of Oxford, Oxford.

Genomic copy number variation (CNV) has recently been recognized as a common form of genomic variation in humans. Hundreds of CNVs can be detected in any individual genome using genomic microarrays or whole genome sequencing technology, but their phenotypic consequences are poorly described. Rare CNVs have been reported as a frequent cause of neurological disorders such as mental retardation (MR), schizophrenia and autism, prompting widespread implementation of CNV screening in diagnostics. For clinical applications it is however essential to reliably distinguish pathogenic from apparently benign CNVs. In previous studies we have extensively studied the genomic content of benign CNVs (Nguyen et al. 2008) and in addition shown that in contrast to benign CNVs MR-associated CNVs are significantly enriched in genes whose mouse orthologues, when disrupted, result in abnormal axon or dopaminergic neuron morphologies (Webber et al. in press). In this study we developed and validated a novel computational method for differentiating between benign and disease-associated CNVs using structural and functional genomic features. After training, we show that a Naïve Bayesian Tree classifier achieves a high accuracy (94%) and negative predictive value (99%) on a blinded test set of CNVs from a large cohort of individuals with unexplained MR. In total 13 features contributed to the classification, with LINE density (-6%) and mouse knockout phenotypes (-5%) having the highest contribution to the classifier’s accuracy. These results indicate that this classification method can be used for objectively prioritizing CNVs in clinical research and diagnostics. Results from this method can easily be combined with information on CNV frequency and inheritance, thereby greatly enhancing clinical interpretation of this important class of genomic variations. Nguyen et al. “Reduced purifying selection prevails over positive selection in human copy number variant evolution”. Genome Research 18: 1711-23 (2008). Webber et al. “Forging links between human mental retardation-associated CNVs and mouse gene knockout models” PLoS Genetics (in press).

## 2687/F/Poster Board #235

**Detection of parent-of-origin effects mediated by SNPs which generate or destroy CpG sites.** *B.M. Herrera[1,2], C.A. Anderson[1], J.C. Randall[1], W. Yuan[1], K. Elliott[1], R. Mott[1], A.P. Morris[1], C.M. Limdgren[1,2].* 1) Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, UK, OX3 7BN; 2) Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford University,Oxford, UK OX3 7LJ.

Parent-of-origin effects (POEs) such as imprinting and histone acetylation, result in deviations from Mendelian inheritance and modify gene expression in a manner determined by the sex of the parent from which the locus is inherited. POEs are often mediated by epigenetic mechanisms like methylation at CpG islands. To establish the presence of SNPs which affect gene expression through *cis*-acting mechanisms, in particular those SNPs which may mediate POE through the creation or destruction of CpG islands (meth-SNPs). We used publically available gene expression and SNP genotype data from HapMap families. Out of 47,294 transcripts in 30 Caucasian (CEU) and 30 Yoruba (YRI) trios, we considered only genes expressed with a standard deviation $\sigma$0.5 (CEU=1,013 and YRI=1039). A linear regression method was employed to detect POEs. In order to detect *cis*-effects, only SNPs located 500Kb upstream and 50Kb downstream of the transcript were considered. CEU and YRI trios were analyzed separately and then meta-analysed, and a genome-wide significance threshold of $p \leq 10^{-5}$ applied. The lists of genes obtained were then queried using a gene candidacy program (genesniffer) to generate imprinting-candidacy scores for each gene. In YRI families, we observe POEs for 96 genes (annotated by 345 SNPs, of which 21.1% are meth-SNPs). In CEU families, we observe POEs for 73 genes (annotated by 392 SNPs, of which 22.7% are meth-SNPs). The meta-analysis detected a total of 106 genes with POE effects (annotated by 324 SNPs of which 24% are meth-SNPs). Among the genes identified by the meta-analysis, we observed one known imprinted gene *NDN*, adjacent to one of the 15q11 imprinting centres. In addition, we observed POEs for 13 genes overlapping between our CEU and YRI sets, including *TJP1* (15q13) and *DPYD* (1p22) which obtained high genesniffer scores further suggesting that their expression is regulated by specific POE. Further replication of findings from the meta-analysis and the CEU results will be carried out in a larger Northern European cohort. These results constitute a potentially novel set of imprinted genes for which expression may be mediated via methylation mechanisms.

## 2688/F/Poster Board #236

**Improved Multiplex Ligation-dependent Probe Amplification Analysis (MLPA) with BioNumerics®.** *K. Janssens[1], D. Michielsen[1], B. Pot[1], J. Goris[1], B. West[2], P. Vauterin[1], L. Vauterin[1].* 1) Applied Maths NV, Sint-Martens-Latem, East-Flanders, Belgium; 2) Applied Maths Inc., Austin, TX.

Copy number variations in genomic DNA have been linked with pathogenic mutations in many diseases. These variations are usually not detected by PCR-based sequence analysis because a normal copy is still present. Traditionally, techniques as Southern blot hybridization or FISH are used to detect these types of mutations, with the disadvantage that these are both time-consuming and require a high amount of DNA. Multiplex ligation-dependent probe amplification (MLPA) is a relatively simple method that allows relative copy number estimation of up to 45 nucleic acid sequences in one single reaction. Introduced by MRC-Holland, MLPA has become a promising technique for the rapid detection of copy number variations in genomic DNA, mRNA profiling and for identification of microbial communities. The BioNumerics database and analysis platform was extended with a novel iterative normalization algorithm with subsequent scoring, embedded in the existing scripting environment. Sequencer data coming from different hardware manufacturers can be imported in batch as peak-tables or as raw curve files. Due to the variations of PCR efficiencies within and between samples and probes, traditional normalization methods do not perform very well. We developed a unique rapid iterative normalization method that uses both the control probes peaks as well as the peak intensities. The use of this iterative approach improves the accuracy and lowers the false positive rates, compared to traditional normalization techniques. Once the automated normalization step is completed, each probe peak is scored according to adjustable thresholds. The scoring is subsequently stored in the database. Extensive statistics and reports possibilities are embedded in the tool for individual patient reports as well as overall data mining on results obtained from MLPA combined with data from other techniques or information fields about the patients. The use of the MLPA-plugin in BioNumerics allows the user to easily and rapidly detect large genomic rearrangements in new samples and perform meta-analysis on the results. The general BioNumerics platform allows overall data management and advanced data mining. Reports for each gene or region of interest can be generated for rapid detection of candidate samples with copy number changes. The analysis can be set up with a high degree of automation, making a high throughput approach feasible.

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## 2689/F/Poster Board #237

**Insertion of small pyrosequencing reads into a core tree using parsimonious insertion.** *D. Knox[1], A. Gonzalez-Peña[1], K. Ramirez[2].* 1) Computer Science, University of Colorado at Boulder, Boulder, CO; 2) Ecology and Evolutionary Biology, University of Colorado at Boulder, Boulder, CO.

Understanding the full breadth and depth of microbial diversity between different communities provides valuable insight into global processes ranging from biogeochemical processes to human health. With the advent of high throughput sequencing technology, there has been a flood of sequence data. Successfully handling and analyzing this data has proved difficult and has hindered the ability of researchers to fully synthesize results. One goal of analyzing unknown sequences is to align and classify each sequence into a taxonomic group, and then find a relationship between sequences using phylogenic analyses. There are many tactics available to classify unknown sequences. One method, the use of parsimony insertion into a core tree, has proven successful and accurate, but difficult and time consuming on a large number of sequences. The goal of this research was to create a program to insert unknown 16S ribosomal RNA sequences into a “core” phylogenetic tree using parsimony insertion. This research was approached in two steps: (1) an alignment model was created to align sequences and prepare them for insertion into a core tree. The alignment model was created using the program Infernal and representative sequences chosen from the well-annotated Greengenes database. Using a gapping method short read sequences were aligned to representative sequences, and then rapidly ungapped for parsimonious insertion into the core tree. (2) A parsimony algorithm was created to insert sequences into the core tree. This algorithm has been tested on both short and full-length sequences. In tests, the algorithm has a ~95% accuracy for placing both short and full length sequences into the core tree. Further when sequences are designated by percent similarity, the algorithm has up to 75% accuracy when ≥ 85% sequence similarity is used. The benefit of efficiently inserting sequences into a core tree is two fold. First, it aids in the organization and visualization of sequence data. Second, and more importantly, parsimony insertion gives a highly accurate method of classifying sequences and determining phylogenetic relatedness.

## 2690/F/Poster Board #238

**Investigation of the patterns of Linkage Disequilibrium in the human genome by comparing HapMap and genome-wide association scans.** *W. Lau, N. Maniatis.* Research Department of Genetics, Evolution & Environment, University College London, London, United Kingdom.

Over the recent years, association mapping of disease genes has developed into one of the most dynamic research areas of human genetics. Establishing the Linkage Disequilibrium (LD) structure of the Human Genome was extremely important for the effective application of association mapping. It was also instrumental for the advancement of Genome Wide Association (GWA) mapping. Metric LD maps in LD units are analogous to linkage maps in that map distances are additive but for disease association mapping they provide a much higher resolution. From the International HapMap Project and other sources, millions of SNP genotypes are now available for constructing such high resolution maps. Here we compare the LDU maps that were constructed from the 60 CEU individuals of the Phase II HapMap data and the combined 17,000 individuals from the Welcome Trust Case Control Consortium (WTCCC). We investigate the patterns of LD in HapMap and WTCCC and their relationship to the second generation linkage map. We finally examine the use of LD maps as a tool in identifying disease genes.

## 2691/F/Poster Board #239

**The CIDR Affymetrix® Analysis Pipeline: An Automated Analysis Pipeline for the Affymetrix Genome-Wide Human SNP Array 6.0.** *D.R. Leary, M.W. Barnhart, J.L. Goldstein, K.M. Roberts, K.N. Hetrick, G.W. Lowe, B.D. Craig, S.M.L. Griffith, E. Hsu, K.F. Doheny, L. Watkins, Jr.* Center for Inherited Disease Research, Johns Hopkins University, Baltimore, MD.

The Center for Inherited Disease Research provides high quality genotyping services and statistical genetics consultation to investigators working to discover genes that contribute to common disease. The CIDR Affymetrix Analysis Pipeline, a Java application tightly integrated with the CIDR Affymetrix LIMS, is a completely automated analysis pipeline built around the Affymetrix Power Tools (APT) software suite provided by Affymetrix. Multiple Affymetrix scanners output GeneChip data to a single, multi-terabyte, network-attached storage location. The pipeline monitors the network location and, upon determining when each GeneChip’s scan is complete, archives that GeneChip’s data. The archiving process organizes the data by project, and also creates file redundancy for disaster recovery purposes. After file archiving, data concerning the scanner and the fluidics station are extracted from the AUDIT file and stored to CIDR’s Affymetrix LIMS database. Next, an eight-node Apple XServe compute cluster simultaneously analyzes the CEL file using three APT programs: apt-geno-qc, apt-copynumber-workflow, and apt-probeset-genotype. Each compute node has 32GB RAM, 350GB disk space, and dual quad-core Intel® Xeon® processors. Up to 64 APT jobs can be executed concurrently. When each of these jobs finishes, the pipeline software parses the resulting output files and stores these data into the database: quality control metrics and gender estimates from apt-geno-qc, copynumber metrics from apt-copynumber-workflow, and birdseed analysis results from apt-probeset-genotype for quality control purposes. In addition, the apt-geno-qc DM SNP genotypes are stored to aid in sample tracking and identification, and HapMap concordance is calculated for CIDR control samples from the apt-probeset-genotype output and is also stored. All of these data are then available via several user-customizable reporting applications. To date, CIDR’s pipeline has analyzed over 4.2 billion genotypes across more than 4,600 GeneChips, providing real-time quality control metrics on each of these. This has allowed lab managers to make quick, informed decisions regarding lab processing in a high-throughput environment.

## 2692/F/Poster Board #240

**Interrogation of SNPs in duplicated gene loci: true polymorphisms or false identities.** *W. Lin[1,2], M. Ho[1,2].* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Institute of Biomedical Informatics, National Yang-Ming University, Taipei, Taiwan.

In our previous study, we have established a concise gene oriented ortholog database in order to improve gene transcript complexity examination and orthologous gene assignment. Due to the complex alternative splicing transcripts in human genome, the use of traditional protein primary sequence similarity best-hit search methods could have difficulties in resolving complicated orthologous relationships between many alternative spliced isoforms. By clustering transcripts with the origin of all biological mechanismsthe genomewe will be able to better decipher gene locus information as well as summarize gene isoform information. Compared with other ortholog databases, gene-oriented ortholog database provides about 80% coverage of reference sequences and yields higher accuracy. Intriguingly, around 15% of human reference genes, that could not assigned by our pipeline, are mapped to multiple genomic regions. These duplicated gene loci (DGL) might be generated from an error in homologous recombination, a retrotransposition event, or from duplication of a large chromosomal segment. Many of these multiple location DGLs are putative genes with copy number variations (CNVs) in their duplicated regions. Due to the extremely high sequence similarity between the DGL gene loci, it is likely that any given mismatch between DGLs could be mistakenly annotated as SNPs. In fact, they are merely sequence differences among various genomic copies. This provides possible explanations for numbers of SNPs with multiple genomic locations. In order to further understand the expression of these DGL or CNV genes, we tried to use dbSNP dataset (17,804,034 rsSNPs) to map and distinguish different genomic loci of DGL genes. In this study, we interrogated 2,090 DGL genes of high sequence similarity (95%) with multiple genomic locations (distributed at 7,160 genomic loci). After mapping rsSNPs to these genomic locations, we found 148,897 rsSNPs located within the boundaries of these DGL or CNV gene regions. Further examination reveals that most of these single nucleotide sequence variation sites should not be annotated as single nucleotide polymorphism. Utilization of these SNPs in genotyping practices could generate inaccurate information and impair anticipated genotyping calculations. Therefore, our study suggests that in-depth characterizations and re-annotation of these DGL or CNV associated SNP records should be performed to improve the SNP based genome-wide association studies.

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## 2693/F/Poster Board #241

**ALFRED: A resource for research and teaching.** *H. Rajeevan[1], K.-H. Cheung[2], U. Soundararajan[1], S. Stein[1], A.J. Pakstis[1], J.R. Kidd[1], K.K. Kidd[1].* 1) Dept Genetics, Sch Med, Yale U, New Haven, CT; 2) Center for Medical Informatics, Sch Med, Yale U., New Haven, CT.

ALFRED (the ALlele FREquency Database, http://alfred.med.yale.edu), has been accumulating gene frequency data on human populations from published literature, collaborators, high throughput data sources such as HGDP-CEPH website and Human Genome Diversity Project at Stanford University, and the host laboratory. ALFRED currently has nearly 500,000 tables (one population sample typed for one site) of allele frequencies involving over 18,000 polymorphisms with data on nearly 700 distinct populations from around the world. 23 polymorphisms in ALFRED have allele frequency data on more than 100 population samples. 58 population samples in ALFRED have allele frequency data on more than 4000 polymorphisms. There are several complete data sets in ALFRED, some with partial overlap. They include uploads from the host lab (638 SNPs on 40 population samples), and from published literature; 2810 SNPs on 51 HGDP populations (PMID: 18513279), 11,555 SNPs on 12 diverse populations (PMID: 16004724). Another 77 populations (combination of host lab and HGDP populations) in ALFRED, all have data on at least 791 SNPs that are a subset of the Illumina HumanHap 650Y Beadchip array panel. The remaining tables from the panel are being uploaded into ALFRED. In addition to existing downloading formats, tools to select and extract specific complete data sets in different formats are also being developed. Among multiple graphical display modes for allele frequencies for a polymorphism, pie-charts can be viewed on Google Map and Google Earth. These display modes allow users to utilize all the functions available from the Google Map, Google Earth applications. Fst and average heterozygosity values for bi-allelic SNPs displayed in tabular format and organized by chromosomes can now be sorted by locus, Fst, average heterozygosity and number of populations typed. A table mapping dbSNP's unique identifier, "rs-number" and ALFRED's unique identifier has facilitated ways to have reciprocal URL links with dbSNP, PharmGKB and Genopedia HuGE Navigator. To involve the community in collaborative authoring and curation tasks we created ALFREDWiki (http://alfred.med.yale.edu:8080/wiki/index.php/About_ALFRED). The application includes pages to edit population description and a feedback section. ALFRED is supported by U.S. NSF BCS0840570.

## 2694/F/Poster Board #242

**e-GenoType: A Rapid and Accurate Method for *in silico* Genotype Characterization.** *J. Reid, M. Bainbridge, D. Deiros, L. Nazareth, D. Muzny, R. Gibbs.* Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX.

With the rapid increase in density and read-length on NexGen sequencing platforms there has also been a significant increase in the run-time required for the analysis necessary to map reads and produce variant calls. To address this we have developed a method (available at http://www.e-genotype.com/) of rapidly screening read data using in silico probes designed to provide genotyping information on any sample almost immediately after base-calling is complete. Because of the simplicity of generating in silico probes, as the list of known variants rapidly grows (as with the 40 million new variants identified by the thousand genomes project) so will the probe list used for screening. With this method genotype calls can be produced from raw read data in minutes or hours instead of days or weeks, an essential feature necessary for addressing time-sensitive problems such as sample verification and contamination screening.

Based on analysis of HapMap deep-coverage sample data generated at the HGSC for the thousand genomes deep-sequencing pilot project, we have demonstrated that e-GenoType can rapidly produce genotype calls at over 700,000 known polymorphic sites with a miscall rate of less than 2%. We will also show that high-coverage miscalls can be improved by screening probe sets for non-specific probes, discuss an approach to e-GenoTyping in/dels and structural variants of any size, and show how rapid identification of putative somatic alleles can be found in tumor/normal pairs.

## 2695/F/Poster Board #243

**Combination of genotyping platforms to develop a haplotype map in Mexican Mestizo and Amerindian populations.** *G. Richard[1,2], C. Rangel-Escareno[1], J.C. Fernandez-Lopez[1], I. Silva-Zolezzi[1], G. Jimenez-Sanchez[1].* 1) National Institute of Genomic Medicine (INMEGEN), Mexico City, Mexico; 2) National Institute of Applied Sciences (INSA) of Lyon, Villeurbanne, France.

The aim of the Mexican Genome Diversity Project (MGDP) is to characterize genetic diversity and linkage disequilibrium structure to generate a ~1.5 million SNP haplotype map. This will allow the implementation of efficient tagging and imputation strategies for association studies in Mexicans. To increase SNP density and hence genome coverage, we merged genotype data of same individuals from the following platforms: Affymetrix 500K, Illumina HumanHap550 and Illumina HumanExon510S. We analyzed genotypes generated for the MGDP from 300 Mexican Mestizos and 150 Amerindians from three populations (Mayas, Tepehuanes and Zapotecas). Combined raw data provided a total of 1,427,627 SNPs. We evaluated genotyping assignment consistency between all platforms on the 139,033 overlapping SNPs representing 9.4%; of the total. Of these, 25.3%; were consistently reported across platforms, the rest (103,832) had either one of the following problems: strand inversion 25%;, allele differences but same strand 24.7&%;, strand differences same alleles 25%;, and allele mismatch 0.00072%;. To evaluate genotype concordance in all samples across platforms, we first solved these problems by allele or strand re-assignation for each SNP, achieving complete consistency. SNP genotype frequencies were used in a principal component analysis (PCA). Objects relative to the first two principal components were plotted as clusters using the Partitioning Around Medoids algorithm, based on the search for representative clusters among the data that minimize dissimilarities. Resulting clusters were drawn based on the average and covariance matrix of each of them. Observed clustering patterns in Mestizos and Amerindians were consistent with those obtained from our previous Affymetrix 100K analysis in the same populations (Silva-Zolezzi I. et al., PNAS 2009). However, it also showed clear platform-related differences. Affymetrix 500K includes random evenly-distributed markers, whereas Illumina 510K has a trend related to the selection of gene-centered tagSNPs and coding SNPs. Our results describe the feasibility of combining SNP panels from different platforms, a necessary step towards the construction of a haplotype map, which will be relevant for population genetics studies in Mexicans.

## 2696/F/Poster Board #244

**Predicting heart transcription enhancers in the human genome.** *N.J. Sakabe[1], L. Narlikar[2], M.A. Nobrega[1], I. Ovcharenko[2].* 1) Human Genetics Department, University of Chicago, Chicago, IL; 2) NCBI, NLM, NIH, Bethesda, MD.

Regulation of gene expression during heart development involves promoters and distal regulatory elements spread across the genome up to megabase-long distances from genes. To identify these elements based solely on their genomic sequence, we developed a classifier that learns features from an experimentally validated set of embryonic heart regulatory elements and scans the human genome for regions with similar features. Our training set was composed by 50 validated enhancers and promoters from the literature and other sources. The classifier was built to learn enrichment of known and de novo motifs. We then employed a linear regression method (LASSO) that selects features that are important in the training set and learns a linear formula comprising the selected features with different weights. As a negative set, we generated a random set of 1000 sequences with matching GC content and length. Among the features selected as important, MEF2, SRF and other 18 motifs are known to play an important role in heart development. Our method outperformed others on both sensitivity and specificity, with an area under the ROC curve of 0.92 versus 0.77 of the best competing method. We then used the trained classifier to score non-coding elements in the human genome conserved in mouse (CNE) in windows of 250 bp. Out of a total of 363,765 CNEs, 6,097 were scored as putative heart enhancers. The majority (93%) of the putative heart enhancers lie at least 2kb away from the nearest TSS. The predicted enhancers are associated with genes highly expressed in the heart and significantly correlate with chromatin modifications and DNase I hypersensitive sites, indicative of active regulatory elements. Finally, about half of the 20 putative enhancers tested in zebrafish reporter assays were validated as heart enhancers. We also tested 2 sequences on transgenic mice and both were positively validated.

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## 2697/F/Poster Board #245

**A Computational System to facilitate efficient discovery of disease genes.** *T.E. Scheetz[1,2], K. Cribben[2], T.L. Casavant[1,2,3], T.A. Braun[1,2], J.H. Fingert[1], E.M. Stone[1,4], V.C. Sheffield[1,4,5].* 1) Dept Ophthalmology, University of Iowa, Iowa City, IA; 2) Dept Biomedical Engineering, University of Iowa, Iowa City, IA; 3) Department of Electrical and Computer Engineering, University of Iowa, Iowa City, IA; 4) Howard Hughes Medical Institute, Iowa City, Iowa; 5) Department of Pediatrics, University of Iowa, Iowa City, IA.

We have developed and successfully utilized a system biology strategy for disease gene prioritization. This system utilizes multiple networks of correlated gene expression to prioritize candidate disease genes. This system extended the original NPCE algorithm (Non-Positional Correlation of Expression; Scheetz et al. 2006) by: (1) incorporating a network-based analysis strategy, (2) supporting the integration of multiple expression data sets, and (3) utilizing comparative methods to integrated data among multiple species. The performance of the system was assessed using the traditional area under the curve (AROC) measurement. For genes known to cause retinitis pigmentosa, we achieved an AROC of 0.896. Of interest, just over half of the RP genes are prioritized very well, falling in the top few percent in a prioritization of all genes. The remaining genes are less specifically prioritized, although the "worst" prioritization was in the top half of all prioritized genes. In contrast the genes known to cause Bardet-Biedl syndrome were all prioritized very well with this enhanced system, with an AROC of 0.954. All of the BBS genes are prioritized in the best 15%, with most in the best 1% of all prioritized genes. We are currently pursuing mutation discovery in prioritized candidate retinal degeneration genes in previously mapped intervals in which the gene has not been identified. Scheetz et al. (2006) Regulation of Gene Expression in the Mammalian Eye and its Relevance to Eye Disease. Proc Natl Acad Sci USA, 103(39):14429-34.

## 2698/F/Poster Board #246

**Which Comes First - Lab or LIMS? The Tandem Implementation of a Laboratory Process and a Laboratory Information Management System (LIMS).** *M. Singleton[1], T. Tait[2], C. Hull[3].* 1) Laboratory Management, Axial Biotech, Salt Lake City, UT; 2) Information Technology, Axial Biotech, Salt Lake City, UT; 3) Administration, UNIConnect, Sandy, UT.

Axial Biotech was founded by top spine surgeons and geneticists focused on developing genetic tests and treatments for spinal disorders. The research developed into a clinical genetic test providing a prognosis for the advancement of Adolescent Idiopathic Scoliosis, ScoliScore. This test required a software system to track and regulate the lab and maintain quality control. Axial faced an issue similar to most labs: Do they develop the lab around LIMS, or LIMS around the lab? Axial elected to implement a LIMS early in the process that enabled a focus on customization in tandem with the development of lab processes. The UNIFlow platform was selected for its rapid adaptability. UNIFlow also offered the ability to be configured by non-programmers at Axial. An Axial team comprised of the VP of Clinical Operations, the IT Director, and the Lab Scientist attended a 3 day training session to understand the system's potential. As the ScoliScore test got closer to a launch, the VP created the LIMS vision, the Scientist outlined the process, and the IT Director configured it. With the addition of new equipment or a new assay, LIMS was configured to adapt to new processes including quality control, and physicality of performing the assay. The process of design-create-test continued over the next 9 months culminating in a LIMS that tracked the complete process. In Sept. of '08, the lab was inspected under CLIA/CAP guidelines as part of funding requirements. The auditors found very few issues surrounding the testing process that were not controlled or tracked by the LIMS. In time, the LIMS became an exact reflection of their processes, having been designed and built by Axial personnel, those who perform the lab processes on a daily basis, all built within the UNIFlow platform. In Jan. of '09, Axial went through a CAP inspection. The auditors were impressed with LIMS management and accounting for the lab processes and the inspection resulted in zero deficiencies. Conclusion: Which came first - Lab or LIMS? In the case of Axial, both. The development of a process specific LIMS greatly impacted the quality of the lab. The success of the lab in terms of daily sample processing, regulatory audits, and overall quality has been driven by the use of a customizable and flexible LIMS without having to bend and shape the lab processes to fit a preexisting mold. UNIFlow put the power to improve the system in the hands of the technician, rather than a hired programmer.

## 2699/F/Poster Board #247

**Comparision of Next Gen sequencing technologies and bioinformatic analysis tools - Case studies on different projects.** *K. Stangier.* GATC Biotech AG, Konstanz, Germany.

Objectives: Next Generation sequencers, e.g. Roche GS FLX and Illumina Genome Analyzer II, have been on the market for quite some time. A variety of different projects now reveal the pros and cons of each technology. The projects performed by GATC show that the use of one technology alone does not deliver the best results for all projects. Rather a combination of two or three technologies provides a more complete, cost-effective analysis. In addition to sequencing, bioinformatic analysis is critically important for gaining an in-depth understanding of the biological significance of the sequence data. The combination, analysis and visualisation of these data are key challenges to the successful application of the Next Generation sequencing technologies. Methods and results: Having performed many projects, each with a different set of questions and goals. GATC presents sequencing data using single and paired end sequencing methods on different systems. Sequencing of mate pairs, especially using libraries with large inserts, helps tremendously to improve assemblies and alignments. GATC uses a wide range of bioinformatic solutions for genome assembly and alignment, transcriptome analysis and other studies. We will present data comparing different bioinformatic tools which are available on the market. Conclusion: To ensure a successful sequencing project and to maximise the information obtained, it is necessary to choose the best Next Generation technology or combination of technologies followed by bioinformatic analysis using a pipeline consisting of state-of-the-art analysis tools.

## 2700/F/Poster Board #248

**The role of microRNA in the development of oxLDL-induced atherosclerosis.** *Y. Wang, S. Juo.* Graduate Institute of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan.

Background: The migration and the proliferation of vascular smooth muscle cells (VSMCs) are critical to the initiation and development of atherosclerosis. Evidence suggests that microRNAs (miRNAs) might be involved in atherosclerosis. The aim of the present study is to investigate the role miRNAs in oxidized low density lipoprotein (ox-LDL) induced atherosclerotic responses in human VSMC and the differential expression after an anti-atherosclerotic chemical compound, saponin. Methods: VSMCs were treated with ox-LDL with and without saponin for 4 hrs and total RNA was extracted. miRNAs expression profiling was measured using the TaqMan Array Human MicroRNA. Expression level of each miRNA was determined with $\Delta$CT by subtracting CT of endogenous control RNU48. Differentially expressed miRNAs were calculated by StatMiner software. Results: After treating VSMC with ox-LDL, we found that 85 miRNAs decreased expression levels by at least 2-fold. Treating VSMC with saponin in the presence of ox-LDL, the cell proliferation was reduced compared with the cells only treated by ox-LDL. In addition, we found 7 miRNAs increased expression by at least 2-fold in the saponin treated group compared with the group with oxLDL. Three of the seven miRNAs with increased expression were in the miR-15 family. miRNAs in this family have been speculated to be involved in cell progression, inflammation, cell death and angiogenesis according to the information from the Sanger miRBase. Conclusions: Our results show miRNAs are involved in ox-LDL induced atherosclerosis, and some of these miRNAs may account for anti-atherosclerotic drug effect.

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## 2701/F/Poster Board #281

**Data Storage & Archiving for High-Throughput Genotyping.** *L. Watkins, G. Lowe, E. Hsu, K. Roberts, J. Goldstein, M. Barnhart, D. Leary, K. Hetrick, B. Craig, E. Pugh, C. Ongaco, M. Zilka, J. Romm, K. Doheny.* Center for Inherited Disease Research, Johns Hopkins University, Baltimore, MD.

The Center for Inherited Disease Research (CIDR) began offering GWAS services in 2006, ramping up to full-scale production in 2007, expanding in 2008 to include both Affymetrix® and Illumina® platforms. A major consequence has been a vast increase in the rate and volume of data production. This requires a flexible and rapidly-evolving strategy for data storage and archiving. Starting with a few Illumina BeadArray® Readers, existing CIDR informatics infrastructure was initially able to handle all data produced. Scanner raw data was written to a network folder on a Windows fileserver connected to a Storage Area Network System (SANS), which also stored all downstream data. All of this data was retained and manually curated within a highly structured Windows/SANS filesystem. The advent of large-scale GWAS required several adaptations as more data was produced much faster. So we developed a combined genotype calling & data archiving pipeline to address these issues. Analytical data and QC metrics were automatically moved into project-specific SANS folders, with raw data files compressed on disk and copied to tape. By mid-2007 archiving was re-directed to 4TB Linux-based storage appliances. Soon even this solution became inadequate, unreliable and difficult to manage. For archival storage we then adopted CAStor, an easy to manage, highly reliable but arbitrarily large global filesystem. Still, active analytical data often exceeded the 2TB Windows/SANS limit. So in 2008 we added a large Isilon network-attached storage cluster for both raw and analytical data. By fall 2008 higher-capacity scanners and multi-sample chips pushed data production up to 12TB/week - unsustainable by almost any measure. Thus, in early 2009 we began intensive consultation among CIDR staff and external experts to redesign our storage/archiving scheme. We concluded that raw images (TIFF files) could be deleted if bead-level intensity files were preserved, bringing data output back down around 2-3TB/week. We also developed a data triage protocol whereby classes of files are archived and/or deleted at each production phase, thereby reducing the net rate of data growth. We continue to pursue every avenue to meet the data storage & archiving challenge, including 10-gigabit networking; data compression and de-duplication; filesystem virtualization to separate physical from logical storage, allowing change and growth without disruption; and ever-increasing storage density and efficiency.

## 2702/F/Poster Board #282

**Snipper: a research tool for extracting and searching biological annotations on genes near SNPs.** *R.P. Welch[1], C.J. Willer[2], L.J. Scott[2], M. Boehnke[2].* 1) Bioinformatics Program, University of Michigan, Ann Arbor, MI; 2) Department of Biostatistics, University of Michigan, Ann Arbor, MI.

Genome-wide association studies (GWAS) have provided a flurry of disease-associated loci, many of which span or are located near multiple genes. It is often unclear which of these genes may be influencing the disease or trait of interest. Functional studies can shed some light on the problem, however only a small number of genes can be interrogated as the cost and time for such studies are typically high. Therefore, prioritizing genes for functional follow-up work is an important task. To this end, we present Snipper, a command-line software utility for locating genes near associated SNPs, downloading their biological annotations from NCBI databases, and searching these annotations for information relevant to the disease or phenotype under study. Snipper is able to perform these functions simultaneously on all associated SNPs from a GWAS study, typically in a matter of minutes. The program then sorts the genes by various criteria, such as the distance to the associated SNP, the number of PubMed articles linked to the gene matching search terms, and the number of biological annotations from NCBI Entrez Gene that match search terms. This information is then exported to a formatted report file. The researcher can use this wealth of information when considering genes for future study. In contrast, collecting this information by manually navigating the NCBI databases takes hours, and searching it requires considerably more time. We present examples of Snipper's usage, including analysis of novel loci discovered by a recent meta-analysis of type 2 diabetes genome-wide association studies as part of the DIAGRAM consortium. We also discuss future additions to Snipper, including searching public eQTL, gene/protein and protein/protein interaction databases. Snipper is open-source and freely available software, written in Python and able to run on Windows, Mac OS X 10+, and most variants of Unix and Linux.

## 2703/F/Poster Board #283

**Integration of gene expression and SNP data in pathway based analyses.** *Q. Xiong, S. Mukherjee, T.S. Furey.* IGSP, Duke University, Durham, NC.

We propose a framework to integrate gene expression and genotype assays for pathway based association analysis. The central idea is to extend increasingly popular pathway based gene expression analysis, also known as gene set analysis, to incorporate genotype or SNP data. Pathway based analysis using gene expression data aims to find concerted differential expression between case and control samples over a set of a priori defined gene sets. We extend this approach to identify pathways associated with clinical phenotypes in terms of gene expression, SNP variation, or both genomic assays. The integration of biological evidence both across genes in defined pathways as well as across genomic assays can result in the inference of associations that are more robust and comprehensive. In addition, significant variation associated with a phenotype for both genetic and gene expression variation provides greater information on the possible mechanism underlying a disease. This integrative analysis may also suggest whether cis-regulation or trans-regulation is more relevant for the disregulation of a particular pathway. We apply this method to simulated genotypic and expression data to illustrate the modeling principles and interpretation of the data. We further validate the method on gene expression data and genotype data from 14 cancer cell lines with sensitivity/resistance to docetaxel as phenotypes. The 379 gene sets in the molecular signatures database (MSigDB) were used as the pathway database. Three differential pathway enrichment analyses were evaluated: differential enrichment of expression data alone (GSEA); differential association with respect to SNPs mapped to the genes defining these gene sets (GSEA_SNP); and integrative analysis of evidence for differential association in expression and with respect to SNPs (GSAA). We found that a large number of phenotype-associated genes were enriched in the top-ranked pathways. We also can identify two important genes, AKT1 and PIK3CA in the phosphatidylinositol 3-kinase(PI3K)-AKT pathway, which has been proved to play an important role in the resistance to docetaxel or other chemotherapeutic agents. These analyses required the development of a novel gene set enrichment methodology we call gene set association analysis (GSAA) that integrates expression and SNP data in a gene set framework. These analyses also involved the adaptation of gene set enrichment analysis (GSEA) to genotype data that we call GSEA_SNP.

## 2704/F/Poster Board #284

**GenePipe: A high performance bioinformatics pipeline for large-scale human genomic variation studies.** *A. Yao[1,2], C.K. Liu[2], Y.H. Chen[2], Y.J. Lin[2], C.Y. Tang[2], Y.C. Cheng[2], M.F. Tsai[2], Y.T. Chen[1,2].* 1) Biomedical Sciences, Academia Sinica, Taipei, Taiwan, Taiwan; 2) National Genotyping Center, Academia Sinica, Taipei, Taiwan, Taiwan.

With the flood of information generated by the new generation of sequencing technologies, there is a great need for more efficient bioinformatics tools with which to organize and analyze the tremendous amount of information regarding genetic variations. As a result, we have developed GenePipe 3.0 (http://genepipe.ngc.sinica.edu.tw/ngwelcome.do), a tool capable of processing hundreds of candidate markers (or regions) and thousands of neighboring variations in a matter of minutes to facilitate genome-wide association studies as well as studies of other diseases involving novel variations, such as cancer. GenePipe has three major components: VarioWatch, PrimerZ, and QualiSeq. VarioWatch not only integrates important information from various databases such as dbSNP, UniProt, KEGG, Gene Ontology, etc. for browsing but also incorporates in-depth functional analysis information on both known and novel variations. For greater user convenience, VarioWatch accepts four common query types such as gene name, SNP ID, and physical position in either single or batch query mode. Returned results are classified into four specially designed Views. The first of these, Genome View, shows an overview of the positions of queried markers on chromosomes and their possible functional impacts. Clicking on an interesting variation will lead to Gene View, which displays genes and associated variations close to the marker. In addition, annotations for each gene of gene function, tissue-specific expression, diseases, sub-cellular location, pathway, and Gene Ontology are also included here. At the gene transcript level, Transcript View shows gene structure and SNP distribution. Clicking on SNP directs one to Variation View which provides very detailed information of the variation such as risk type, risk level, allele change, amino acid change, codon position, amino acid position and describes the functional impact analysis flow. If interesting genes or variations are found, their primers can be easily designed by the second component, PrimerZ. Moreover, their flanking sequences can be directly downloaded by the third component, QauliSeq, for further bioinformatics analysis. The use of GenePipe as a high performance bioinformatics pipeline provides a very efficient tool for large-scale genomic variation studies. Currently, GenePipe mainly covers point variations but will be extended to cover copy number variations and micro RNAs by the end of 2009.

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## 2705/F/Poster Board #285

**The Global Problem of Mapping Gene Identifiers to HUGO Gene Symbols and Responsibility for Dissemination of Errors.** *N. MacLennan, A. Presson, E.R.B. McCabe.* University of California, Los Angeles, Los Angeles, CA.

Background: Genetic databases contain a variety of annotation errors that often go unnoticed, especially in microarray analysis with large data sets where investigators are removed from the raw data by algorithms that are not always accurate. Correct mapping of identifiers (IDs) such as microarray probe (PIDs), genbank (GB), and entrez gene (EG) IDs to HUGO gene symbols (GS) is an important but reputedly trivial task. We show that third party pathway analysis software contributes to the annotation problem by not mapping probe sets to GS according to the chip manufacturer's recommendation. Objective: To study annotations and assess whether this is a more global problem. Methods: We compared the Affymetrix (Affy) GS annotations for 678 Mouse 430 v.2.0 PIDs to Ingenuity Pathways Analysis (IPA) software annotations for four IPA releases (2008 to 2009). We analyzed the full Affy mouse 430 2.0 array using IPA, GeneGO and Pathway Studio and compared PID, GS, GB, and EG ID annotations. Results: There were notable mapping changes among the four IPA releases. In March 2008, 25/678 probes were not annotated by Affy (Mouse430_2.na24.annot.csv), 24/678 were not annotated by IPA (v6.0) and 13 were not mapped by either. In October 2008, about the same number of PIDs were unmapped (24/678) by Affy (Mouse430_2.na25.annot.csv), but the number unmapped by IPA (v6.5) more than tripled (90/678). A comparison of IPA, GeneGO and PathwayStudio for the Affy mouse 430 v2.0 showed that all programs mapped different numbers of the four identifier types (PID, GS, GB, EG), and these differed from the corresponding Affy annotation numbers. Conclusions: Researchers must be aware of potential annotation errors that can occur when identifiers are translated or mapped to other identifier types. We provide suggestions to mitigate this problem. Recommendation: Companies should inform their customers of mapping discrepancies and any annotation errors in the software. However to this date, nothing has been disseminated about the mapping issues we experienced in 2008 and 2009. Therefore, we propose a web site dedicated to sharing and discussing experiences with scientific software and databases. This web site would centralize information on experiences with software tools and alert others to scientific issues, problems encountered in biological research, troubleshooting websites and recommendations for corporate responsibility to prevent propagation of errors.

## 2706/F/Poster Board #286

**Spectral error correction and de novo assembly for bacterial genomes from short mate pair reads.** *E. Spier, D. Brinza, Z. Zhang, H. Tu, N. Mullaken, C. Cummings.* Bioinformatics R&D, Applied Biosystems, Foster City, CA.

It is generally considered impossible to generate de novo assembly from short reads if the error rate is > 3% [1]. Spectral error correction was previously used for short sequencing reads to decrease error rates prior to assembly [1]. We describe spectral error correction method for two base encoded (2-BE, aka color-space) SOLiD reads and show that it decreases error rate for 50-mers from 3-4% to less than 1%. We use color-call (phred-like) quality values (QVs) to build the spectrum: a list of k-mers (we found that k=17 works the best for bacterial genomes) that frequently occur in reads. We implemented a dynamic programming algorithm to correct color-calls in reads based on their QVs and the spectrum. One of the challenges for base-based error correction is that it is prone to overcorrection in duplicated regions. A single base difference show as two adjacent color call changes in 2-BE reads and we implemented a rule not to correct adjacent "errors" thus dramatically decreasing the risk of overcorrection. Decreased errors enable meaningful de novo assembly for both 2x25 and 2x50 long mate pairs: We used velvet_de [2] to assemble 98% of the Listeria (2.7 Mb) genome into 8 scaffolds (N50=1.4 MB for scaffolds and 6Kb for contigs) from 140x coverage 2x25 long mate pair data set. 200x coverage for a 2x50 long reads from E.coli produced assembly of similar quality. 2-BE enables error correction in diploid data because two SNP alleles differ by two color calls and therefore we cannot overcorrect from one allele into another. We demonstrate that spectral error correction decreases false positive and false negative SNP calls in human targeted resequencing data. We think that spectral error correction will be an integral part for next generation sequencing data analysis pipelines for both de novo and resequencing applications.

1. M. Chaisson, D. Brinza, P. Pevzner De novo fragment assembly with short mate-paired reads: does read length matter? Genome Research 19, 336-46 (2009) 2. D. Zerbino, E. Birney Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. Genome Research 18, 821-9 (2008).

## 2707/F/Poster Board #287

**SCAN: SNP and Copy number Annotation.** *E. Gamazon[1], S. Duan[1], A. Konkashbaev[1], W. Zhang[1], E. Kistner[4], D. Nicolae[1,2], M.E. Dolan[1], N. Cox[1,3].* 1) Department of Medicine, University of Chicago, Chicago, IL; 2) Department of Statistics, University of Chicago, Chicago, IL; 3) Department of Human Genetics, University of Chicago, Chicago, IL; 4) Department of Health Studies, University of Chicago, Chicago, IL.

The large-scale genotype data available in the HapMap project has revolutionized our ability to understand human genetic variation, including patterns of linkage disequilibrium (LD). Lymphoblastoid cell lines (LCLs) from the individuals included in the HapMap project have been used extensively in gene expression studies, and the combination of the large-scale genotype and transcript level information in the same set of individuals has facilitated genome-wide association of expression phenotypes. The SCAN database leverages these dual successes of the HapMap project in order to facilitate a novel approach to annotating genetic variation with information useful in prioritizing follow up studies at the level of SNPs, CNVs and genes. SCAN encompasses a large-scale extensible database with a web interface and a set of methods and algorithms; it includes not only physical and functional annotations currently distributed across several public databases, but also summary data not available elsewhere, including multilocus linkage disequilibrium annotations as well as annotations showing association of a variant to gene expression at a user-specified threshold. For each SNP, SCAN is intended to provide: 1. summary information from analyses conducted to characterize HapMap SNP associations to gene expression in the full set of HapMap LCLs derived from individuals of European (CEU) and African (YRI) ancestry for over 13,000 transcript clusters evaluated using the Affymetrix GeneChip ® Human Exon 1.0 ST Array 2. LD information, including what genes have variation in strong LD (pairwise or multilocus) with the variant, and how well the SNP is interrogated (i.e. multilocus LD measure) by SNPs on each of the high-throughput genotyping platforms. For each gene, SCAN provides annotations on: 1. all expression quantitative trait loci (eQTLs) that predict the expression of the gene (at user-specified thresholds for cis- and trans-regulators) 2. how well all variants in the HapMap at the gene are interrogated on each high-throughput platform (using several multilocus LD coefficients for each SNP within and up to 2 kb from the gene). For each region, SCAN provides: 1. physical and functional annotations of all SNPs, genes, and known CNVs within the region 2. all genes regulated by eQTLs within the region at a user-specified threshold.

## 2708/F/Poster Board #288

**Type 2 Diabetes Mellitus Genetic Association Database.** *J.E. Lim[1], H-S. Jin[1], K-W. Hong[1], J.T. Woo[2], H.K. Park[1], B. Oh[1].* 1) Department of Biomedical Engineering, School of Medicine, Kyung Hee University, Seoul, Korea; 2) Endocrinology and Metabolism, School of Medicine, Kyung Hee University, Seoul, Korea.

The prevalence of type 2 diabetes mellitus has been epidemic worldwide with the greatest increase in Asia, Africa, and South America. The life-threatening complications of diabetes sometimes devastate patients from the retinopathy, nephropathy, lower-limb amputation caused by the continued exposure of tissue to the high glucose. Since the hyperglycemia could be prevented and reversed greatly by changing the life style including the exercise and nutrition, the diabetes risk tests, such as one provided by American Diabetes Association, have been used to alarm the high risk group. However, the increase of diabetes incidence has not been stopped for the last decade, demanding new approaches. Following the growth of genomics, the disease susceptibility of human genetic variations has successfully been explored to provide the better understanding for the development of diabetes mellitus. Since the discovery of TCF7L2 gene in 2006, more than 15 genes have been confirmed to be replicated from diverse populations. "Type 2 Diabetes Mellitus Genetic Association Database" has been constructed to collect published results of genetic association studies related to type 2 diabetes mellitus. HuGE Navigator (version1.3, http://hugenavigator.net) was used to ascertain the publications for the database. The information of the database was extracted from 572 publications of diabetes association studies. Each entry of a gene contains 12 attributes, such as gene, rs number, PubMed ID, population, sample size, odds ratio, p-value, association study result etc. The data was graphically displayed to provide the association results better to researchers. Eventually, the diabetes genetic association database will be utilized to make a computer program which provides health care providers with the individual susceptibility to diabetes for the personalized medicine, and could be expanded for the selection of high risk group for the preventive medicine.

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## 2709/F/Poster Board #289

**On design of deep-sequencing experiments.** *A. Bashir[1], V. Bansal[2], V. Bafna[1].* 1) Computer Science, University of California, San Diego, La Jolla, CA; 2) Scripps Genomic Medicine - Scripps Translational Science Institute, The Scripps Research Institute, La Jolla, CA.

New technologies have made it possible to sequence populations of individuals. The abundance of technologies and applications of sequencing pose a number of design challenges, regarding the technology to be used and the depth of sequencing. Here we describe a number of analytical and empirical results to address these questions. Specifically, we focus on detection of structural variations, transcript abundance and haplotype assembly. Our results provide explicit trade-offs between detection and resolution of rearrangement breakpoints, and the optimal mix of paired-read insert lengths. Specifically, we prove that optimal detection and resolution of breakpoints is achieved using a mix of exactly two insert library lengths. Furthermore, we derive explicit formulae to determine these insert length combinations, enabling a 15% improvement in breakpoint detection at the same experimental cost. On empirical short read data, these predictions show good concordance with Illumina 200bp and 2kb insert length libraries. For transcript sequencing, we determine the sequencing depth needed to detect rare transcripts from a small pilot study. With only 500,000 reads, we can derive corrections that enable nearly perfect prediction of the underlying expression pdf, and use this to to predict the sequencing depth required to detect low expressed genes with greater than 95% probability. For haplotype assembly, we empirically estimate the N50 haplotype lengths for a range of sequence read lengths and insert library lengths. We demonstrate how paired-end sequencing with a mix of insert sizes currently available for the Roche 454 platform can enable the reconstruction of long haplotypes with modest sequencing depth. For very short reads, using an appropriate mix of insert sizes, we can obtain N50 lengths of 200 kb at ~ 50x sequence coverage. Our results provide platform independent guidelines for designing sequencing experiments (amount of sequencing, choice of insert length, library mixtures) that will continue to be applicable as sequencing technology rapidly evolves. They also reveal interesting trade-offs; structural variation is best detected with low variance in insert lengths, but the reverse is true for haplotype assembly. Additionally, the transcriptomic results help direct the number of different samples to pursue in a large-scale sequencing survey. Together, these form a generic framework for many design considerations related to high-throughput sequencing.

## 2710/F/Poster Board #290

**Accurate Follow-up SNP Selection.** *E. Kostem[1], J. Lozano[3], E. Eskin[1,2].* 1) Computer Sci Dept, UCLA, Los Angeles, CA; 2) Human Genetics Dept, UCLA, Los Angeles, CA; 3) Computer Sci Dept, University of the Basque Country.

Genome-Wide association studies aim to identify regions associated with complex human diseases by collecting genetic variation data, such as SNPs, from case and control individuals. An informative subset of SNPs, called tagSNPs, are selected to serve as proxies for the rest of the SNPs. Once these tagSNPs are genotyped, an association statistic is calculated for each. Significantly associated tagSNPs indicate a potential causal variant in their linkage disequilibrium block. Once these regions are detected, there is substantial interest in identifying all of the associated SNPs in these regions so that the causal variant can be determined. A cost effective approach is to perform a follow-up study which collects additional SNPs, called follow-up SNPs, and genotypes them in the same case/control individuals. In this work, we address the problem of selecting the follow-up SNPs which are more likely to be associated based on the information available from observed tagSNPs and reference data on human genetic variation such the HapMap. We analyze the problem in a statistical framework based on the causal relationship between SNPs, evaluate the performance of the traditional SNP selection approach is in terms of its accuracy and statistical power. A selection method is proposed that is optimal in accuracy and benchmark results on simulated data from the ENCODE region are presented.

## 2711/F/Poster Board #291

**Computational identification of transcription factors involved in obesity identifies variation in SPI1 affecting body mass index.** *J.M. Lane[1], L.D. Parnell[1], J.P. Fortin[2], A.S. Kopin[2], J.M. Ordovas[1].* 1) Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA; 2) Molecular Pharmacology Research Center, Molecular Cardiology Research Institute, Tufts University School of Medicine, Boston, MA.

Obesity is a complex disease that involves multiple tissues. Research in human populations is especially difficult because of gene-by-environment interactions. Here, we show that starting with data from a fat storage candidate gene screen in *C.elegans*, a novel bioinformatics approach can be used to generate candidate transcription factors (TFs) involved in obesity. In brief, human homologs of worm fat storage genes were identified. The human homologs were grouped according to function and biological process as evidence suggests that TFs control groups of genes with similar function. Then, to determine if the genes in each functional group are coordinately regulated, the upstream region of each gene in the group was scanned for putative common transcription factor binding sites (TFBS). TFBS motifs that occurred in genes within a group but not in random sets of genes were of interest. The TFs that bound to the TFBS of interest then became candidate TFs for involvement in obesity. Computationally identified TFs were validated by microarray expression analysis of differentiating preadipocytes. Our novel method identified SPI1 as a candidate TF involved in obesity. In addition, we identified variants of SPI1 that associate with obesity related traits. The single nucleotide polymorphism (SNP) rs4752829 associates with BMI in a White population. Recently, it has been demonstrated that SPI1 is expressed in white adipose tissue (Wang F et al. 2008) and that overexpression of SPI1 inhibits adipogenesis. Our studies confirm these findings in OP9 preadipocyte cells. We are currently examining the effects of SPI1 depletion on adipogenesis. Overall, this approach demonstrates the value of utilizing publicly available data and model organisms in order to gain insight into a complex human condition.

## 2712/F/Poster Board #292

**Characterizing Gene-Gene Interaction by Transitional Dynamics in Times Series Microarray Data.** *P. Song[1], X. Gao[2], Q. Pu[2].* 1) Biostatistics, Univ Michigan, Ann Arbor, MI; 2) Mathematics and Statistics, York University, Toronto, Ontario, Canada.

Gene-Gene dependency plays a very important role in system biology as it pertains to the crucial understanding of different biological mechanisms. Time-course microarray data provides a new platform useful to reveal the dynamic mechanism of gene-gene dependencies. Existing interaction measures are mostly based on association measures, such as Pearson or Spearman correlations. However, it is well known that such interaction measure can only capture linear or monotonic dependency relationships but not for nonlinear combinatorial dependency relationships. Further, when computing correlation measures, the observations across different time points have to be considered as independent replications, which ignores the dependencies across time points. With the invocation of hidden Markov models, we propose a new measure of pairwise dependency based on transition probabilities. The new dynamic interaction measure essentially checks whether or not the joint transition kernel of the bivariate state variables is the product of two marginal transition kernels of each of two state variables. This new measure enables us not only to evaluate the strength, but also to infer the details of gene dependencies. It reveals nonlinear combinatorial dependency structure in two aspects: between two genes and across adjacent time points. We conduct a bootstrap-based chisquare square test for presence/absence of the dependency between every pair of genes. Simulation studies and real biological data analysis demonstrate the application of the proposed method.

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## 2713/F/Poster Board #293

**Genome-Wide Noncoding RNAs Discovery and Characterization.** *J. Li[1], H. Tseng[2], B. Zhang[1], X. Zhang[1], J. Hansen[3], W. Ruzzo[2], L. Zhao[1].* 1) Quantitative Gen Epidem, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Computer science and Engineering , University of Washington, Seattle, WA; 3) Clinical division, Fred Hutchinson Cancer Research Center, Seattle, WA.

The recent waves of genome wide association studies (GWAS) on complex human diseases such as heart diseases, cancers and mental disorders have led to many discoveries of genetic markers, many of which fall in poorly annotated noncoding regions triggering great interests in functional annotation of noncoding RNAs (ncRNAs). While several methods have been used to discover and annotate ncRNA, we propose a two-stage approach to systematically screen for ncRNAs throughout the human genome. The first stage is to screen for all the ncRNA candidates, based on Watson-Crick base-pairing principle that has been shown to be a universal characteristic for all experimentally studied miRNAs and siRNAs. The second stage involves secondary structure prediction and statistical analyses with Hidden Markov Model (HMM) and logistic regression model to determine the candidacy of ncRNAs and their annotations. As a proof-of-principle, we have applied this strategy to our recent GWAS results, and have identified ncRNAs, their targets and associated signaling pathway which may be associated with acute graft-versus-host disease. In this presentation, we will report the genome-wide ncRNAs discovery and characterization results, results from which will be made available to public via QGE web site at Fred Hutchinson Cancer Research Center (http://qge.fhcrc.org/).

## 2714/F/Poster Board #294

**A Bayesian approach to significant analysis of dual channel RNAi data with replications.** *C.Y. Chen[1], I.S. Chang[1,2], C.H. Chen[1], C.A. Hsiung[1].* 1) Division of Biostatistics and Bioinformatics, National Health Research Institues, Miaoli, Taiwan; 2) Institute of Cancer Research, National Health Research Institues, Miaoli, Taiwan.

RNAi (RNA interference) is a conserved biological mechanism to silence gene expression on the level of individual transcripts. Cell-based RNAi screens can be used to identify genes whose mutation leads to an altered phenotype relevant to a specific pathway. Rapid reverse genetics by RNAi allows the systematic screening of a whole genome whereby every single transcript is depleted by siRNAs, dsRNAs or shRNAs. Genes with unknown functions can then be classified according to their phenotype. However, issues such as interferon response and off-targeting effect on essential genes induced by RNAi vectors justify the incorporation of viability into the analysis. We therefore consider an RNAi high throughput screening experiment in which two phenotypes are monitored; one measures cell viability and the other measures the phenotypic value representing the activity of a particular pathway. We are interested in genes that do not affect the cell viability but are involved in this particular pathway. For each shRNA, the experiment is replicated in 2J wells in which J of them are used to measure the cell viability and the other J replicates are used to measure the activity of the pathway. We propose a Bayesian data analysis method that utilizes this data duplication. Both simulation studies and validation studies are carried out to indicate its usefulness and performance. Simulation studies indicate that this Bayesian approach is capable to utilize the duplication to take care of both false discovery rate and false negative rate in identifying the interested genes. In an RNAi experiment having 6552 RNAi wells and 426 controls wells, using 281 of these controls for normalization purposes, we find none of the remaining 145 controls in the top 900 shRNAs showing significant phenotypic effects.

## 2715/F/Poster Board #295

**Genome-wide characterization of genetic variants using SIFT web server.** *P. Kumar[1], P. Ng[1,2].* 1) Department of Genomic Medicine, J. Craig Venter Institute, San Diego, CA; 2) Fred Hutchinson Cancer Research Center, Seattle, WA.

Human genetic variation data will increase in size as a result of high-throughput next-generation sequencing platforms that enable whole genome sequencing, exome sequencing and targeted gene re-sequencing for multiple individuals. The 1000 Genomes Project for example, aims to sequence the genomes of at least one thousand individuals from different ethnic groups over the next three years. Therefore, there is an increasing need to characterize and predict the effects of coding genetic variants on protein function and potentially on the phenotype of an individual.

Non-synonymous single nucleotide polymorphisms (nsSNPs) are the types of genetic variants that cause an amino acid substitution (AAS) in the corresponding protein. “Sorting Tolerant From Intolerant” (SIFT) is an algorithm that uses sequence homology and evolutionary conservation of amino acids in a protein to predict the effects of AASs on protein function. Recognizing researchers’ need for high throughput analysis of genetic variants, we have improved SIFT by adding new ‘Batch Tools that characterize and provide predictions for variants occurring at multiple genomic coordinates or in multiple proteins. SIFT can perform high throughput genome-wide variant analysis within a short time, enabling researchers to prioritize their variants for further analysis. SIFT is available as a web-tool at http://sift.jcvi.org.

## 2716/F/Poster Board #296

**Bioinformatic scoring of structural genomic variations on a universal scale of allele-specific functional difference.** *J.L. Rodriguez-flores[1], T. Gaasterland[2].* 1) Bioinformatics Graduate Program, Univ California, San Diego, La Jolla, CA; 2) Laboratory of Computational Genomics, Univ California, San Diego, La Jolla, CA.

Guame (phonetic goo-ah-meh) is Puertorrican slang for an easy task. Bioinformatically predicting genetic polymorphism function is NOT a guame because: (1) Most GWAS associated SNPs have unknown function, (2) recently discovered structural variations and epigenetic modifications expand the list of functional possibilities, (3) no universal scoring method exists. Functional prediction methods for SNPs such as SIFT (Ng02) and RAVEN (Andersen08) construct a PSSM from experimentally-verified homologous sequences to represent the probability distribution of amino acids or nucleotides at each position in a sequence or motif. We construct PSSMs for copy number variations using hundreds of CNV hotspots in 20 chimpanzees (Perry06) and 100 humans (Wong07), and integrate PSSM-based scoring for polymorphism classes (nonsynonymous, regulatory and structural) into a universal scoring method and webserver called Guame (http://snp.ucsd.edu/guame). This work demonstrates that any polymorphism can be represented using a PSSM and scored (including microRNAs, splicing, and CpG methylation) in a three-step process: (1) Classification of the polymorphism based on the molecular genomic change (SNP, indel, CNV), (2) selection of the appropriate PSSM library based on the known cellular function of the affected genomic sector (coding, regulatory, intronic), and (3) sub-class by functional change (amino acid substitution, premature stop codon, motif destruction, CNV doseage, alternative splicing). In addition to presenting the method and webserver, we demonstrate how the method works using polymorphisms, including a Gly->Ser nonsynonymous amino acid substitution in catestatin, a G->A SNP in the PNMT promoter which disrupts a mammalian-conserved G-rich Sp1 binding site, and a CNV hotspot in CCL3L1 correlated with HIV-1 infection.

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## 2717/F/Poster Board #329

**How should orthologous sequences be used to predict the impact of amino acid substitutions on protein function? An experimental test of substitutions in human MTHFR.** *N. Marini[1], P. Thomas[2], J. Rine[1].* 1) California Institute for Quantitative Biosciences, University of California, Berkeley, CA; 2) Evolutionary Systems Biology Group, SRI International, Menlo Park, CA.

Comparative sequence data can potentially provide two types of information useful for predicting the effect of a nonsynonymous coding variant in a human gene. Evolutionary conservation of an amino acid is indicative of negative selection against impaired variants. Conversely, the presence of a divergent amino acid at a homologous site in an orthologous protein is generally taken to be indicative of a functional variant fixed by neutral drift or positive selection. In practice, most sites in a group of related proteins are both partially conserved and partially divergent, yet little is known about how these two signals should be balanced in predictions. We examined this question by evaluating nonsynonymous substitutions in a prototypical enzyme, human methylenetetrahydrofolate reductase (MTHFR), in a cell-based assay based on yeast complementation. The results demonstrated that, as expected, substitutions in human MTHFR at sites that are conserved in orthologs from all domains of life result in an impaired enzyme, while fixed substitutions in very recently diverged sequencesincluding a five-site mutant that "resurrects" the human-chimp common ancestorresult in a functional enzyme. Assays of a number of substitutions at sites conserved for varying lengths of time indicated that, whenever conservation is observed among all known deuterostome orthologs (most recent common ancestor ~500 million years) amino acid substitutions are nearly always impairing, even though these substitutions have been fixed at that site in a more distant ortholog. When conservation is observed for shorter periods, nearly all substitutions we tested were functional, suggesting that at these sites amino acid conservation may be due not only to strict negative selection, but also insufficient opportunity to fix other functional variants. We conclude that for human MTHFR, orthologous genes from other deuterostome species are consistently informative for predicting the effects of amino acid substitutions, while more distant orthologs are not.

## 2718/F/Poster Board #330

**Transposases phylogeny outlying clasic taxonomy.** *J.A. Morales[1], E. Borrayo-Carbajal[2], S.E. Lopez-Ruiz[1].* 1) Computer Sciences, Universidad de Guadalajara, Mexico; 2) Molecular Medicine Division, CIBO, CMNO-IMSS, Mexico.

Background: Transposases have been extensively studied ever since Barbara McClintock described the transposition phenomena more than half a century ago. Transposases are proteins related with horizontal transport, where one domain recognizes its transposon sequence, another motif homodimerizes with other transposase bound to its palindrome, and the third domain cuts the sequence and "paste" it somewhere else on the same DNA chain, on other chain on the cell or even another cell or organism. Even though it has been discovered a great deal about transposition, many details still remain unknown. Traditional phylogenetic trees are built by main branching the different central conserved domain and posterior measurement of the distances between the rest of the protein or gene sequence. These trees usually rely upon taxonomy to evidence where those sequences are biasing or misinterpreted. In this work we present the preliminary results of the analysis for transposases phylogeny. Methods: Transposase nucleotide sequences were downloaded from KEGG database. Since this database is already curated and annotated, sequences were aligned and compared as is. Jukes-Cantor algorithm was applied for distance measuring and the phylogenetic tree was assembled by average method. Data mining and statistics were done using Matlab® (v7.7). Results: 27'152 sequences were downloaded from KEGG database from 612 bacterial organisms. To evaluate the relationship between any given member of the reported families we selected a sample of random entries from each organism. The resulting phylogenetic tree shows seven distinct branches clustering four different classes of *proteobacteria* (average distance: 0.52), two of *firmicutes* (avg. dist. 0.78), and *actinobacterias* (avg. dist. 0.64) respectively. When the distances for transposase protein family members were measured, there were no statistical significance for any specific branch distribution along the tree. Conclusion: Traditionally, protein families are clustered upon single main conserved domains, however when several different conserved domains occur in a protein, modularity combination may occur which shall be considered for the classification; and given the possibility of transposases for horizontal transport even between species, the resulting tree does not match the classic taxonomy.

## 2719/F/Poster Board #331

**MetaBioME: Exploring Commercially Useful Enzymes in Metagenomic Datasets.** *T.D. Taylor, V.K. Sharma, N. Kumar, T. Prakash.* MetaSystems Res Team, RIKEN Adv Sci Inst, Yokohama, Kanagawa, Japan.

Microbial enzymes have well known applications as biocatalysts in several industries like biotechnology, agriculture, medical, etc. However, only a few enzymes are currently employed for such commercial applications due to the limited number of sequenced microbes. In this scenario, metagenomic data provide a unique resource for discovering commercially useful enzymes (CUEs) from yet unidentified microbes belonging to complex microbial communities from diverse ecosystems. Therefore, the onslaught of metagenomic data from several worldwide projects provides a new unexplored ocean of genomic wealth which cannot only enhance the enzyme repertoire by the discovery of novel CUEs, but can also reveal better functional variants of the existing CUEs.

We curated a catalogue of ~500 CUEs using text mining of PubMed abstracts and publicly available information. We classified these CUEs into nine broad application categories based on their function. Further, in order to identify novel homologs of these CUEs, we used our in-house metagenomic analysis pipeline 'iMetaSys' to identify potential ORFs in all publicly available metagenomic datasets. We then developed the comprehensive Metagenomic BioMining Engine (MetaBioME) to bring all of this information together.

Using MetaBioME, we have already successfully identified several novel homologs to the known CUEs which can serve as leads for further experimental verification. MetaBioME is an intuitive search engine for accessing the manually curated data on CUEs stored in a relational database system, along with several options for identifying their homologs from metagenomic dataset and for validating the results. Further analysis of these potential CUEs may lead to the discovery of more efficient, less costly, and environmentally friendly alternatives to the current repertoire of medical and other important biocatalysts.

## 2720/F/Poster Board #332

**Genetic variation in Matrix Metalloproteinase-2, 9 in Patients with Osteonecrosis.** *C. Cho[1,2], H. Kim[1,2], K. Kim[3], K. Yoon[1,2].* 1) Department of Biochemistry and Molecular Biology (BK21 project); 2) Medical Research Center for Bioreaction to Reactive Oxygen Species and Biomedical Science Institute; 3) Department of OrthopaedicSurgery East-West Neo Medical Center, School of Medicine, Kyung Hee University, Seoul, Korea.

Osteonecrosis is a disease caused by reduced blood flow to bones in the joints. It is resulting from the temporary or permanent loss of the blood supply to an area of bone. With too little blood, the bone starts to die and may break down and bone collapses. If avascular necrosis involves the bones of a joint, it often leads to destruction of the joint articular surfaces. The disorder is characterized by progressive pain in the groin, mechanical failure of the subchondral bone, and degeneration of the hip joint. Matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as bone remodeling, resorption as well as osteoclast recruitment. Among them, MMP-2,-9 are assumed the development of osteonecrosis. In previous reports, the variations of MMP2 and MMP9 promoter have been researched. Interestingly, the -1306T and -735T haplotype displays an even lower promoter activity and mRNA expression compared with the haplotype consisting of only one T allele at the -1306 or -735 site, indicating an interactive effect of these two SNPs on MMP2 transcriptional function. We investigated that a genetic variant of the MMP-2,-9 determines the risk of osteonecrosis in Korean patients, using PCR, sequencing and TaqMan 5' allelic discrimination assay. Genomic DNA from 218 patients with nontraumatic FHON and 221 control subjects was analyzed for MMP-2(-1306C/T; rs243865, -735C/T; rs2285053) and MMP-9(-1562C/T; rs3918242, -90(CA)n; rs3222264) SNPs. The allele distribution of the -1306C/T in the patients (C=91.3%, T=8.7%), controls (C=89.8%, A=10.2%) and the -735C/T in the patients (C=71.6%, T=28.4%), controls (C=73.5%, T=26.5%) was detected. These findings suggest that MMP polymorphism may be a genetic risk factor in osteonecrosis.

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## 2721/F/Poster Board #333

**Relationship Between Femoral Head Osteonecrosis and Gene Polymorphism related to the Coagulation and Fibrinolytic Systmes.** *H. Kim[1,2], K. Kim[3], K. Yoon[1,2].* 1) Department of Biochemistry and Molecular Biology (BK21 project); 2) Medical Research Center for Bioreaction to Reactive Oxygen Species and Biomedical Science Institute; 3) Department of OrthopaedicSurgery East-West Neo Medical Center, School of Medicine, Kyung Hee University, Seoul, Korea.

Femoral head osteonecrosis (FHON) frequently leads to progressive collapse of the femoral head followed by a degenerative arthritis of the hip joint. Besides an increased bone marrow pressure with reduced blood supply, an enhanced coagulation has been considered. Plasminogen activator inhibitor type 1 (PAI-1) is the primary inhibitor of both tissue- and urinary-type plasminogen activators. A reduced plasma fibrinolytic activity, mainly attributable to increased plasma levels of PAI-1, has been associated with osteonecrosis. The human PAI-1 gene is mapped on chromosome 7q21.3-q22 and contains nine exons and eight introns. It has been reported that there are several variations within the PAI-1 gene. We investigated that a genetic variant of the PAI-1 determines the risk of osteonecrosis in Korean patients, using PCR, sequencing and TaqMan 5' allelic discrimination assay. Genomic DNA from 218 patients with nontraumatic FHON and 221 control subjects was analyzed for four SNPs (rs2227631, rs1799889, rs6092, rs11178) located at promoter, exon2 and 3'flanking region. The femoral head osteonecrosis patients had higher frequency of rs2227631 A allele and rs11178 C allele than controls (43.8% versus 36.7%, P=0.031; 55% versus 46.2%, P=0.008). We found that rs2227631 and rs11178 polymorphism in the PAI-1 was associated with an increased susceptibility of FHON in the Korean population. The results of this study suggest that coagulation abnormalities in the forms of PAI-1 polymorphism might play a role in FHON.

## 2722/F/Poster Board #334

**Distinct Regulatory Architectures Controlling Gene Expression in Embryogenesis and Post-natally.** *I. Aneas, F. Arimura, A. Blanski, M.A. Nobrega.* Department of Human Genetics, University of Chicago, Chicago, IL.

Developmental genes often rely on multiple widespread regulatory elements to regulate their fine spatial-temporal expression. While these genes are also expressed post-natally, nothing is known about the regulatory architecture coordinating their expression in adulthood. To address this, we characterized the regulatory architecture of TBX20, a gene involved in heart development, during embryogenesis and post-natally. Using a combination of bioinformatics, mouse and fish transgenic assays, we scanned 1,200,000 bp of genomic sequence that spans the Tbx20 locus and its intergenic regions. We identified 10 embryonic enhancers that, together, recapitulate TBX20 endogenous expression. Six of these tissue-specific enhancers drive expression in discrete and overlapping domains within the developing heart. In order to assay these enhancers in their endogenous genomic context, we engineered a 278-kb human BAC harboring all heart enhancers by recombining LacZ onto the TBX20 translation start site. BAC-transgenic mouse embryos showed LacZ expression corresponding to a sum of all individual enhancers identified. LacZ activity in the heart was also observed postnatally (P0) and 21 days after birth (P21). Deletion of the 6 enhancers from the BAC resulted in abrogation of LacZ expression in the embryonic heart, showing that those sequences are essential and sufficient to control TBX20 expression during development. Interestingly, a longitudinal analysis of mice harboring this enhancer-deletion BAC shows that by 21 days after birth lacZ expression is restored to the heart, indicating the presence of a different set of enhancers within this BAC control TBX20 expression in the adult heart. We scanned to saturation the 500 kb TBX20 locus using a reporter system in primary cardiomyocytes to uncover these adult-specific regulatory sequences. Our findings suggest that developmental genes are endowed with distinct sets of regulatory elements to orchestrate their embryonic and post-natal expression. Mutations in these elements may lead to vastly different phenotypic consequences, ranging from developmental defects to adult, late-onset diseases. These insights will be of importance in the follow-up of GWAS that have repeatedly pointed at noncoding sequences surrounding developmental genes as harboring variations associated with adult-onset diseases.

## 2723/F/Poster Board #335

**Comparative Genomics of the Primate Subtelomeric regions.** *Y. Kuroki[1], A. Toyoda[2], A. Fujiyama[1,2,3].* 1) Computational Systems Biology Research Group, RIKEN Advanced Science Institute, Yokohama, Kanagawa, Japan; 2) Comparative Genomics Laboratory, National Institute of Genetics, Mishima, Shizuoka, Japan; 3) Principles of Informatics Research Division, National Institute of Informatic, Chiyoda-ku, Tokyo, Japan.

Human subtelomeric regions have unique character as for the genome structure, in other words, these regions are curious that the subtelomeric region-specific repeats are highly duplicated among the most of human chromosomes and form a complex patchwork structure. It is known that some genes or gene families have identified in the human subtelomeres, however the functional and systematic analyses of the genes have not yet conducted. In addition, the characteristics of the genome structure, such as the gene contents, components of DNA elements, and copy number of the duplicated subtelomeric region-specific repeats, have not fully analyzed. To clarify the structural characterization of the primate subtelomeres, we carried out genome sequencing and comparative analysis of the human and chimpanzee subtelomeric regions. High quality genome sequence data provided us the exact aspect including the number of the subtelomere genes, the duplicated segments, and the repeat contents for the two chromosomes, chromosome 19 and 21. The genomic structure of subtelomere was highly diverged between the closely related two species, human and chimpanzee. We will present the systematic genome analysis of primate subtelomeres, and discuss about the biological meaning and evolutionary aspects of this regions.

## 2724/F/Poster Board #336

**Analysis of phylogenetically reconstructed mutational spectrum in human mitochondrial DNA.** *N. Volodko, N. Eltsov.* Dept Molecular and Cell Biology, Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russian Federation.

Mutations in mtDNA are extremely important in studies of human evolution. They play significant role in forensic investigations and are responsible for various clinical phenotypes. Therefore, analysis of mitochondrial genomes from patients and normal controls becomes increasingly common, which leads to rapid accumulation of complete mtDNA nucleotide sequences. In this study we analyzed distribution of mtDNA mutations in global phylogeny. All complete nucleotide sequences of human mtDNA (5475 mtDNAs) currently available in GenBank were extracted and their global mtDNA phylogeny was reconstructed using recently developed software tool mtPhyl v.2.010 (http://www.eltsov.org/mtphyl.aspx). As a result hotspots and "cold" regions were revealed. A total of 5740 segregating sites were identified of which 703 were in control region, 4168 in protein-coding genes and 869 in RNA genes. In the group of protein-coding mutations 2753 synonymous, 1406 nonsynonimous and 9 nonsense mutations were detected. The total number of homoplasic mutations (i.e. which occurred more than once in phylogeny) among the 5475 mtDNAs was 2958 (54% of segregating sites). This is greatly exceeds previous estimates (17%). The most common homoplasmic mutation in coding region was 709 in the 12S rRNA gene. It occurred 102 times in human mtDNA evolution. The most common nonsynonimous mutation was 5460 in the ND2 gene which occurred 52 times. We furthermore analyzed the relation between the number of homoplastic mutations and the number of DNA sequences studied. The distribution of mutations within protein-coding genes was explored and possible role of selection is discussed.

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## 2725/F/Poster Board #337

**Discovery and Reconstruction of Copy Number Variations.** *N. Furlotte[1], D. He[1], E. Eskin[1,2].* 1) Department of Computer Science, University of California Los Angeles, Los Angeles, CA; 2) Department of Human Genetics, University of California Los Angeles, Los Angeles, CA.

Structural variations such as copy number variations (CNVs) account for a large portion of human genetic variance. The accurate discovery and reconstruction of the genomic regions containing CNVs is important for the understanding of phenotypic variation. There exist array-based methods for discovering CNVs, but these methods are unable to determine the regions in copy number with high resolution. The rapidly decreasing cost associated with next generation sequencing technologies offers an alternative for finding CNVs. In this project, we describe a computational method that utilizes the properties of paired-end read sequences to identify CNVs. Our method first determines the genomic regions that likely contain CNVs by clustering discordant paired-end reads. Each potential CNV is assigned a confidence score and probabilities corresponding to the number of copies in the region. Once an estimate of the region has been obtained, we are then able to reconstruct the exact sequences making up the CNV using un-mapped reads, namely reads that span the junctions between copies and that do not match anywhere in the reference genome. Given the high coverage of next generation sequencing technologies, all junctions are covered by un-mapped reads with high probability. Copies that differ from the original reference region will do so by having different starting and ending positions, which will result in different prefixes and suffixes for these copies. Therefore, by using un-mapped reads we are able to recover the exact start and end positions of each copy within the original CNV region by mapping their prefixes and suffixes back to the reference sequence. Candidate positions can be identified when both prefix and suffix match the CNV region in the reference sequence. SNPs in different copies can then be used to recover the order of these copies. For experimental evaluation, we tested our method on both simulated random sequences and real genomic sequences. Specifically, we generate real genomic sequences by randomly sampling and concatenating large sub-sequences from an actual chromosome. This process maintains the repeat structure found in real genomes, which does not exist in completely random sequence. CNVs of differing lengths were inserted at various spacings then simulated reads are created and mapped with a widely used mapping program. The results indicate that our method is able to re-construct the CNVs accurately for both datasets.

## 2726/F/Poster Board #338

**CNV analysis of systemic-onset juvenile idiopathic arthritis.** *H. Tadaki[1,2], T. Saistu[2], H. Sakai[2], N. Miyake[2], S. Yokota[1], N. Mastumoto[2].* 1) Pediatrics, Yokohama City University, Yokohama, Japan; 2) Human Genetics, Yokohama City University, Yokohama, Japan.

Background: Systemic-onset juvenile idiopathic arthritis (JIA) is a subtype of chronic childhood arthritis, manifested by spiking fever, erythematous skin rash, pericarditis, and hepatosplenomegaly. Approximately 7% of these children are fatal because of the disease transition to macrophage activation syndrome (MAF). However, the cause of systemic JIA remains poorly defined. Although IL-6 and MIF polymorphism are reported to be associated with JIA, there are no studies analyzing copy number variations (CNVs) in systemic JIA. Method: A total of 50 patients with systemic JIA who had disease refractory to conventional treatment and were given tocilizumab were studied. We used two different commercially available SNP array platforms, the Affymetrix Genechip Human Mapping 500K array (23 patients) and the Genome-wide Human SNP array 6.0 (27 patients) , to detect CNVs. When chromosomal structural abnormalities were detected, further analysis was done using quantitative PCR, and/or fluorescence *in situ* hybridization (FISH). Result: We found 6 deletions: 2 larger than 1 Mb in size, 2 from 100 kb to 500 kb, and 2 smaller than 100 kb. The largest deletion detected was 5.5 Mb. FISH demonstrated that the mother also carried the 5.5-Mb deletion. Thus the deletion containing approximately 50 genes is unlikely to be pathogenic. Six duplications were also detected within the size from 100 kb to 1 Mb. All of the duplications were inherited from either of the parents. Conclusion: Although we have performed CNVs analysis using high density microarrays in systemic JIA, no significant pathogenic CNVs have been detected so far. Further study is absolutely necessary to identify the gene for systemic JIA.

## 2727/F/Poster Board #339

**Copy number variation in candidate regions in extended families with autism spectrum disorder.** *D. Salyakina, H. Cukier, D.Q. Ma, A. Griswold, I. Konidari, J. Jaworski, P. Whitehead, H.H. Wright, R. Abramson, M.L. Cuccaro, J.R. Gilbert, M.A. Pericak-Vance.* MIHG, Univ Miami Miller Sch Med, Miami, FL.

Recent studies have identified several copy number variations (CNVs) that are associated with increased risk of autism spectrum disorder (ASD). Most of these findings describe rare CNVs and have not been replicated by other research groups. Recently Glessner et al.(Nature, 2009) reported novel and known CNV candidate regions for ASD. Their positive findings were validated in an independent cohort of 1336 ASD cases and 1110 controls and of European ancestry. We have conducted analysis of these validated candidate regions in a cohort of 69 extended ASD families (3-6 affected ASD individuals per family) with 117 individuals diagnosed with ASD and 354 healthy relatives. We examined if any of these CNVs can account for any of the familial ASD. We tested the hypothesis that a consistent CNV contributing to ASD risk is shared in the affecteds in the family. Samples were genotyped using the Illumina Human 1M Beadchip. We used the PennCNV algorithm for CNV calling in our cohort. For the 26 candidate regions listed in the paper, we identified CNVs in 16 of these regions. 14 of them were inherited and two could be de novo: one individual had a deletion on chromosome 16p11.2 and another had a duplication on chromosome 4q31.21. Both individuals were diagnosed with ASD. Both CNVs did not segregate among relative individuals with ASD in these two families. The most CNV rich region was on chromosome 15q11-13. 80 individuals (healthy and affected) had various deletions and duplications with different breakpoints in this locus; however, no segregation in affected individuals within families could be shown. One locus on chromosome 3q26.31, including the neuroligin 1 (NLGN1) gene, had a duplication overrepresented in ASD affecteds. In total, there were 10 individuals with ASD (8.5%) and 19 healthy relatives (5.3%) that have the duplication. 15 of 19 healthy carriers of this duplication were female and 8 of 10 affected individuals with the duplication were male. Most affected relatives (62%) of the carriers of the CNV on chromosome 3q26.31 were not carriers, suggesting that this CNV probably does not contribute to ASD risk. CNVs in remaining candidate regions also did not show segregation in affecteds with ASD. In conclusion, no one candidate region mentioned by Glessner et al.(Nature, 2009) showed segregation in multiple affected individuals within our families. These CNVs probably do not contribute to risk of ASD.

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## 2728/F/Poster Board #340

**Utilisation of a Whole-Genome Approach to Characterize a Novel Immunodeficiency Disorder and Implicate IL25.** *L.R. Griffiths[1,2], M.R. Green[1,2], M.K. Gandhi[2,3], J. Peake[4].* 1) Genomics Res Centre, Griffith Institute for Health and Medical Research, Griffith University Gold Coast, Southport, Australia; 2) Griffith Medical Research College, GU-Queensland Institute of Medical Research, Herston, Brisbane, Australia; 3) Queensland Institute of Medical Research (QIMR), Clinical Immunohaematology Laboratory, Herston, Brisbane, Australia; 4) Queensland Pediatic Immunology and Allergy Service, Royal Children's Hospital, Herston, Brisbane, Australia.

Primary immunodeficiency disorders (PIDs) affect ~2 people in 100,000 and predispose affected individuals to recurrent infections and the development of other disorders such as lymphoma. Diagnosis of PIDs uses case history information, immunological interrogation of cellular repertoires and immunoglobulin isotypes, as well as genetic tests to detect mutations. However, when an individual does not fit the immunological characteristics of defined disorders, and genetic tests for common immunodeficiency syndromes yield no diagnosis, there is no protocol for characterization of the disorder. In this study, we have used genome-wide SNP and gene expression microarrays to provide insight into the etiology of one such disorder. DNA copy number analysis using Affymetrix 250K Sty SNP arrays revealed quadraploidy of chromosome 14q11.2 mapping over an area of approximately 280Kbp. This amplification was confirmed by MassArray-based DNA copy number analysis of SNPs within the candidate region. The genetic locus harbouring the copy number alteration is rich in coding sequences but only two of these genes had a role in lymphocyte signalling - T-cell Receptor Delta-Alpha (TRD$\alpha$) and Interleukin-25 (IL25). From microarray data, TRD$\alpha$ showed down-regulation, however IL25 showed increased expression. Using qPCR analysis, IL25 exhibited a 2.49 fold increased expression compared to control lymphocytes following anti-CD3 T-cell activation. Microarray data supported the hypothesis of an aberrant Th2 switch resulting from the IL25 over-expression, with down regulation of expression of TBX21 (0.24 fold) and IRF2 (0.28 fold) which induce the expression of Th1-associated genes. There was also significant up-regulation of genes associated with a Th2 phenotype, as well as down-regulation of genes associated with a Th1 phenotype. Overall our results thus identified hyperploidy of a genetic region centromeric to 14q11.2, which causes over-production of IL25 in response to T-cell stimulation, and is associated with a gene expression pattern indicative of a Th2 switch. Recurrent infections associated with this disorder may therefore be associated with the inadequate clearance of pathogens that are normally addressed with Th1 responses. This disorder may provide further insight into the function of IL25 and the regulation of Th1 and Th2 responses. In conclusion, we have used a whole-genome approach to characterize the molecular etiology of a novel immunodeficiency disorder.

## 2729/F/Poster Board #341

**Detection of copy number events in patients with eye development disorders using SNP arrays and multiple detection algorithms.** *I. Ragoussis[1], L. Winchester[1], S. Ugur[2], N. Ragge[2].* 1) Genomics, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, UK; 2) Dept of Physiology, Anatomy and Genetics, Le Gros Clark Building, South Parks Rd, University of Oxford, Oxford, OX1 3QX, UK.

Human developmental eye anomalies, including anophthalmia, microphthalmia and coloboma are responsible for around 25% of all childhood blindness. Our recent research has demonstrated that these abnormalities can be caused by disruption to single genes not only through mutations, but also through whole gene or exon deletions, detected through techniques such as MLPA (e.g. SOX2 OTX2). Surprisingly, we found the prevalence of whole gene deletions was almost as great as mutations. Here we used Illumina 1M Duo arrays to assay 40 patient trios with developmental eye disorders, mainly anophthalmia and microphthalmia. We investigated multiple analysis methods for using SNP data to detect copy number (CN) events and describe the use of several statistical models for CN detection in germline samples. We examined a number of algorithms designed to detect CN changes through the use of signal intensity data and consider methods to evaluate the changes found. We applied these algorithms to a set of well characterised CEPH samples to give an indication of detection accuracy and success of CN change prediction on Affymetrix and Illumina data. We found an overlap of between 20-49% between events detected by algorithm and previously published data covering both copy number variations and polymorphisms. We applied the QuantiSNP and PennCNV algorithms to the patient dataset. From our QuantiSNP dataset we found a total of 18,229 events, 48.7% of these were in genes found in the RefSeq sequence collection. We included a patient with a known 6p deletion in the TFAP2A gene as a control which was successfully identified by both PennCNV and QuantiSNP detection algorithms. We checked our data for events in genes known to be related to eye developmental disorders and found events in 26 genes of interest, including deletions of MAF and PAX2. We found a large novel deletion on the X chromosome in a child, disrupting 35 genes including HCCS which has been linked to microphthalmia. After filtering of our detected event list, we found a series of 52 novel events in genes not previously linked to eye developmental disorders, but some of which are expressed in retina or implicated in axon guidance. The latter provide excellent candidate genes for further investigation. We used the secondary set of detected events from PennCNV to confirm events of interest and clarify breakpoints. Finally coding SNPs were analysed for the prevalence of rare alleles in candidate genes.

## 2730/F/Poster Board #342

**CNstream: A method for the identification and genotyping of copy number polymorphisms using Illumina microarrays.** *A. Alonso, S. Marsal, A. Julià.* Grup Recerca Reumatologia, Institut de Recerca Hospital Vall d'Hebron, Barcelona, Barcelona, Spain.

Background: Copy Number Polymorphisms (copy number variants with a prevalence >1%), are actually drawing much interest as new genetic variations associated to disease risk. For this reason, Illumina Beadchip arrays, originally designed for SNP genotyping, are now starting to be used to extract copy number information. However, one of the present challenges is to develop algorithms that are able to deal with the reduced sensibility of this type of technology. Current methods available for Illumina CNP discovery and quantification are based on either a per-sample analysis of LogRatio and B-Allele Frequency parameters (i.e. PennCNV) or on a multiple-sample analysis of the two channel intensities (i.e. SCIMM - only deletions). Methods: We propose a new method specific for Illumina arrays that can identify deletions as well as amplifications using a multiple-sample approach. CNstream uses Illumina two-channel intensities as input data and the markers are successively analyzed starting with a genotyping step. This genotype information is used to robustly initialize an algorithm that indepently analyzes each intensity channel and assigns to each sample two scores that are proportional to the number of copies. In this algorithm, a Gaussian Mixture Model is fitted to the intensity data and the final scores are computed based on the likelihood of the data with respect to each GMM component. Finally, in order to avoid false positives, CNstream applies a filtering step over the scores of each sample that takes into account the distance between markers. Another strength of CNstream is that it includes two normalization steps (per-plate and per-genotype) which highly contribute to reduce the technical variability of the data and standardize the distribution of all the markers. Results: We have compared CNstream against PennCNV over a set of 488 samples genotyped with Illumina HumanHap300. PennCNV identified a total of 73 CNP regions from which 55 (75%) were also found by CNstream. Importantly, CNstream was able to identify 30 CNP regions with frequencies up to 9% that PennCNV was not able to detect, and that are located in previously described CNV regions (Database of Genomic Variants). We propose CNstream as a useful and powerful method for the identification and quantification of CNPs in Genome Wide Association Studies. CNstream will be available as an open-source R package.

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## 2731/F/Poster Board #343

**Copy Number Variations (CNVs) in Biliary Atresia.** *M.J. Braskett[1], R.S. Venick[1], S.V. McDiarmid[1, 2], J.H. Vargas[1], D.G. Farmer[2], R.W. Busuttil[2], E.R.B. McCabe[1, 3, 4], S.A. McGhee[1].* 1) Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA; 2) Surgery, Dumont-UCLA Transplant Center, Los Angeles, CA; 3) Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA; 4) Bioengineering, Henry Samueli School of Engineering and Applied Science, Los Angeles, CA.

Rationale: Biliary atresia, the leading cause for pediatric liver transplantation, usually presents as an isolated finding, although up to 10% of affected individuals have a constellation of other anomalies. Reported observations describe copy number variations (CNVs) in a small subset of patients with multiple congenital anomalies. Specifically, karyotype analysis has been used to identify duplications at 22q11 associated with cat eye syndrome and 10q11.2q22.3 associated with multiple congenital anomalies, der (22) t(11; 22)(q23; q11) associated with the supernumerary der (22) syndrome, as well as full trisomies of 18 and 21. We hypothesized that microarray analysis may increase the detection of CNVs at these regions in individuals with isolated biliary atresia. However, as CNVs are also found in healthy individuals, controlled studies are warranted to investigate this hypothesis. Methods: A trio was analyzed as an index case study. Subsequently, five case subjects with biliary atresia were genotyped using the 6.0 Affymetrix platform, and data for 10 control subjects was obtained from the HapMap project. Cases did not have clinical manifestations suggestive of chromosomal abnormalities. Analysis was performed using R with the Oligo and DNAcopy packages. CRLMM was used to normalize array data and calculate intensity ratios. We modified the CRLMM algorithm to permit estimates of CNVs. Results: No shared CNVs were noted among cases except for 4 polymorphisms on chromosomes 1, 14 and 22. The index case demonstrated a de novo 791kb duplication at 10q11.22 consistent with a description in the literature, but this finding was not reproduced in the other cases. Moreover, other CNVs found in case subjects did not correspond to regions described in reported cases. Data for case subjects did not demonstrate an increased prevalence of CNVs on 10, 11, 18, 21 and/or 22. Conclusion: The role of CNVs in the pathology of biliary atresia remains unclear. One patient was identified that shared duplication on 10q11.22 with a previously described case. Of note, unlike the reported case, this individual lacks syndromic features. This suggests that high density oligonucleotide mapping array data may identify CNVs associated with biliary atresia in non-syndromic patients. These findings may indicate considerable genetic heterogeneity for biliary atresia.

## 2732/F/Poster Board #344

**Differential Detection of Copy Number Variation of Complement Component C4 Family Using TaqMan Copy Number Assays.** *I.R.L. Casuga[1], A.J. Broomer[1], F. Wang[1], N. Majumdar[1], Y. Wang[1], R. Charlab[1], T.H. Karlsen[2], A. Franke[3], C. Chen[1], K. Li[1].* 1) Applied Biosystems part of Life Technologies, Foster City, CA; 2) Medical Department, Rikshospitalet University Hospital, Oslo, Norway; 3) Institute for Clinical Molecular Biology, Kiel, Germany.

Copy number variation (CNV) is a widespread phenomenon in the human genome. CNVs are part of the normal variability among healthy individuals, but some CNVs may also increase disease susceptibility. For instance, CCL3L1 CNVs are associated with susceptibility of HIV infection. The ability to detect accurate copy number changes is important in copy number analysis, and is especially critical for isoforms within a gene family such as the complement system component C4. Located within the MHC at chromosome 6p21, the C4 gene family is part of this highly polymorphic region associated with numerous autoimmune diseases. Although they differ in only 5 nucleotides, C4A copy number but not C4B is associated with the susceptibility of systemic lupus erythematosus (SLE). In addition, the C4 gene has 2 isoforms (C4L and C4S) of different length due to the insertion of the retrovirus HERV-K(C4). Differential detection of copy number for different C4 isoforms is especially challenging but critical in understanding their association to diseases. To differentially detect copy number differences among C4 isoforms, we designed TaqMan Copy Number Assays to specifically target each isoform (C4A, C4B, C4L, & C4S). The assays were tested first on an internal panel of 94 gDNA samples from African American and Caucasian populations. Distinct copy number profiles and population differences were observed. To test accuracy of the assays, positive samples of known copy number were also tested; the results show TaqMan assays are 100% consistent with the expected copy number. To measure C4 copy number frequency and further examine the population differences, we tested the assays on the HapMap panel of 270 individuals from 4 different populations. The copy number profiles among the HapMap population are strikingly different for each C4 isoform, and copy number frequency in different populations is statistically significant. This is the first time copy number profile population differences in C4 isoforms on HapMap samples have been reported. This study indicates population difference in copy number profiles for C4 isoforms should be considered in any disease association studies and understanding the difference may help explain the population difference in disease susceptibility. The study also demonstrates that TaqMan Copy Number Assays can differentially detect challenging targets like closely related gene family members.

## 2733/F/Poster Board #377

**Segmental copy number variation shapes tissue transcriptomes.** *E. Chaignat[1], C.N. Henrichsen[1], E. Ait Yahya-Graison[1], N. Vinckenbosch[1], S. Zollner[2], F. Schutz[1], M. Ruedi[3], H. Kaessmann[1], A. Reymond[1].* 1) CIG, University of Lausanne, Lausanne, Vaud, Switzerland; 2) University of Michigan, Ann Arbor, MI; 3) Natural History Museum, Geneva, Switzerland.

Copy number variation (CNV) of DNA segments has recently been identified as a major source of genetic diversity, but a comprehensive understanding of the phenotypic effect of this type of variation is only beginning to emerge. We have generated an extensive map of CNV in wild mice and classical inbred strains. Copy number variable regions cover a total of ~340 megabases (~11%) of their autosomal genome. Genome-wide expression data from 6 major organs and 4 developmental times in six different strains reveal that expression levels of genes within CNVs positively or negatively correlate with copy number changes in approximatively 35 and 15% of the cases, respectively. Consistently, CNV genes are significantly overrepresented amongst the genes that differ in their spatiotemporal pattern of expression between strains. Our experiments also show that CNVs influence the expression of genes in their vicinity - an effect that extends up to half a megabase. These controls over expression are effective throughout mouse development, however some genes appear to be under compensatory loops at specific time point. Interestingly, genes within CNVs show lower expression levels and more specific spatial expression patterns than genes mapping elsewhere in the genome. Furthermore, genes expressed in the brain are significantly underrepresented in CNVs compared to genes with expression in other tissues, suggesting differential selective constraint on copy number changes of genes expressed in different tissues. Our study provides initial evidence that CNVs shape tissue transcriptomes on a global scale and thus represent a significant source for within-species phenotypic variation.

## 2734/F/Poster Board #378

**Genome-wide array scan identified several Copy Number Variants loci associated with adolescent idiopathic scoliosis (AIS).** *R. Chettier, L. Nelson, J.W. Ogilvie, R.A. Macina, K. Ward.* Axial Biotech, Salt Lake City, UT.

Developmental disorders are often associated with genetic variants. Copy number variations (CNVs) are recently documented micro DNA insertions and deletions that may be ten times more frequent than point mutations. Many believe that CNVs may be more likely to cause disease that point to frequently benign mutations. We performed a genome wide scan to find CNVs associated with Adolescent Idiopathic Scoliosis (AIS). A total of 879 Caucasian individuals with severe AIS and 1486 Caucasian controls were evaluated for CNVs using the Affymetrix 6.0 HUSNP array. After the implementation of quality filters the data were quantile normalized. Copy Number analysis was performed using Helix Tree (Golden Helix, Bozeman, MT). The copy number segments were determined using the Golden Helix's Univariate Segmentation Algorithm. Statistically different segments were extracted using mean Log2 ratio intensity for that segment to highlight deletions, neutrals and duplications. We then performed association analysis on those segments. A p-value of <10-7 was considered evidence of significance. We found 143 significant segments/regions associated with idiopathic scoliosis. 94 of those regions showed gains of copy while 49 had deletions. Sixty-three of these significant regions map to known genes. Biological functions of the genes identified reveal complex groups associated to embryonic development, nervous system development and function, and tissue development. These groups present an extensive overlap with the gene biological function groups that were generated using associated Single Nucleotide Polymorphism (SNP) data from the same set of individuals. For the first time we show significant copy number loss or gain in several genomic regions associated to severe scoliosis patients as compared to a control population. We are testing CNVs in mild patients to determine whether they further improve the performance of the current AIS prognostic Test (AIS-PT).The identification of novel/rare CNVs in severe cases of AIS could not only lead to the enhancement of the current AIS-PT but could also help us identify specific biological pathways that cause AIS and/or accelerate AIS progression.

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## 2735/F/Poster Board #379

**Whole genome distribution and ethnic differentiation of copy number variation in Caucasian and Asian populations.** *H. Deng[1,2,3,4], J. Li[1], T. Yang[1,2], L. Wang[1,2], H. Yan[1,2], Y. Zhang[2], Y. Guo[2], F. Pan[2], Z. Zhang[1,2], Y. Peng[2], Q. Zhou[2], L. He[2], X. Zhu[2], H. Deng[1], S. Levy[5], C. Papasian[1], B. Drees[1], J. Hamilton[1], R. Recker[6], J. Cheng[7].* 1) School of Medicine, Univ Missouri, Kansas City, Kansas City, MO; 2) The Key Laboratory of Biomedical Information Engineering of Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, Shanxi 710049, P R China; 3) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P R China; 4) College of Life Sciences and Bioengineering, Beijing Jiaotong University, Haidian District, Beijing 100044, P R China; 5) Vanderbilt Microarray Shared Resource, Vanderbilt University, Nashville, TN 37232, USA; 6) Osteoporosis Research Center, Creighton University, Omaha, NE 68131, USA; 7) National Engineering Research Center for Beijing Biochip Technology, 18 life Science Parkway, Changping District, Beijing 102206, P.R.China.

Although copy number variation (CNV) has recently received much attention as a form of structure variation within the human genome, knowledge is still inadequate on fundamental CNV characteristics such as occurrence rate, genomic distribution and ethnic differentiation. In the present study, we used the Affymetrix GeneChip® Mapping 500K Array to discover and characterize CNVs in the human genome and to study ethnic differences of CNVs between Caucasians and Asians. Three thousand and nineteen CNVs, including 2381 CNVs in autosomes and 638 CNVs in X chromosome, from 985 Caucasian and 692 Asian individuals were identified, with a mean length of 296 kb. Among these CNVs, 190 had frequencies greater than 1% in at least one ethnic group, and 109 showed significant ethnic differences in frequencies ($p<0.01$). After merging overlapping CNVs, 1135 copy number variation regions (CNVRs), covering approximately 439 Mb (14.3%) of the human genome, were obtained. Our findings of ethnic differentiation of CNVs, along with the newly constructed CNV genomic map, extend our knowledge on the structural variation in the human genome and may furnish a basis for understanding the genomic differentiation of complex traits across ethnic groups.

## 2736/F/Poster Board #380

**Characterization of CNV Detection in Autism Genomes Using Next-Generation Sequencing, Oligo aCGH, and SNP Microarrays.** *B. Doan[1], C. Stewart[2], A. O'Connor[1], T. Turner[1], G. Marth[2], A. Chakravarti[1].* 1) Inst Gen Med, Johns Hopkins Med, Baltimore, MD; 2) Dept of Biology, Boston College, Boston, MD.

To improve our ability to identify genomic regions sensitive to dosage, we have focused on the study of 2 Autism genomes across multiple technological platforms. Two probands were selected with validated regions of duplication (SNRPN) or deletion (16p11.2). Whole genome sequencing data was generated using AB SOLiD technology. To assess the CNV data quality of next-generation sequencing compared to existing technologies, we generated data on the probands and their parents on three additional platforms: NimbleGen 2.1M (N2m) and Agilent 1M (A1m) oligo aCGH arrays, and Affymetrix SNP 6.0 (Affy6) microarrays. Data for each individual were processed for CNV discovery by: existing technology-provided software, Bioconductor tools, and newly developed algorithms. AB pair-end reads were analyzed with our CNV detection tool, Spanner. Because such reads are susceptible to false negatives in regions of genomic repeats, a complementary algorithm focusing on read depth was also developed. Segmentation algorithms and hidden Markov models were applied to the oligo aCGH and SNP microarray based data, respectively. As expected, sequencing data identified the most variants, with a total of ~1400 identified in both probands, compared to only 429 (A1m), 552 (N2m), and 550 (Affy6) on the arrays. Only 75 of the regions, including SNRPN and 16p11.2, were found with all technologies, and ~20% of the variants identified by sequencing overlapped the 372 CNVs previously identified (ASRD) for autism spectrum disorders. One heterozygous deletion of ~1kb (at position 51.08Mb) on chromosome 2 was identified in both sequenced genomes, overlapping NRXN1. This deletion was further validated and replicated by PCR, and found to have a frequency of 10.8% in the parents of 419 autistic trios. Although none of the array probes overlapped this deletion, a heterozygous deletion was found nearby in only one individual across the array data (at position 52.6Mb, with a deletion of size ~25kb in Affy6, ~32kb in A1m, and ~1kb in N1m). Such preliminary results suggest that the overlap among all the technologies is small, and that sequencing is able to identify additional CNVs at a greater resolution than the existing technologies. With a more detailed comparison, we hope to provide a useful map of the level of genomic variation that can characterize diseased individuals and better understand the limits of technologies to discover such variations.

## 2737/F/Poster Board #381

**A multilevel hidden Markov model of DNA copy number variation.** *S.W. Erickson[1,2].* 1) Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR; 2) Department of Biostatistics, University of Arkansas for Medical Sciences, Little Rock, AR.

DNA copy number variation (CNV) has received increasing attention over the past several years, and multiple studies have revealed both common inherited variants and unique de novo variants in healthy, as well as diseased, subjects. CNVs have been associated with several disease phenotypes, as well as with expression quantitative trait loci (eQTLs). Most existing methodologies for inferring copy number from microarray data have focused on analyzing arrays one at a time or, if analyzing multiple arrays, on a post-hoc basis. A multilevel hidden Markov model (mHMM) of CNV, however, simultaneously accounts for both the correlation of CNV state at different loci within an individual (i.e. along chromosomes) as well as between individuals. This is accomplished by distinguishing between common, or shared, variants and de novo, or unique, variants. Common variants are usually shared by multiple, but not all, subjects. Modeling common variants across multiple subjects allows for flexible and coherent inferential results, such as heritibility or association with case/control status. I will introduce a general inferential framework, examine computational and inferential challenges, and present results on simulated and actual data.

## 2738/F/Poster Board #382

**Neuronal Calcium Ras Signaling in Schizophrenia Impacted by Copy Number Variation.** *J.T. Glessner[1], K. Wang[1], C.E. Kim[1], C. Hou[1], J.P. Bradfield[1], H. Zhang[1], P.M.A. Sleiman[1], M. Imielinski[1], E.C. Frackelton[1], R. Chiavacci[1], K. Annaiah[1], K. Thomas[1], F.G. Otieno[1], J. Doran[1], M. Garris[1], J.H. Flory[1], S.F.A. Grant[1], R.E. Gur[2], H. Hakonarson[1,3].* 1) Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Psychiatry, University of Pennsylvania, Philadelphia, PA; 3) Pediatrics/Genetics, Children's Hospital of Philadelphia, Philadelphia, PA.

Schizophrenia is a late adolescence onset psychiatric disease typically characterized by delusions, hallucinations and thought disturbances. To identify CNVs increasing risk of schizophrenia, we performed a whole-genome CNV study on a cohort of 912 schizophrenia cases and 2,000 healthy children of European ancestry who were genotyped with 800,000 SNP probes and 800,000 CN probes. Positive findings were evaluated in an independent cohort of 644 schizophrenia cases and 1,684 controls of European ancestry. *KCNMB4* and *CACNA1B*, calcium signaling genes responsible for neuronal excitation, were deleted in 20 cases in our discovery cohort and 7 cases in the replication cohort which was overrepresented in comparison to controls ($P=2.74x10^{-4}$). In addition, *RET* and *RIT2*, ras related genes important for neural crest development, were found significantly impacted by CNV. RET deletion was exclusive to 8 cases ($P=1.46x10^{-3}$) and RIT2 deletions were overrepresented common variant CNVs in cases ($P=2.61x10^{-4}$). Although these variants may be individually rare, they target genes involved in calcium dependent cascades and Ras neuronal signaling which is involved in most aspects of normal brain function. Our results indicate that variations involving calcium Ras gene networks of the brain contribute to the genetic susceptibility of schizophrenia.

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## 2739/F/Poster Board #383

**Genome-wide analysis of copy number alterations in colorectal cancer.**
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Genomic alterations such as amplifications and deletions play important roles in the pathogenesis of human cancer. In the current study we performed a genome-wide analysis of copy number alterations (CNAs) in colon tumors using two different single nucleotide polymorphism (SNP) genotyping platforms, the Affymetrix Genome-Wide Human SNP Array 6.0 and the Illumina Human 1M-Duo BeadChip. DNA derived from 19 frozen colon tumors were analyzed by Affymetrix arrays containing 1.8 million probes, including 906,600 SNP probes and more than 946,000 invariant probes for detection of copy number changes, and 8 tumors were analyzed by Illumina arrays containing 1.1 million SNPs. Eight of the samples were run on both platforms. To determine whether the detected alterations were somatic or constitutional, we analyzed 8 matched genomic DNA samples from normal tissues of the same patients. Copy number changes were identified using PennCNV software. We compared our results with those from two previous studies of genome-wide copy number changes in colon tumors using less dense arrays (Leary et al. 2008, Ji et al. 2006), and found many CNAs in regions previously reported, including amplifications in regions containing the genes *MYC*, *EGFR*, and *IRS2*, as well as deletions in regions containing the genes *MAP2K4*, *SMAD2*, and *SMAD3*. We identified 8,037 alterations (total CNA size 1.9 Gb) in the tumor samples (7460 amplifications and 577 deletions) compared to 224 alterations (total CNA size 11.2 Mb) in the matched genomic DNA samples (103 amplifications and 121 deletions). The mean number of CNAs per tumor sample was 1,010 (median = 696). The average CNA size was significantly larger in tumor samples (mean=245,036 kb, median= 119,785 kb) than genomic controls (mean=50,342 kb, median= 15,634 kb, p = 0.0000018). Additionally, we detected alterations as small as 173 kb in tumors. Tumor samples had a greater number of amplified copies (more than 3 copies, n=20) as well as homozygous deletions (zero copies, n=30) than genomic controls (more than 3 copies, n=4 and zero copies, n=8). We identified several previously unreported CNAs that contain genes with known oncogenic properties such as *CDK8* and *PIK3CA*, as well as amplification of a region harboring *KRAS* in a single tumor without a *KRAS* mutation. Our study demonstrates that SNP arrays can detect chromosomal alterations at a high resolution and that these CNAs point towards candidate genes for further study.

## 2740/F/Poster Board #384

**Screening for gene deletions using the GenomeLab GeXP technology.**
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Large genomic duplications and deletions have been recognized as pathogenic mutations for many years. These types of mutations are thought to represent about 5% of all reported mutations. Therefore the determination of gene dosage becomes very important in clinical medicine. One well established method for the detection of deletions in single genes is the multiplex ligation-dependent probe amplification (MLPA). However there are a lot of genes with clinical relevance where no MLPA-Kit is available for testing or the kits are not detecting all exons. In this study we adopted the GenomeLab Gene Expression Profiling (GeXP) technology for the detection of copy number variations in the human genome. The GeXP technology was originally developed for gene expression analysis and can now also be used for the detection of deletions and duplications at genomic DNA level. The GeXP method uses a universal priming strategy to overcome potential bias in amplified targets that are typically associated with other types of multiplexed assays. For validation of the GeXP method 20 patients where tested, of which 6 were known to have copy number variations of the low-density lipoprotein receptor (LDLR) due to MLPA analysis. Validation samples included deletions and duplications of several exons of the LDL receptor as well as deletions of single exons. The results showed that the GeXP method is a highly efficient technique to detect copy number variations of genes or of single exons. All deletions or duplications found with an MLPA analysis could also be detected with the GeXP method. In contrast to MLPA, the setup for a GeXP assay can be easily performed in any molecular genetics laboratory and all exons of the target gene can be included with no limitations. Data presented here demonstrate that the GeXP method is a new technique for copy number quantification at genomic DNA level.

## 2741/F/Poster Board #385

**Genome Wide Copy Number Variation Study Associates Metabotropic Glutamate Receptor Genes with Attention Deficit Hyperactivity Disorder.**
*H. Hakonarson[1,2], J.T. Glessner[1], K. Wang[1], X. Gai[3], N. Takahashi[4], P.M.A. Sleiman[1], J.H. Flory[1], H. Zhang[1], C.E. Kim[1], J.P. Bradfield[1], M. Imielinski[1], C. Rabin[5], P. Shaw[5], T.H. Shaikh[6,7], S.F.A. Grant[1,6,7], J.D. Buxbaum[4], J.L. Rapoport[5], M. Devoto[6,7,8,9], P.S. White[3,7,10], J. Elia[11,12].* 1) Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Pediatrics/Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 3) Center for Biomedical Informatics, The Children's Hospital of Philadelphia, Philadelphia, PA; 4) Laboratory of Molecular Neuropsychiatry, Department of Psychiatry, Mount Sinai School of Medicine, New York, NY; 5) Child Psychiatry Branch, National Institute of Mental Health, Bethesda, MD; 6) Division of Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA; 7) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA; 8) Dipartimento di Medicina Sperimentale, University La Sapienza, Rome, Italy; 9) Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA; 10) Division of Oncology, The Children's Hospital of Philadelphia, Philadelphia, PA; 11) Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA; 12) Department of Child and Adolescent Psychiatry, The Children's Hospital of Philadelphia, Philadelphia, PA.

Attention-Deficit, Hyperactivity Disorder (ADHD) is a common, heritable neuropsychiatric disorder of unknown etiology. Recently, we identified an enrichment of rare variants in genes involved in learning, behavior, synaptic transmission and central nervous system development in autism, suggesting that rare inherited structural variants play an important role in the etiology of ADHD. In an attempt to comprehensively identify CNVs conferring susceptibility to ADHD, we performed a whole-genome CNV study on a cohort of 556 ADHD cases and 2,118 healthy children of European ancestry who were genotyped with 550,000 SNP markers. Positive findings were evaluated in an independent cohort of 770 ADHD cases and 1,987 controls of European ancestry. GRM5 (glutamate receptor, metabotropic 5) was deleted in eight cases total and deletions were not observed in controls (p=1.24x10-5). In addition, DPP6 was duplicated in five cases and GRM7 was deleted in 3 cases, both with a control frequency of zero. All variants were experimentally validated using quantitative PCR. Although these variants may be individually rare, they target genes involved in glutamatergic neurotransmission which is an important mediator for the developing brain and normal brain function. Our results indicate that variations involving glutamatergic gene networks of the brain may contribute to the genetic susceptibility of ADHD.

## 2742/F/Poster Board #386

**Combining next-generation sequencing, arrays and population genetics to characterize genome structural variation in human populations.**
*R.E. Handsaker[1], J. Nemesh[1], J.M. Korn[1], S.A. McCarroll[1,2].* 1) Broad Institute, Cambridge, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA.

Next-generation sequencing technologies provide new opportunities for characterizing human genomic variation. In deeply sequenced individual genomes, structural variation has been revealed by analysis of end-sequence pairs (Tuzun et al.; Korbel et al.) and read depth (Chiang et al.). A more common application of next-generation sequencing in the coming years may be to perform shallower sequencing (0.5-4x) on much larger numbers of individuals, as planned by the 1000 Genomes Project and contemplated in other disease-focused resequencing projects.

We describe a set of statistical approaches for discovering and characterizing structural variation from population-based resequencing data and genotyping array data, which exploit many additional relationships and properties of structural variation in populations, including: (1) population-genetic observations that a large fraction of structural variation arises from common, segregating structural alleles; (2) the observation that many such polymorphisms are in linkage disequilibrium with SNPs; (3) additional, orthogonal information provided by array-based copy-number analysis in the same samples; (4) additional population-statistical footprints that segregating polymorphisms are predicted to leave in next-generation sequencing data.

We show that an integrated, population-genetics-based approach that incorporates array data can yield clear improvements (relative to the use of sequencing data on its own and in one individual at a time) in detecting and characterizing structural polymorphisms, in estimating their breakpoints, and in precisely measuring their integer copy number each of the genomes in a population. We will present a detailed characterization of segregating structural polymorphisms on chromosome 5 based on roughly 4X sequencing coverage of 132 unrelated individuals from the 1000 Genomes Project.

Precise characterization of structural polymorphisms in a reference population, including breakpoint locations and relationships to other markers, will help enable the creation of an integrated reference map of structural variations and SNPs that can be used as a resource for imputation in future studies.

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## 2743/F/Poster Board #387

**Comparative analysis of CNV detection performance of the Affymetrix 6.0 and NimbleGen HD2 platforms.** *R.R. Haraksingh[1], A.E. Urban[1,2], M. O'Huallachain[1], S. Weissman[2], M. Snyder[1]*. 1) MCDB, Yale University, New Haven , CT; 2) Genetics, Yale University, New Haven , CT.

Determining the functional consequences of CNV in the human genome requires genome-wide, high resolution mapping of the locations of these events. Current methods for mapping CNVs include SNP genotyping, array CGH and sequencing based methods. One of the most popular and successful high-resolution platforms for detecting CNVs is the Affymetrix SNP 6.0 array. An alternative high-performance platform for genome-wide CNV analysis that is now becoming available is the NimbleGen HD2 whole genome tiling array. Here we report a systematic comparison of CNV detection performance of the two platforms. The Affymetrix array simultaneously measures 906,600 SNPs and copy number at 1.8 million genomic loci. The copy number probes are spaced densely in regions of known CNV but are at low density in other regions, especially segmental duplications. While the NimbleGen array does not detect SNP genotype at all it measures copy number at 2.1 million loci spaced evenly ~1kb apart throughout the non-repetitive genome, including in areas of segmental duplication where CNV have been shown to be enriched (Korbel et al., Science 2007b). Other potential advantages of the HD2 platform could include the ability of 60mer probes to perform with less cross-hybridization and hence result in more accurate CNV detection, and the NimbleGen 2-channel protocol, which has been optimized for CGH whereas the Affymetrix protocol is adjusted to allow for both SNP and CNV detection. The expectation is that the NimbleGen platform can detect even novel CNV evenly and at high resolution throughout the genome and not just in regions previously known to contain CNVs. Conversely, one main advantage of the Affymetrix platform might become apparent when integer CNV genotypes are to be obtained. The relative performance of these two CNV detection methods is unknown. We compare the array's performances by their abilities to detect, at high resolution, a set of 1000 CNVs which are known to occur in HapMap genome NA12878, based on independent sequence-based prediction and validation using PCR and custom array CGH. We will present a comparison, against this 'gold standard', of NA12878 CNV calls from the Affymetrix SNP 6.0 array (McCarroll et al., Nat. Genet. 2008c) and NimbleGen HD2 high confidence CNV calls observed in multiple technical replicates of NA12878 hybridized against a pool of seven females and analyzed with several different algorithms and parameters.

## 2744/F/Poster Board #388

**Definitive SNP/CNV haplotyping of Asian genomes using DNAs derived from complete hydatidiform moles.** *K. Hayashi[1], Y. Kukita[2], T. Tahira[1], K. Yahara[3], K. Yamamoto[4], K. Kato[5], N. Wake[5]*. 1) Div. Genome Analysis, Med Inst Bioreg, Kyushu University, Fukuoka, Japan; 2) Research Institute, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan; 3) Life Science System, Fujitsu Kyushu Systems, Fukuoka, Japan; 4) Division of Molecular Populatin Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; 5) Department of Gynecology and Obstetrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Segmental copy number variations (CNVSs) in the human genome can confer various phenotypic variations such as risk to complex disease. Because there is an abundance of CNVSs with possible population differentiation, cataloging CNV regions for each ethnic group is important. Available CNV data is far from complete, due to the limitations of detection methods and samples used. We determined a genome-wide high resolution SNP/CNV map, using a collection of 100 Japanese complete hydatidiform moles (CHMs) as samples, and analysing by high-density DNA array hybridization. CHM has duplicated haploid genome, and is the material advantageous in CNV detection, because the relative difference of the hybridization signal caused by the change of unit copy number is expected to be two-fold larger than when diploid samples are analyzed (larger S/N ratio). Another advantage of using CHMs in CNV detection is that it is free from the complication of resolving overlapped CNVSs when they appear as heterozygote in diploid materials. The array platforms we used were Affymetrix SNP Array 6.0 and Illumina 1M-duo, and combined numbers of markers were 1.8 million for SNP detection and 3 million for CNV detection (before q.c. filtering). The data analysis is in progress, and tentative results suggested that there may be more common CNVSs than registered in public databases such as Database of Genomic Variants (Toronto), and many so-called CNPs are likely to be divided into different ancestral CNV events. Our data also reveals definitive linkage disequilibrium structure of SNPs and CNV regions, because the materials are haploid, and the genotypes are always genome-wide definitive haplotypes.

## 2745/F/Poster Board #389

**Using Mouse models for Smith-Magenis and Potocki-Lupski syndromes to study the impact of CNV on weight and metabolism.** *M. Heney[1], W. Gu[1], J. Yan[1], W. Bi[1], P.K. Saha[2], L. Chan[2], J.R. Lupski[1,3,4]*. 1) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX; 3) Dept of Pediatrics, Baylor College of Medicine, Houston, TX; 4) Texas Children's Hospital, Houston, TX.

Potocki-Lupski syndrome (PTLS; MIM #610883) is associated with microduplication in chromosome 17p11.2, and it is characterized by congenital and neurobehavioral abnormalities, developmental delay, low muscle tone, poor feeding, and failure to thrive. The reciprocal 17p11.2 microdeletion is associated with Smith-Magenis Syndrome (SMS; MIM #182290), a well-characterized multiple congenital anomaly disorder with features of metabolic syndrome, including obesity and hypercholesterolemia. We have generated mouse models for PTLS, *Dp(11)17/+,* and SMS, *Df(11)17/+*, that harbor either a duplication or deletion of a ~2 Mb region syntenic to the PTLS/SMS region. These mouse models recapitulate some of the physical and neurobehavioral phenotypes seen in patients, including metabolic phenotypes. This unique mouse model system allows the study of copy number variation (CNV) in relation to specific physical, neurobehavioral, and metabolic phenotypes, because *Df(11)17/+, Df(11)17/Dp(11)17, Dp(11)17/+,* and *Dp(11)17/Dp(11)17*, mice can be analyzed to evaluate the effect of one, two, three, and four copies, respectively, of the dosage-sensitive SMS critical region. The metabolic phenotypes of these mice were studied to determine if CNV of the SMS critical region can be linked to metabolic deregulation. *Dp(11)17/+* mice are significantly underweight at the time of weaning, and throughout their lifespanIn contrast, Df(11)17/+ mice are obese. Histology of adipocytes & liver, plasma chemistries, food intake, glucose tolerance, and body composition were also studied both early and later in the lifespan, and indicate a general failure to thrive in these mice. Copy number normalization in *Df(11)17Dp(11)17* mice is able to partially correct this phenotype; these results indicate that dosage imbalance in the SMS region leads to errors in metabolism, suggesting presence of gene(s) functioning in the regulation of metabolic pathways in this region. Our mouse model thus provides an opportunity to study not only the molecular mechanisms for the multiple features in SMS and PTLS, but also common traits such as obesity and metabolic disorder.

## 2746/F/Poster Board #390

**The Normal and Tumour spectrum of Copy Number Variation: Copy Number alterations correlate with changes in gene expression in tumour transcriptome.** *F.C.L. Hyland[1], X. Xu[1], R. Gottimukkala[1], B.B. Tuch[1], M. Muller[1], C. Barbacioru[1], C. Bormann-Chung[1], C. Monighetti[1], J. Brockman[2], J. Schageman[2], J. Gu[2], S. Kuersten[2], R. Setterquist[2], H. Peckham[3], Y. Fu[3], A. Siddiqui[1], D.I. Smith[4], F.M. De La Vega[1]*. 1) Applied Biosystems, Foster City, CA; 2) Applied Biosystems, Austin, TX; 3) Applied Biosystems, Beverly, MA; 4) Mayo Clinic, Rochester, MN.

Copy number variations (CNVs) have been widely observed in normal humans and in tumor genomes, and are increasingly implicated in common disease (for example, mental retardation and autism), and in cancer progression. Massively parallel sequencing allows powerful, unbiased genome-wide interrogation of CNVs. In contrast to array methods, sequencing data allows measurement of genomic coverage at single base resolution, allowing more precise breakpoint resolution. We sequenced genomic DNA from matched tumor/ normal samples of three patients with tongue/tonsillar cancer using the SOLiDTM System. Using a modified version of the SegSeq algorithm and controlling the false discovery rate, we compared the numbers of sequence reads from tumor samples to those from normal samples in 100kb windows; we identified at least 300 significant copy number changes (ranging from 1 kb to 71,000 kb and from 0 to 9 copies) per genome. Genome-wide discovery of CNVs is robust at low coverage (1x). Using a new total RNA-based protocol, we performed SOLiD sequencing of the whole transcriptome of the tumor and normal samples, and examined the correlation between genomic copy number variation and changes in gene expression between tumor and normal samples. We found a significantly positive correlation (0.76) between CNV and gene expression in a patient. Some genomic segments with big increases in copy numbers in the tumor genome also show significantly elevated expression levels in the tumor transcriptome. These CNV segments offer insight into genes associated with the initiation or progression of cancer. In parallel, we developed an algorithm to detect copy number variation in a single sample. We calculate coverage in variable-sized genomic windows that are selected to contain a constant number of mappable positions. Within these windows, we normalize coverage based on predicted mappability and GC content. We then use a Hidden Markov Model for segmentation, and we apply empirically derived filters to the contiguous segments to call copy number variants. Of CNVs above 5kb, 89% are in the Toronto CNV database, suggesting a very high true positive rate. When we compare to an orthogonal data set, we detect 72% of predicted CNVs above 2kb in the same sample. These results are robust even at 5x coverage. Hypothesis-free genome-wide CNV detection at low coverage opens the way for CNV genotyping of many samples to elucidate the role of CNVs in complex disease.

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## 2747/F/Poster Board #391

**High Resolution X-Chromosome Copy Number Variation in Autism.** *M. Ikeda[1], S. Warren[1, 2].* 1) Human Gen, Emory Univ, Atlanta, GA; 2) Pediatrics, Emory Univ, Atlanta, GA.

Autism spectrum disorder (ASD) is a broadly defined developmental disorder with impairments in reciprocal social interaction and communication and stereotyped behaviors and interests. Twin and family studies demonstrate a substantial genetic component underlying ASD. Although some rare Mendelian forms of ASD exist, most cases have a complex etiology of both genetic and environmental factors. A 4- to 10-fold male preponderance of ASD suggests the existence of sex-specific risk alleles and raises the possibility of a recessive susceptibility locus on the X-chromosome. Recently, copy number variation (CNV) has been appreciated as a rich source of both inherited and de novo human genomic variation. We have exhaustively surveyed CNV of the entire X-chromosome in a series of 100 ASD males from the AGRE collection, utilizing high-density arrays consisting of 2.1 million oligonucleotide probes specific to the X-chromosome at a resolution of one probe per 50 bp of non-repetitive sequence. We identified 200 variant loci of which 90 were deleted, 102 were duplicated, and 8 were deleted or duplicated in different individuals. The median size of the deletions was 1,550 bp, 2,063 bp for duplications, and 4,807 bp for loci with both deletion and duplication calls. Over half of these loci involve genes, including 10 genes previously identified to cause X-linked mental retardation (XLMR). When resulting in XLMR, the mutations are predicted to be severe, often null mutations. In contrast, our CNV of these loci suggest more subtle effects, affecting promoters regions or other non-coding genic regions. Our hypothesis is that loci capable of causing MR with highly penetrant alleles may also be ASD susceptibility loci when the allelic variation is more subtle. On validated CNV involving genes, we are assessing functional changes conferred by the CNV allele.

## 2748/F/Poster Board #392

**An accurate genome-wide map of copy number variants of NA10851, a widely used reference sample for array CGH.** *Y. Ju[1,2], H. Park[2,3], J. Kim[1,2,4], J. Seo[1,2].* 1) Department of Biochemistry, College of Medicine, Seoul National Univ, Seoul, Korea; 2) Genomic Medicine Institute, Medical Research Center, Seoul National Univ, Seoul, Korea; 3) Brigham and Women's Hospital, Boston, MA, USA; 4) Psoma Therapeutics Inc, Seoul, Korea.

Identifying copy number variants (CNVs), including deletions, insertions and duplications, has been actively pursued over the past couple of years using a variety of different technology platforms. Amongst these, array comparative genome hybridization (aCGH) represents one of the most powerful methodologies for detecting CNVs which requires a 'reference' sample for comparative analysis. However, owing to this need for reference, it is impossible to distinguish a loss in the test sample from a gain in the reference sample, or vice versa. In order to elucidate the direct relationship between CNVs and susceptibility to disease, and also to enable utilization of CNV information in the personalized medicine, it is necessary that the absolute copy number state of certain genomic regions (rather than relative value) to the reference sample be known. Here we report the first accurate genome-wide CNVs map of NA10851, which has been widely used as a reference for aCGH experiments in the past. Three differing strategies were systematically applied to accurately identify CNVs of the reference sample in the current study. Firstly, experiments utilizing high resolution custom-designed 24 million probe set aCGHs (Agilent Inc.) and 42 million probe set aCGHs (Nimblegen Inc.) were performed on the test sample (AK1) and NA10851. Secondly, the whole genome shotgun sequencing data obtained by Illumina Genome Analyzer for both AK1 and NA10851 genome were analyzed to an average depth of 24.8x and 5.7x, respectively, and subsequently cross-compared with the data obtained through aCGH. Lastly, we reexamined the CNV regions previously reported on the Database of Genomic Variants utilizing the massively parallel sequencing data of NA10851. Through these combined approaches, we were able to elucidate 446 copy number losses and 213 copy number gains, totaling 5.76 Mb and 8.84 Mb, respectively, on NA10851 genome. Moreover, our preliminary data suggests the majority of copy number gains identified were overestimated due to copy number losses of NA10851. Overall, about 340 genes were on CNV regions of NA10851 genome. Our CNV map from NA10851 will enable unbiased and more accurate interpretation of future CNV results using aCGH. Absolute copy number of genes for test sample will also be made available. Findings from present study will help us uncover the direct relationship between the CNVs and the phenotypes, and move us one step closer to the era of personalized medicine.

## 2749/F/Poster Board #425

**Databases for copy number variations in Japanese Integrated Database Project.** *A. Koike[1], N. Nishida[2], D. Yamashita[2], I. Inoue[3], S. Tsuji[4], K. Tokunaga[2].* 1) Cent Res Lab, Hitachi, Ltd, Kokubunji, Japan; 2) Dept Human Genetics, Univ. Tokyo; 3) Dept. of Mol. Life Sci. and Mol. Med., Tokai Univ. Sch. Med; 4) Dept Neurology, Univ. Tokyo,.

Recent advances in measurement technologies for copy number variations (CNVs) on a genome-wide scale have gradually revealed disease-associated CNVs. Especially microarray-based CNV analyses have enabled us to detect CNVs in a high-throughput manner. So far, our organization has created the repository database for genome wide association study (GWAS) (https://gwas.lifesciencedb.jp/) to achieve continuous and intensive management of GWAS data and data sharing among researchers and has widely called for data submission. In this study, we have newly constructed a repository CNV database for normal samples and a repository CNV-GWAS database for CNV association studies in addition to extending the GWAS DB function to call up CNV-case control results with SNP case control analysis results. The CNV control DB and CNV-GWAS DB provide hierarchical view of CNV regions, CNVs, CNV frequencies, and genotype patterns consisting CNVs by tables and graphs. The CNV-GWAS DB also accumulates case control statistical analysis results for duplication, deletion, and alteration of genomic segments. The comparison among studies and that among different analysis results and the superposition with DGV (Database of Genomic Variants) data and gene structures are also available.

In this presentation, in addition to the introduction of these CNV databases' structures, the analysis results of currently registered CNVs of Japanese normal individuals (Affymetrix 6.0: 150 individuals, Affymetrix 500K: 150 individuals) are reported. These CNVs were detected by multiple methods such as Circular Binary Segmentation (Olshen et al. 2004), Gaussian process with adaptive criteria (Picard et al. 2004), Hidden markov model based method (Korn et al. 2008), and were carefully selected based on the thresholds, which were determined using HapMap data (Affymetrix 6.0 and 500K ) and their CNV data validated by other experiments. The comparisons of these CNVs with known CNVs in inter- and intra-populations and the characteristics of CNV-located gene functions and the bias of CNV-locations on the genome are also discussed.

Acknowledgement: This work was supported by the contract research fund "Integrated Database Project" from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

## 2750/F/Poster Board #426

**Population Genetics of Rare Copy Number Variants, and Implications for Studies of Phenotype Association.** *J.M. Korn[1,2,3,4,5], J. Nemesh[3], M.J. Daly[3,5], S.A. McCarroll[3,4], D. Altshuler[3,4,5].* 1) Dept Biophysics, Harvard Univ, Boston, MA; 2) Bioinformatics and Integrative Genomics, HST, Cambridge, MA; 3) Broad Institute, Cambridge, MA; 4) Dept Genetics, Harvard Medical School, Boston, MA; 5) Center for Human Genetic Research, MGH, Boston, MA.

Copy number variation (CNV) is increasingly analyzed alongside SNPs in whole genome association studies for association to phenotypes. A fundamental question in interpreting such data is the extent to which rare CNVs (<1% MAF) observed at the same locus in different individuals arise from a shared ancestral mutation, rather than a collection of mutations occurring at the same site (and are private or *de novo* CNVs in each individual). A more general question, relevant to association studies for both rare CNVs and rare sequence variants, involves whether ancestry matching using common variants correctly controls for association of rare variants, which as a class may be more sensitive to population substructure.

Using data from several thousand individuals whose genomes were analyzed using the Affymetrix SNP 6.0 array, we first demonstrate that the majority of rare CNVs are of the type that have a shared ancestral origin. Such CNVs have coherent breakpoints and tend to lie on long shared haplotypes (as expected of recent mutations); we use phasing and imputation algorithms to define such haplotypes. Since most rare CNVs in the general population arose from a common ancestor, the demonstration that a rare CNV arose multiple times in affected individuals carries implications for both the association statistic and the interpretation of the results. Multiple mutation events can indicate negative selection at the locus and increase confidence in an association. Furthermore, the lack of a shared haplotype implies the CNV itself is likely the causal mutation.

The tendency towards shared origin of rare CNVs ascertained in the general population is common regardless of other features of the CNV, such as size, local sequence context, and allele frequency. We demonstrate that rare CNVs can have shared haplotypes even across continental populations, although haplotype length varies both within and across populations. We show that the typical correction for ancestry as determined by common variants may not address population substructure as effectively as is required to assess the disease association of low-frequency segregating polymorphisms without inflation of association statistics. Finally, we revisit several published associations of phenotypes to rare CNVs to determine which are likely arising from an ancestral mutation (and thus potentially confounded by population substructure), and which are not.

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## 2751/F/Poster Board #427

**Genome-wide analysis of structural variations in different ethnic groups.** *V. Kumar, AM. Hilmer, P. Nuwantha, C. Lee, JHH. Tan, Y. Zhu, F. Yao, SWK. Ken, Y. Ruan, M. Seielstad.* Genome Institute of Singapore, Singapore.

Abstract: Structural variation (SV) can be defined as all genomic alterations other than single base-pair substitutions. A number of studies have shown that SV is abundant in human populations. Systematic analysis of this variation will provide fundamental knowledge to understand human diversity and to distinguish from pathogenic alterations. However, identifying them comprehensively with high resolution remains a technical challenge. We have taken a multi-pronged strategy, including Paired-end Mapping (PEM), SNP-Chip (Illumina IM and Affymetrix 6.0) and aCGH (Nimblegen 2.1M and Agilent 1M) to characterize SVs. We have applied these methods to 8 individuals comprising 2 trios of African and European descent and, 2 Asians. For PEM we fragmented genomic DNA extracted from lymphoblastoid cell lines to a size of 10 kb. This was followed by 25 base-pair sequencing of the fragment-ends using the ABI SOLiD, and mapping of these paired-ends to the reference assembly hg18 to identify SVs. The SNP and CGH microarray platforms were used to identify copy number variation (CNV). To maximize the resolution of the SNP chips we combined the Illumina and Affymetrix datasets and normalized the log-ratios. Based on the analysis of the data generated by PEM and the various microarrays we are able to accurately identify a large number of SVs, both novel and reported, as well as precisely map the break points.

## 2752/F/Poster Board #428

**An Optimization Framework for Unsupervised Identification of Rare Copy Number Variation from SNP Array Data.** *T. LaFramboise[1,2,3], G. Yavaş[3], M. Koyutürk[3], M. Özsoyoğlu[3], M. Gould[1].* 1) Genetics, Case Western Reserve University, Cleveland, OH; 2) Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH; 3) Electrical Engineering & Computer Science, Case Western Reserve University, Cleveland, OH.

Until fairly recently, it was believed that essentially all human cells harbor two copies of each locus in the autosomal genome. However, studies have now shown that there are segments of the genome that are polymorphic with regard to genomic copy number. These copy number variations (CNVs) have a role in various diseases such as Alzheimer disease, Crohn's disease, autism schizophrenia, and others. In the effort to scan the entire genome for these gains and losses of DNA, single nucleotide polymorphism (SNP) arrays have emerged as an important tool. As such, CNV identification from SNP array data is attracting considerable attention as an algorithmic problem, and many methods have been published over the last few years. In this work, we address the problem from a novel perspective. We frame CNV identification as an objective function optimization problem. The objective function is ideally designed so that its optimal solution is the most accurate set of CNV calls. Our method, termed ÇOKGEN, finds the best solution using a variant of the well-known heuristic simulated annealing. We apply ÇOKGEN to data from hundreds of samples, and demonstrate its ability to detect known CNVs at a high level of sensitivity without sacrificing specificity. Furthermore, we show that it performs better than other publicly-available methods. ÇOKGEN is implemented in an R package, freely available from the authors, that converts raw Affymetrix 6.0 SNP array .CEL files into CNV inferences.

## 2753/F/Poster Board #429

**Identification of Copy Number Variation in high-risk African-American men with prostate cancer.** *E. Ledet[1], X. Hu[2], M. Li[2], D. Mandal[1].* 1) Dept Genetics, Louisiana State Univ HSC, New Orleans, LA; 2) Hayward Genetics Center, Tulane University School of Medicine, New Orleans, LA.

Prostate cancer is a complex multi-allelic disease and the most common malignancy in men throughout the world. In the United States, a lifetime risk of mortality from prostate cancer is 3% for white men and 4% for African-American men. Thus far, disease susceptibility loci have been identified for this cancer but no definite locus-specific information is established due to the tremendous amount of genetic heterogeneity. Genomic copy number variations (CNVs), the most prevalent form of structural variation in the human genome, has been detected in prostate tumors and several other cancers, but changes in copy number have not been studied in germ-line DNA from hereditary African-American prostate cancer cases. In order to identify prostate cancer associated CNVs, we have identified ten high-risk African-American families with at least 3 affected individuals. From these families, 30 individuals, including 21 affected males and 9 unaffected males, were selected for CNV analysis. Array comparative genomic hybridization (aCGH) technology was used to determine the common or case specific CNVs that may predispose African-American men with family history to prostate cancer. We have used a combined targeted/whole-genome array system using the Agilent 4 X44 K format. Fifty percent of the probes were selected from the Agilent eArray system that targets more than three hundred fifty oncogenes, tumor-suppressor genes, and known cancer-associated chromosome regions, including over thirty reported prostate cancer associated genomic regions. Intervals between above genes or regions were filled relatively evenly with the remaining 50% probes to cover the whole genome. DNA extracted from the patients was co-hybridized with DNA from pooled normal individuals to the arrays. Scanned data from aCGH experiments were analyzed using CGH Analytics 3.5 software (Agilent Technologies) and genomic copy number changes were identified using aberration detection method 1. All CNVs were compared with the standard reference database to rule out known polymorphisms. Novel CNVs were identified on chromosomes 1, 13, and 20 in prostate cancer cases. These CNVs may represent a component of genetic variability which contributes to the high prevalence and high mortality of prostate cancer in African American men. Detailed analysis of our array CGH data and validation study will be presented.

## 2754/F/Poster Board #430

**Validation of High-resolution Copy Number Variation Discovery by TaqMan Assays.** *K. Li, F. Wang, I. Casuga, A. Broomer, B. Ching, X. You, N. Majumdar, C. Chen.* Applied Biosystems, Foster City, CA.

Copy number variation is a recently appreciated, widespread form of polymorphism in the human genome and can be associated with genomic disorders and complex diseases. Array-based comparative genome hybridization (aCGH) has been a commonly used methodology for CNV discovery. The Structural Genomic Variation Consortium (http://www.sanger.ac.uk/humgen/cnv/) published a first generation, genome-wide copy number variant (CNV) map in 2006 (Nature 444:444-454, 2006) and reported that the copy number variation regions (CNVRs) cover as much as ~12% of the human reference genome. This study suggested that thousands of CNVs existed in the human genome, but technological limitations precluded the discovery of more CNVs. In 2007, the consortium launched its phase II CNV discovery to develop a more comprehensive, higher resolution CNV map for the human genome - at a level of 100-fold finer than reported previously. A 42- million probe set that tiled across the human genome was designed for NimbleGen's 2.1 million probe (HD2) arrays. Forty HapMap samples (20 CEU and 20 YRI) were then analyzed. The study was designed to capture common CNVs in these individuals that were 500 bp and larger, with a frequency of at least 2.5 percent. To validate the CNV calls made on the NimbleGen's arrays, orthogonal technologies were employed. Here, we report the TaqMan validation results of ~100 randomly chosen CNVR from this project. TaqMan assays were selected for each target from a set of ~1.7 million, pre-designed TaqMan Copy Number Assays. The same 40 HapMap samples were run on both arrays and TaqMan assays. We observed very similar copy number profiles between array and TaqMan across the tested targets and the samples and high concordance between the two platforms. This validation study showed that the CNV calls made with this array platform had a relatively low false positive rate. The validation study also demonstrated that these TaqMan assays were a useful and reliable tool for validating array-based CNV discoveries. The detailed observations and analyses will be presented and discussed. Acknowledgements: Drs. Matthew Hurles and Nigel Carter (Sanger Center, UK), Charles Lee (Harvard Medical School / Brigham and Women's Hospital, Boston, USA), Stephen Scherer (Sick Kids Hospital, Toronto, Canada).

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## 2755/F/Poster Board #431

**Copy Number Variants in Myopia of Singapore Chinese Schoolchildren.** *Y.J. Li[1,2], A.E. Dellinger[1], M. Yu[1], T.L. Young[1,3], S.M. Saw[4,5].* 1) Ctr for Human Genetics, Duke Univ Med Ctr, Durham, NC; 2) Dept. of Biostatistics and Bioinformatics, Duke Univ Med Ctr, Durham, NC; 3) 3Duke - National University of Singapore Graduate Medical School; 4) Dept. of Epidemiology and Public Health, National University of Singapore, Singapore; 5) Singapore Eye Research Institute, Singapore.

Copy number variants (CNVs), duplications or deletions of chromosomal segments, are common in the human genome and can be used as genetic markers for disease studies. CNV data can be generated by multiple platforms, including genome wide single nucleotide polymorphism (SNP) arrays. We examined the characteristics of SNP array derived CNVs among multiple myopia phenotypes. A total of 1116 Chinese participant DNA samples from the Singapore Cohort study Of the Risk factors for Myopia (SCORM) were genotyped using Illumina HumanHap 550 Beadchips. CNVs were detected by Nexus CGH™ and QuantiSNP. A CNV is defined as at least five SNPs within the detected CNV region. Phenotypes examined are quantiative spherical equivalent (SE, n=912) and the binary states of any myopia (SE < -0.50 diopter (D), n=699) and high myopia (SE < -6.00D, n=97). CNVs were excluded if the CNV presented in only one sample for QuantiSNP, or in less than seven samples for Nexus. Linear regression and logistic regression were use to examine the average CNV size per sample and individual CNV size in the three phenotypes. The Fisher exact test was used to test the association of the presence of CNVs (CNV events) with any myopia and high myopia. The average participant age at the final eye examination was 13.35 years (SD=1.9). Nexus generally detected longer and fewer CNVs than QuantiSNP (0.52±1.6MB vs. 0.09±0.37MB). The average CNV length per individual was found to significantly associate with SE ($P=4.19x10^{-8}$ for Nexus, P=0.01 for QuantiSNP) and high myopia ($P=1.38\ x10^{-8}$ ) for Nexus, P=0.03 for QuantiSNP), but not any myopia. CNVs detected by both programs in chromosomes 1, 9, 11, and 16 were significantly associated with at least one phenotype (P<0.05) and map to known gene regions. The top association result was chromosome 3 (from 163,613,386 to 163,796,731) , which shows significant association between CNV sizes and SE in both programs (minimal $P=8.23x10^{-9}$ by Nexus) as well as significant association between the CNV event and SE (minimal P=0.01 by Nexus). Fisher exact test also detected two SNPs in this CNV with significant association to high myopia (P=0.004). However, this region does not map to a gene but a transcription factor binding site Cart-1 could be potentially interesting. In conclusion, our data show that CNV sizes vary among phenotypic groups. CNV size can be a useful surrogate for CNV events for identifying susceptibility genes for myopia.

## 2756/F/Poster Board #432

**Genomic copy number variants in East Asian populations.** *S. Li[1], S. Xu[2], Y. Wang[1], X. Zhang[1], X. Gong[1], Y. Shen[3], Y. Yang[1], H. Wang[1], B. Wu[1,3], J. li[1,2].* 1) MOE Key Laboratory of Contemporary Anthropology and Center for Evolutionary Biology, Institutes of Biomedical Science and School of Life Science, Fudan University, Shanghai, China 200433; 2) CAS-MPG Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China; 3) Children's Hospital Boston and Harvard Medical School, Harvard University, Boston MA, USA.

Genomic diversity between human individuals is more extensive than previously realized. The existence of prevalent genomic copy number variants (CNV), along with single nucleotide polymorphism (SNP), has important implications in understanding human diversity, evolution and disease susceptibility. Characterization of CNV and SNP in the DNA of representative health individual from East Asian populations is the first important effort toward genome-wide association studies in this area. We used Affymetrix SNP 6.0 array for simultaneous interrogation of CNV and SNP. Here we report the initial findings from 100 Han Chinese, 50 Tibetan and 40 other ethnic groups. While many CNVs in East Asian populations could be found in other world populations, novel CNVs were also observed in the samples studied. Some novel CNVs were further confirmed by an independent analysis. We further studied some of the novel CNVs and found that they may reflect not only the mutational history as well as the local adaptation of East Asians. We believe this is the first step towards understanding the unique structural genomic variation of East Asians and their potential functional consequences related to susceptibility to diseases.

## 2757/F/Poster Board #433

**A mutational mechanism for copy number variation.** *L. Liang, L. Deng, O.F. Hashmi, C. Shao, J.A. Tischfield.* Department of Genetics, Rutgers University, Piscataway, NJ.

Discovery of copy number variation (CNV) in the human genome has provided new opportunities to examine the range of genetic variants associated with human diseases. CNVs are changes in copy number of DNA segments ranging from 1 kb to several Mb in size, and can be copy number gains (duplications or insertions), losses (deletions), or complex multi-site rearrangements. The mechanisms underlying the origin of CNV are not known. Several models that were proposed to explain how CNV arises have not been subjected to rigorous test. Nevertheless, molecular analysis of genomic segment alterations either associated with human clinical phenotypes or occurring in healthy individuals has revealed that many CNV events occur at microhomology (2-10 bp) sites. For instance, some deletions spanned one of the two microhomology (2 to 4 nucleotides) sequences and the intervening sequence. The pattern of these deletions resembles genetic consequence of microhomology-mediated end joining (MHEJ), an error-prone repair pathway of DNA double strand breaks (DSBs) that exists in many organisms, including humans. By using a cell-based reporter system for MHEJ, we demonstrated that MHEJ is one of major mutational mechanisms of CNV. Therefore, environmental insults that can induce DSBs can increase the risk for microhomology-mediated CNV, and factors modulating MHEJ can play roles in origin of CNV.

## 2758/F/Poster Board #434

**A genome-wide scan of copy number variation in Moebius syndrome.** *A.C. Lionel[1,2], C.R. Marshall[1,2], I.E. Drmic[3], W.S. Roberts[3,4], S.W. Scherer[1,2].* 1) The Centre for Applied Genomics and Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 3) Autism Research Unit, The Hospital for Sick Children, Toronto, ON, Canada; 4) Child Development Program, Bloorview Kids Rehab, Toronto, ON, Canada.

**Background:** Moebius syndrome, a rare, congenital facial palsy prevalent in 0.002 % of births, is characterized by abnormalities of the abducens (cranial nerve VI) and facial (cranial nerve VII) cranial nerves. In addition to facial paralysis and impaired ocular movement, this syndrome is also associated with a heterogeneous phenotypic spectrum including the increased incidence of limb anomalies, autism spectrum disorder and mental retardation. Familial inheritance of Moebius syndrome has been reported, although a majority of cases are sporadic. Case reports have described several cytogenetic abnormalities such as translocations and inversions as being potentially causal. The purpose of this study is to assess the possible role of copy number variants (CNVs) in the etiology of Moebius syndrome and its co-morbid phenotypes. **Methods:** We conducted a genome-wide CNV scan in 24 unrelated Moebius Syndrome patients using the Affymetrix Human SNP Array 6.0. For CNV detection, we used a multi-algorithm approach incorporating Birdsuite, Partek Genomics Suite, Affymetrix Genotyping Console and iPattern (a novel clustering-based method developed in our lab). High confidence CNVs detected by this approach in the Moebius cases were compared with CNVs found in 2,115 population based controls using the same microarray platform and CNV calling strategy. **Results:** We identified CNVs overlapping genes in the Moebius dataset which were not present in control samples. These included variants which encompassed exons of genes involved in neuronal development and function such as *GRM5*, *PTPRD* and *NRXN1*. The finding of the deletion overlapping exons of *NRXN1*, in a Moebius syndrome patient with autism spectrum disorder, is particularly intriguing, given the previous reports of CNVs at the *NRXN1* locus being associated with autism. We are currently collecting samples from the parents of the Moebius cases in order to study the inheritance patterns of the CNVs of interest and their potential segregation with elements of phenotype. **Acknowledgement:** We thank the children and families who participated and the International Moebius Syndrome Foundation for supporting this work.

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## 2759/F/Poster Board #435

**Data Quality Control in Detecting Copy Number Variations using Illumina SNP arrays.** *E. Lu, Y. Xu, C. Amos.* Epidemiology, UT MD Anderson Cancer Center, Houston, TX.

SNP genotyping arrays have been developed to type DNA copy number variations (CNVs). The quality of the inferences about copy number can be affected by many factors including batch effects, DNA sample preparation, signal processing, and analytical approach. As a part of a genome-wide association analysis of lung cancer susceptibility, we obtained data from the Center of Inherited Disease Research that included 53 CEPH control cell line samples that had been genotyped in duplicate using Illumina Human Map v1.1 BeadChips. These duplicated controls allowed us to evaluate the performance of copy number variation assessments using different analytical algorithms and allowing for intraindividual variability. We analyzed these data using i) QuantiSNP and ii) Penn CNV, both algorithms that use both the log R intensity as well as the B-allele frequencies. Results show that when no constraint is made on inclusion of results from sample reads according to log R intensity, that concordance of CNV calling was higher for QuantiSNP (60% samples have at least 50% identical CNV finding when the Bayes factor was restricted to be >5 for existence of a CNV), and a little bit lower for Penn CNV (50% samples have at least 50% identical CNV findings). When only samples that showed within-subject variability in log R intensity <90% of the entire sample, the repeatability of inferences improved greatly, yielding r=? for Penn CNV. These results show higher repeatibility for the Penn CNV algorithms and the importance of restricting analyses to those samples with tighter variability in log intensities.

## 2760/F/Poster Board #436

**High resolution map of canine copy number variation.** *E.M. Nevalainen[1,2], C. Hitte[3], P. Jokinen[1,2], C. Andre[3], K. Lindblad-Toh[4,5], H. Lohi[1,2], LUPA Consortium.* 1) Department of Medical genetics and Department of Basic Veterinary Sciences, University of Helsinki, Finland; 2) Department of Molecular Genetics, Folkhälsan Institute of Genetics, Finland; 3) Institute of Genetics and Development of Rennes, CNRS-umr6061, University of Rennes, France; 4) Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden; 5) Broad Institute of Harvard and Massachusetts Institute of Technology.

Copy number variations (CNVs) are an important form of structural variation in mammalian genomes. Substantial amount of phenotypic differences between healthy individuals have been attributed to CNVs and they also contribute to disease risk in several human disorders including autism spectrum disorders, schizophrenia and epilepsy. Dog breeds as genetic isolates have recently emerged as a new model for gene mapping of comparative interest for human inherited diseases. Over 400 genetic diseases have been identified in dogs, many of which are similar to human diseases and are likely to have a similar genetic background. CNVs are expected to play a role in several disorders in dogs and contribute to breed-specific characters. This study aimed to catalogue CNVs in 62 dogs from 17 dog breeds (2-10 dogs/breed) and 3 wolves using Nimblegen's comparative genome hybridization (aCGH) arrays with 2.1 million probes. This density gives a high resolution of ≈1 kb median probe spacing across the dog genome. A Finnish Boxer DNA was used as a reference for other breeds. We identified a total of 2665 high confidence CNVs (log2 ratio ≥±0.45, 10 probe coverage) with an average of 72 CNVs per dog. The analysis with less stringent criteria doubled the number of potential CNVs. The size of the CNVs varied from ≈10 kb to 1.9 Mb with an average size of ≈60 kb. X chromosome was excluded from the analysis. The results of this high resolution analysis of the CNVs in a large number of breeds provide important discoveries likely to contribute to disease risk and other phenotypes in dogs and are being followed up in many disease cohorts available in the European canine genomics effort, LUPA consortium.

## 2761/F/Poster Board #437

**High resolution CNV map of Asian population.** *H. Park[1,2], J. Kim[3,4], Y. Ju[2,3], H. Kim[3], C. Lee[1], J. Seo[2,3].* 1) Brigham & Womens Hospital, Harvard medical school, Boston, MA; 2) Genomic Medicine Institute, Medical Research Center, Seoul National University, Seoul, Korea; 3) Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine, Seoul, Korea; 4) Psoma Therapeutics Inc., Seoul, Korea.

The structural genomic copy number changes involve deletions and insertions of segments of genomic DNA spanning from 1 Kb to megabases in length and are present in all individuals. About 12% of the human genome is copy number variable with the CNVs contributing 0.12% to genomic variability seen within humans. Copy number variation (CNV) of DNA sequences is functionally significant but CNV map has yet to be fully established. The specific hypothesis is there remains a need to identify CNV that is smaller than 1Kb. We have constructed a high resolution of CNV map of the human genome through the study of 30 individuals from 3 populations with ancestry in Asia (Korean, Chinese and Japanese of the HapMap collection). We designed twenty four custom 1M feature 60mer in situ synthesized oligonucleotide arrays. Our design consisted of ~23.5M probes with ~100 bp between-probe resolution spaced uniformly across the entire human genome (whole genome tiling array). This high resolution array-based comparative genomic hybridization (aCGH) array set will allow us to interrogate the samples at a level not previously achieved, and will facilitate the identification of smaller CNV events which are often missed in lower resolution aCGH array. High resolution aCGH arrays will be used to identify complete CNV regions including common and different CNV regions from the genomic DNAs of 30 individuals from 3 populations. Approximately, 1,500 copy number variable regions (CNVRs), which can encompass overlapping or adjacent gains or losses, covering 20 megabases were identified in each individual. These CNVRs contained hundreds of genes, disease loci, functional elements and segmental duplications. In conclusion, this strategy will offer insights into the molecular basis and elucidate the role of CNVs.

## 2762/F/Poster Board #438

**Calling and Scoring Copy Number Variants.** *G.A. Satten[1], A.S. Allen[2], M. Ikeda[3], J.G. Mulle[3], S.T. Warren[3].* 1) National Center for Chronic Disease Prevention and Health Promotion, CDC, Atlanta, GA; 2) Dept. of Biostatistics and Bioinformatics, Duke University, Durham NC; 3) Dept. of Human Genetics, Emory University, Atlanta GA.

**Background:** Copy number variation is an exciting new class of genetic diversity currently under intensive investigation. Many platforms and algorithms exist to detect these variants. However, regardless of the technology used, many CNVs detected are false positives. Distinguishing true from false signals requires laborious experimental validation.

**Methods:** We have developed a CNV detection algorithm that addresses the false positive CNV call rate, with a simple score that can be easily calculated for each possible CNV. We show that this score is predictive of the chance that a CNV is experimentally validated. Based on this score, we have developed a new and simple backward elimination algorithm for calling CNVs: starting with a possible jump in log-intensity ratio (LIR) at each probe (where a jump is a putative CNV breakpoint), we remove jumps one at a time (while combining the intensities between jumps) until all remaining called CNVs have a score larger than a user-specified cutoff. To make our algorithm more robust, we use medians rather than means of adjacent probe intensities when combining LIRs. Information on genomic covariates such as GC content could easily be added to our scoring and CNV-calling algorithms if desired. Our approach can also be easily extended to case-parent trio data to only call CNVs that appear in the offspring and at least one parent.

**Results:** We validated our score using samples from males with autism from the Autism Genetic Resource Exchange that were analyzed with a Nimblegen array CGH approach having approximately 2 million probes on the X chromosome. We experimentally validated 91 CNVs called in 41 individuals, of which 43 were validated and 48 were not. The mean score for validated CNVs was 4.5 with standard deviation 2.9. The mean score for non-validated CNVs was 2.3 with standard deviation 1.6.

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## 2763/F/Poster Board #439

**Novel Copy Number Variation Detection in the Asian Population Using a High-Density Array CGH Platform.** *C.J. Shaw, K. O'Moore, K. Hogan, J. Geoghegan, A. Larsen, N. Jiang, T.A. Richmond, V.L. Ott, R.R. Selzer, R. Seibl.* Roche NimbleGen, Inc, Madison, WI.

Array CGH methods have been widely used to investigate genome-wide DNA copy number variation associated with complex disorders. Disease association studies have become increasingly focused on CNVs, and several recent reports show links between CNVs and schizophrenia (Stefansson 2008; Stone 2008), autism (Sebat 2007; Marshall 2008; Glessner 2009), and cancer, among others. CNV frequencies are known to differ among populations, resulting in variable risk factors for individuals of different ethnicities. However, the majority of disease association studies with CNVs have focused on the European and Yoruban populations, while the Asian populations have been under-represented (Kang 2008; Lin 2008). Thus, the currently available databases containing CNV information and the microarray designs used to detect them are somewhat skewed toward the European and Yoruban populations. We sought to discover novel, Asian-specific CNVs using the Roche NimbleGen CNV-focused 3x720K v1.0 CGH array. We studied several individuals from the Chinese, Japanese, and Korean populations, and compared the CNV discovery to that of European and Yoruban individuals. Utilization of the CNV-focused array enabled detection of hundreds of CNVs per individual. When the frequency of CNV detection was compared across the Asian and non-Asian populations, we identified hundreds of novel CNVs that were present in Asian individuals, but absent in non-Asian individuals. Further, when the Database of Genomic Variants was searched for these Asian-specific CNVs, only a fraction were located. Competitive array platforms were also investigated to determine the detection rate of the Asian-specific CNVs we identified. The number of CNVs detected in Asian individuals varied depending on the platform, with the Roche NimbleGen array detecting more Asian-specific CNVs than the other platforms tested. We demonstrate here the variability in CNV detection in Asian individuals based on the platform utilized, and the paucity of Asian-specific CNVs contained in public databases relative to that of the European and Yoruban populations.

## 2764/F/Poster Board #440

**Detecting Copy Number Variations from Whole Genome Resequencing Data at Low Coverage.** *Y. Shen[1,2], I. Pe'er[1,2].* 1) Computer Science, Columbia Univ, New York, NY; 2) Center for Computational Biology and Bioinformatics, New York, NY.

Copy Number Variations (CNVs) are genetic factors crucial for studying human traits and diseases. Whole genome resequencing provides multiple lines of evidence for detecting CNVs. Most obviously, depth-coverage positively correlates with the copy number, therefore can be incorporated in a Hidden Markov Model (HMM) for calculating the emission probabilities of hidden states that represent copy numbers. However, theoretical calculation and practical experience show that at low coverage, methods based solely on depth-coverage lack power to detect small- to medium-size CNVs. Using mate pair information helps to improve the power in such scenarios. Abnormality in mate pair distance, order, or orientation suggests breakpoints, and the distance reflects the size of a deletion or tandem duplication. Additionally, intra-read breakpoints are direct evidence of breakpoints. Conventional CNV detection methods generate and then filter a list of candidate CNVs by ad hoc cutoffs for abnormality of mate pairs and other information. We propose an approach that infers the likelihood of CNVs jointly from multiple mate pairs by a HMM that integrates depth-coverage, mate-pair relationship and intra-read breakpoints. There are two challenges in modeling mate pairs through a HMM: (1) The duration of a state follows exponential distribution whereas the inferred CNV size from a mate pair should follow Gaussian-like distribution; (2) A 1st order Markov chain does not remember inferred CNV size once the path goes beyond break points. To address these issues, we designed a grid of specialized CNV states. Specifically, each row states on the grid represent same copy number. The transition to states representing other copy numbers can only occur at the end of a row. Although the duration of each state still follows exponential distribution, the duration of the row as a whole approximately follows Gaussian distribution based on the central limit theorem. In addition, different rows of states have different transition probabilities designed to accommodate different ranges of CNV size. Intra-read breakpoints increase the emission probabilities of first and last CNV states in each row. By integrating all useful information in such comprehensive model, our method is aimed for projects at low to medium depth-coverage (0.1x to 10x per sample). We apply it to simulated and public-domain individual genomes and demonstrate the accuracy and power with different levels of depth-coverage.

## 2765/F/Poster Board #473

**Functioanl Annotation of copy number variation related genes in Caucasians and Asians.** *H. Shen[1], J. Li[1], L.S. Zhang[1], X.H. Xu[2], F. Chen[2], H.Y. Deng[1], S. Levy[3], C.J. Papasian[1], B.M. Drees[1], J.J Hamilton[1], R.R. Recker[4], H.W. Deng[1,2,5].* 1) Basic Medical Sciences, University of Missouri-Kansas City, Kansas City, MO; 2) The Key Laboratory of Biomedical Information Engineering of Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, Shanxi, China; 3) Vanderbilt Microarray Shared Resource, Vanderbilt University, Nashville, TN; 4) Osteoporosis Research Center, Creighton University, Omaha, NE; 5) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan, China.

Copy number variation (CNV) may affect the gene dosage and expression levels of corresponding genes. Previous studies have suggested that genes with certain biological functions tend to be enriched in CNV regions, such as cell adhesion, chemical stimuli and sensory receptor. In this study, we reported a genome-wide functional annotation of genes overlapping with CNVs in Caucasians and Chinese. CNV genes were examined for biological functions, relation to human genetic disorders and potential effects on human ethnic diversity.

A total of 2796 genes were identified to contain CNVs. These CNV genes are found to be significantly enriched in 29 GO (gene ontology) functional categories, such as multicellular organismal process (P = 6.47E-24, 492 CNV genes), developmental process (P = 2.17E-15, 400 CNV genes), neurological system process (P = 2.09E-09, 172 CNV genes) and reproduction (P = 7.72E-06, 84 CNV genes). In terms of biological pathways, CNV genes were enriched in several neurological pathways, such as neuroactive ligand-receptor interaction pathway and axon guidance pathway, as well as in some cancer related pathways, such as colorectal cancer, melanoma and renal cell carcinoma pathways. In addition, MAPK signaling pathway, calcium signaling pathway, Wnt pathway and insulin signaling pathway also enriched with CNV genes. A total of 317 CNV genes were involved in 392 human genetic disorders. Thirteen CNV genes were related to mental retardation and 6 CNV genes related to obesity. Other diseases, such as schizophrenia, autism, and leukemia, may also be affected by CNV genes.

Further analysis revealed 261 CNV genes showing significant difference in CNV occurrence rates between Caucasians and Chinese. These ethnic differentiate CNV genes are involved in developmental process, response to stimulus, immune system process, reproduction, and growth. A number of human genetic disorders, such as deafness, mental retardation, Cohen syndrome, leukemia, obesity and Parkinson disease, are related to these ethnic differentiate CNV genes, suggesting that CNVs may contribute to the ethnic difference of disease occurrence.

## 2766/F/Poster Board #474

**Association of DUF1220 domain copy number with brain size in human evolution and disease.** *J. Sikela[1], L. Dumas[1], J. Davis[1], M. Sikela[1], S.W. Cheung[2], P. Stankiewicz[2], T. Fingerlin[1].* 1) Dept of Pharmacology, Human Medical Genetics and Neuroscience Programs, Univ Colorado Hlth Sci Ctr, Aurora, CO; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030, USA.

We have previously reported that sequences encoding DUF1220 protein domains are increasingly amplified generally as a function of a species evolutionary proximity to humans, where the greatest number of copies (212) is found. Human DUF1220 domains also show signs of positive selection and are highly expressed in human brain regions thought to be involved in higher cognitive function. These findings suggest that the extreme amplification of DUF1220 domains in the human lineage may contribute to the unique brain-related characteristics, such as increased size, that distinguish the human brain from that of other species. Recent reports found copy number variations (CNVs) in chromosome 1q21.1 present in individuals with microcephaly and macrocephaly, as well as in individuals with schizophrenia (SZ) and autism spectrum disorders (ASD). Compared to unaffected individuals, brain sizes and 1q21.1 copy number in affected individuals with microcephaly and with SZ tend to decrease, or increase in those with macrocephaly and with ASD. Because the majority of DUF1220 domains map to this same region, we tested whether, and to what degree, DUF1220 copy number correlated with brain size differences in a subset of these patients. To accomplish this, QPCR analyses were carried out on genomic DNA from micro- and macrocephalic individuals with 1q21.1 rearrangements (n=33) using primer sets specific for DUF1220 domains and several other 1q21.1 target sequences. Analysis of resulting data indicate that DUF1220 domain copy number and that of a BAC clone flanked by DUF1220-encoding sequences, showed the highest correlation with head circumference (FOC Z-score) ($p<0.0001$), with FOC Z-score increasing on average 0.13 for each one-copy increase in DUF1220 ($p<0.0001$). These data 1) provide support for the view that changes in DUF1220 domain copy number not only may influence evolutionary differences in brain size, but also may influence brain size differences within the human population and underlie some forms of microcephaly and macrocephaly, and 2) suggest that the evolutionary advantage of increasing DUF1220 copy number may have favored retention of the high genomic instability of the 1q21.1 region which, in turn, precipitated a spectrum of human brain and developmental disorders.

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## 2767/F/Poster Board #475

**Joint CNV/SNP haplotype and missing data inference.** *S-Y. Su[1], J. Asher[2], D. Balding[1], A. Blakemore[2], P. Froguel[2], R. Sladek[3], M-R. Jarvelin[1,4], L. Coin[1].* 1) Department of Epidemiology and Public Health, Imperial College, London W2 1PG, UK; 2) Section of Genomic Medicine, Imperial College, Hammersmith Hospital, Du Cane Road, London UK; 3) Departments of Medicine and Human Genetics, McGill University and Genome Quebec Innovation Centre, 740 Ave Dr Penfield, Montreal, QC; 4) Institute of Health, University of Oulu, Oulu, Finland.

With the increasing rate of discovery of copy number variation (CNV) throughout the human genome and its potential impact on human diversity and complex diseases, there is growing interest in investigating CNVs associated with diseases and evolutionary history. Haplotype information is useful for these investigations. However, in most CNV datasets, CNV haplotype information is not directly available and must be inferred from the data. Here, we present a method for imputing missing CNV genotypes and inferring haplotypic phase from both CNV and SNP genotypes in a large-scale population-based study. Our method is based on a haploid hidden Markov model, which models the joint haplotype structure between CNVs and SNPs. Thus, haplotypic phase of CNVs and SNPs are inferred simultaneously. A sampling algorithm is employed to infer haplotypic phase and missing genotypes and to obtain a certainty measurement of each estimate. Our approach is flexible, allowing the copy number (ploidy) vary along the investigated sequence and across the individuals. In order to investigate the effectiveness of this approach, we took genotype data on genotyped male X chromosomes as well as known CNV regions on these samples (from array CGH) and randomly paired these into phased diploid CNV/SNP haplotypes. We then investigated how well we could reconstruct the phase of this data as well as infer missing CNV genotypes. The results of the simulation study suggest that our method provides accurate estimates of missing CNV genotypes and CNV haplotypic phase. With the increasing numbers of the CNV regions identified and the improving technology for genotyping CNV, our program is a very useful tool for the CNV haplotype analysis.

## 2768/F/Poster Board #476

**Integrated study of copy number states and genotype calls using high density SNP arrays.** *W. Sun[1,2], F. Wright[1], Z. Tang[1], S. Nordgard[2,3], P. Van Loo[3,4,5], T. Yu[6], V. Kristensen[3], C. Perou[2].* 1) Department of Biostatistics, University of North Carolina, Chapel Hill, NC, USA; 2) Department of Genetics, University of North Carolina, Chapel Hill, NC, USA; 3) Department of Genetics, Institute for Cancer Research, Oslo University Hospital-Radiumhospitalet, Oslo, Norway; 4) Department of Molecular and Developmental Genetics, Vlaams Instituut voor Biotechnologie, Leuven, Belgium; 5) Department of Human Genetics, Katholieke Universiteit Leuven, Leuven, Belgium; 6) Department of Biostatistics, Emory University, Atlanta, GA, USA.

We propose a statistical framework, named genoCN, to simultaneously dissect copy number states and genotypes using high density SNP arrays. There are at least two types of genomic DNA copy number differences: Copy Number Variations (CNVs) and Copy Number Aberrations (CNAs). While CNVs are naturally occurring and inheritable, CNAs are acquired somatic alterations most often observed in tumor tissues only. CNVs tend to be short and more sparsely located in the genome compared to CNAs. GenoCN consists of two components, genoCNV and genoCNA, designed for CNV and CNA studies, respectively. In contrast to most existing methods, genoCN is more flexible in that the model parameters are estimated from the data instead of being decided a priori. GenoCNA also incorporates two important strategies for CNA studies. First, the effects of tissue contamination are explicitly modeled. Second, if SNP arrays are performed for both tumor and normal tissues of one individual, the genotype calls from normal tissue are used to study CNAs in tumor tissue. We evaluated genoCN by applications to 162 HapMap individuals and a brain tumor (Glioblastoma) dataset and showed that our method can successfully identify both types of CN differences and produce high quality genotype calls.

## 2769/F/Poster Board #477

**Copy number calling in novel human genomic sequences and selected CNV regions by array CGH.** *A. Tsalenko[1], N. Sampas[1], J. Kidd[2], P. Anderson[1], P. Tsang[1], E. Eichler[2,3], L. Bruhn[1].* 1) Agilent Laboratories, Santa Clara, CA; 2) Department of Genome Sciences, University of Washington, Seattle WA; 3) 3Howard Hughes Medical Institute, University of Washington, Seattle WA.

Copy Number Variation (CNV) is now recognized as a prevalent form of structural variation in the genome contributing to human genetic variability. Methods for accurate CNV measurements over a wide dynamic range are needed to fully explore association of CNVs to various Mendelian and complex diseases. We are developing microarray-based methods and data analysis tools to determine absolute copy number states in various copy number variant regions of the genome including regions of segmental duplications and novel genomic sequences discovered by fosmid end-sequencing approaches [Kidd et al. Nature, May 1 (2008)] not yet mapped to the human reference genome assembly. In this study, we designed a 244k feature oligonucleotide microarray with 55k probes targeting non-repeat-masked sections of 3403 distinct novel contigs ranging from 1kbp to 5kbp in size, and with remaining probes targeting selected known copy number variant regions of the genome, including regions of segmental duplications and selected simple deletions. We used a simple ULS-based labeling protocol to characterize 30 HapMap samples using two-color hybridizations with a common reference sample (NA15510). For the novel contig sequences, we confined our analysis to those 2515 (74%) contigs with at least three probes remaining per sequence after removing probes with signals corresponding to less than one copy across the sample set. For these contigs and profiled copy number variant regions, we estimated copy numbers in all samples using the combination of measured log2ratios and signal intensities. Of the 2515 novel sequences present in at least one copy in at least one sample, 95% are estimated to be present in at least a single copy in all 30 samples. 40% of these sequences are estimated to be polymorphic across this sample set. In simple deletion regions, we observed sharp differences in signal intensities in samples with 0, 1 or 2 copies. In addition, in regions with known higher copy numbers signal intensities were consistent with having up to 40 copies in some samples.

## 2770/F/Poster Board #478

**Single-base Pair Breakpoint Resolution Map of Small To Large Indels Using a Split Read Technique with High-throughput Mate-pair and Fragment Sequencing.** *E.F. Tsung[1], H.E. Peckham[1], Y. Fu[1], C.J. Kennedy[1], S.S. Ranade[2], C.C. Lee[1], C.R. Couser[1], J.M. Manning[1], C.L. Hendrickson[1], L. Zhang[1], E.T. Dimalanta[1], T.D. Sokolsky[1], J.K. Ichikawa[1], A.P. Blanchard[2], M.W. Laptewicz[1], J.A. Malek[3], F.C. Hyland[2], F.M. De La Vega[2], G.L. Costa[1], K.J. McKernan[1].* 1) Applied Biosystems, Beverly, MA, 01915; 2) Applied Biosystems, Foster City, CA 94404; 3) Weill Cornell Medical College in Qatar, Doha, Qata.

Insertions and deletions are often classified as small events detectable within a read and large events detectable between mate-pair reads. We demonstrate that we are now able to detect mid-size variants, previously unattainable by either approach, by using 2x50-bp mate-pair libraries with the Applied Biosystems SOLiD™ System. Specifically, we are able to detect variants of up to 500 bp in length throughout the genome with mostly single-base pair resolution using split-reads as well as variants as small as 98 bp with a novel approach for detecting indels between mate-pair reads that are smaller than the standard deviation of the insert size of the library. With modest sequence coverage of 8x for the HapMap individual NA18507, we are able to detect 1156 intra-read deletions of sizes 21 to 500 bp, of which 49.1% have been previously identified in the Venter, Watson, or YanHuang genomes. Of these 1156 variants, 193 are also identified with the 2x50-bp libraries using deviations in the average insert size between reads. We demonstrate that we are able to detect all size ranges of deletions with no gap for medium-sized variants. The 2x50 bp libraries also identified small intra-read indel events of sizes up to 19 bp. Combining this with 50-bp fragment libraries and 2x25-bp mate-pair libraries, we detect a total of 124,936 small deletions and 100,437 small insertions with a dbSNP b129 concordance of 67.2%. Overall, a high prevalence of small indels, even sized indels, and Alu sized deletions (300-350 bp) are found in this genome.

Furthermore, we have called indels in several HapMap individuals and show that we are able to detect small variants even at low sequence coverage (~2x) and others at higher coverage (~10x). We also demonstrate the detection of heterozygous indels and the genome wide frequency of different indel allele sequences, some of which may be in common with biologically significant motifs. Finally, this split-read technique was used to achieve breakpoint resolution of other large structural variations such as inversions. Given the importance of precise placement and the previously unattainable size of these variants, this technique will enable the discovery of a new class of structural variants using ultra-high throughput sequencing.

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## 2771/F/Poster Board #479

**A High Resolution CNV Survey of the GABA Gene Family Candidate Genes in Autism.** *P.L. Whitehead[1], D.J. Hedges[1], H.N. Cukier[1], D. Ma[1], J.M. Jaworski[1], H.H. Wright[2], R.K. Abramson[2], L. Nathanson[1], J.P. Hussman[3], J.L. Haines[4], M.L. Cuccaro[1], J.R. Gilbert[1], M.A. Pericak-Vance[1].* 1) Miami Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) University of South Carolina, School of Medicine, Columbia, SC; 3) Hussman Foundation, Ellicott City, MD; 4) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, NC.

Autism is a common neurodevelopmental disorder with a significant genetic component and locus heterogeneity. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain, acting primarily via the GABA receptors (GABR). Multiple lines of evidence, including alterations in levels of GABA and GABA receptors in autistic patients, indicate that the GABAergic system may be involved in the etiology of autism. Recent findings also suggest that rare copy number variants (CNVs) can play a role in autism risk. We therefore decided to examine the GABR's and genes in related pathways for CNVs that may be associated with autism. For the high resolution CNV screen we employed custom designed high density Comparative Genomic Hybridization (CGH) arrays (Agilent). These 1x244k Agilent CGH arrays covered 23 putative GABRs and 14 other genes involved in GABA metabolism or pathways and allowed the detection of small structural variants that would typically go undetected using standard whole genome CGH arrays. Collectively, our probes spanned a total of 15 Mb with a density of approximately 1 probe every 150 nucleotides allowing a theoretical resolution for detection of CNV's of 700bp or greater. 250 autism cases and 250 control individuals were analyzed for CNVs. Preliminary findings have identified CNVs affecting multiple GABA related genes. Notable results to date include a deletion removing 3 exons from the GABRB3 gene detected in one autism case and no controls and one intronic deletion within the X-linked GABRA3 gene observed in 18 cases and 3 controls. A deletion removing 2 exons from the GABRR1 gene was observed in 2 autism cases and 0 control individuals. Finally, a deletion removing 2 exons of the SLC6A1 gene was observed in 1 autism case and 0 controls. These observations provide further support for the possible role of genetic variation within GABA genes in autism. These data also demonstrate that important structural DNA variation exists at size ranges below the current resolution of standard genome-wide array CGH and SNP-based arrays.

## 2772/F/Poster Board #480

**A Quantitative Real-Time PCR Method for Detection of UGT2B17 Copy Number Variants in Studies of Bone Disease.** *S.G. Wilson[1,2,3], S. Chew[1], B.H. Mullin[1], J. Lewis[1,2], T.D. Spector[3], R.L. Prince[1,2].* 1) Endocrinology & Diabetes, Sir Charles Gairdner Hosp, Nedlands, Australia; 2) School of Medicine & Pharmacology, The University of Western Australia, Crawley, Western Australia; 3) Department of Twin Research & Genetic Epidemiology, King's College London, London, UK.

Osteoporosis is characterized by low bone mineral density (BMD) and deterioration in bone microarchitecture. Genetic variation is a major determinant of bone structure. Yang *et al.* (Am J Hum Genet 2008 83:663-7) recently reported that a DNA sequence copy number variation (CNV) including the *UGT2B17* gene, was significantly associated with the risk of osteoporotic hip fracture. This gene encodes an enzyme with a role in glucuronidation of steroid hormones, particularly testosterone and dihydrotestosterone. We developed a high-throughput quantitative real time PCR (qRT PCR) method for genotyping candidate CNV polymorphisms. Subjects were genotyped using 30ng of genomic DNA, custom primers (UGT2B17: Fwd 5'CCAGATGAGTATGGGCACTACA, Rev 5'GGTCTCAGGTAATCCTCCACCT; Control: Fwd 5'TCATCTGGGTCCTAGCCATC, Rev 5'AGGGGAGCCAGGTAGATGTT) and a QuantiFast SYBR Green PCR Kit (Qiagen). The genotype of the CNV was determined using the Pfaffl equation. This approach accounts for the respective amplification efficiencies of CNV and control primers and threshold cycle (Ct) values for test and control DNA. By this technique subjects were categorized as having zero, one or two copies of the *UGT2B17* CNV. The technique was validated by genotyping the CNV in 1347 subjects from a population-based cohort of Caucasian women aged 70 - 85 years. Multiple linear regression (MLR) was used to assess the association, with BMD regressed against age, weight and *UGT2B17* CNV. The genotype frequency and mean Ct ratio for homozygotes, heterozygotes and wild-type homozygotes was 12.0% (0.01 ± 0.01), 45.5% (0.56 ± 0.08) and 42.5% (1.00 ± 0.16; mean ± SD), respectively. Further validation was performed using gel electrophoresis of the PCR products. The CNV genotype distribution was consistent with the expected Hardy-Weinberg distribution ($\chi^2$ test, $P > 0.05$) and not different from frequencies reported by Yang *et al.* Data from MLR showed that total hip BMD was associated with weight ($P < 0.001$) and *UGT2B17* CNV genotype ($P = 0.039$). Individuals homozygous for the deletion of *UGT2B17* had higher mean total hip BMD compared with individuals with 2 copies of the gene (0.819 ± 0.01 vs 0.806 ± 0.01, mean ± SE respectively).These data suggest that qRT PCR is a rapid, high-throughput technique for determination of candidate CNVs. This technique should be useful for the determination of genotypes for *UGT2B17* CNV, and other CNVs. This study provides further evidence of association of the *UGT2B17* CNV with BMD.

## 2773/F/Poster Board #481

**Comparing CNV detection methods for SNP arrays.** *L.M. Winchester[1], C. Yau[2], J. Ragoussis[1].* 1) Genomics Laboratory, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX11 8JY, UK; 2) Henry Wellcome Centre for Gene Function, Department of Statistics, University of Oxford, Oxford, UK.

Data from whole genome association studies can now be used for dual purposes, genotyping and copy number (CN) detection. We present a comparison of the methods for using SNP data to detect CN events and describe the use of several statistical models in CN detection in germline samples. We examine a number of algorithms designed to detect CN changes through the use of signal intensity data and consider methods to evaluate the changes found. We apply a selection of proprietary and academically developed algorithms on a set of well characterised CEPH samples to give an indication of detection accuracy and success of CN change prediction on Affymetrix and Illumina data. For a single HapMap sample, NA12156, we found an overlap of 20-49% between events detected by algorithm and previously published sequencing data. Algorithms in the comparison include Birdsuite, GADA, PennCNV and QuantiSNP which detect both CNVs and CNPs. We conducted a secondary comparison on a sample, NA15510, characterised by three different studies and found an overlap of below 23% between detected events and the published validated results for each algorithm. Few events were common to all three published studies (~7%, 3% and 3% respectively). We observed similar variation between different publications as between detected events in our data and published results, suggesting this is a problem in all comparisons and not unique to the algorithms we tested. Comparison of the events detected between software directly on NA10861 showed a low level of overlap between most algorithms and platforms. This ranges from 0 overlapping events (between GADA for Illumina and CNAT for Affymetrix) to 100% of detected events overlapping (between CNAT for Affymetrix and other Affymetrix targeted detection algorithms). We show the expected similarities between data generated by the same platform and also some of the striking differences between lists of detected events from the same data using different algorithms. We conclude by suggesting a number of factors for other users to consider when picking an algorithm for their own data analysis.

## 2774/F/Poster Board #482

**Copy Number Variation prediction using Bayesian Hidden Markov Model.** *Z. Zhang[1], R. John[1], C. COGA[2], C. COGEND[3], C. GENEVA[4].* 1) Dept. of Psychiatry, Washington University in St. Louis, Saint Louis, MO; 2) COGA project; 3) COGEND project; 4) GENEVA project.

Taking the Copy Number Variant (CNV) status as the hidden states and the hybridization intensity measurements from microarrays as the observed states, we applied a Bayesian Hidden Markov Model (HMM) to the Gene Environment Association (GENEVA) alcohol addiction GWAS data. The method uses the Metropolis-Hastings algorithm for sampling with the CNV considered in a time-series analysis. Compared to other existing Bayesian models, the method offers a promising approach for CNV distribution prediction. Further investigation of the model will be applied and a user-interface program will be made available.

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## 2775/F/Poster Board #483

**Rare pathogenic microdeletions and tandem duplications are microhomology-mediated and stimulated by local genomic architecture.** *L.E.L.M. Vissers[1], S.S. Bhatt[2], I.M. Janssen[1], Z. Xia[2], S.R. Lalani[2], R. Pfundt[1], K. Derwinska[2,3], B.B.A. de Vries[1], C. Gilissen[1], A. Hoischen[1], M. Nesteruk[2,3], B. Wisniowiecka-Kowalnik[2,3], M. Smyk[2,3], H. Brunner[1], S.W. Cheung[2], A. Geurts van Kessel[1], J.A. Veltman[1], P. Stankiewicz[2].* 1) Dept Human Genetics 855, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA; 3) Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland.

Genomic copy number variation (CNV) plays a major role in various human diseases as well as in normal phenotypic variation. For some recurrent disease-causing CNVs that convey genomic disorders, the causative mechanism is meiotic non-allelic homologous recombination between breakpoint regions exhibiting extensive sequence homology (e.g. low-copy repeats). For the majority of recently identified rare pathogenic CNVs, however, the mechanism is unknown. Recently, a model for CNV formation implicated mitotic replication-based mechanisms, such as (alternative) non-homologous end joining and fork stalling and template switching, in the etiology of human pathogenic CNVs. The extent to which such mitotic mechanisms contribute to rare pathogenic CNVs remains to be determined. In addition, it is unexplored whether genomic architectural features such as repetitive elements or sequence motifs associated with DNA breakage stimulate the formation of rare pathogenic CNVs. To this end, we have sequenced breakpoint junctions of 38 rare pathogenic CNVs, representing the largest series of such CNVs examined to date in this much detail. Our results demonstrate the presence of (micro)homology ranging from 2 bp to over 75 bp, in 79% of the breakpoint junctions. This indicates that microhomology mediated repair mechanisms, including the recently reported fork stalling template switching and/or microhomology-mediated break-induced replication prevail in rare pathogenic CNVs. In addition, we found that the vast majority of breakpoints (81%) were associated with at least one of the genomic architectural features evaluated. Moreover, an additional 8% of breakpoints were associated with the presence of two novel sequence motifs, specifically enriched in duplication breakpoint regions. These data suggest that rare pathogenic CNVs do not occur at random genome sequences, but are stimulated and potentially catalyzed by various genomic architectural features.

## 2776/F/Poster Board #484

**Comparison of copy number calling algorithms and integrated analysis of gene expression in cancer cell lines.** *A. Oudes, A. Hoover, S. Vega.* Biosoftware, Rosetta Biosoftware, Seattle, WA.

Genomic copy number variations (CNV) may play a role in modifying gene expression patterns in diseases such as cancer. Integrated analysis of gene expression and CNV data can provide insight into the relationship of gene dose and transcript level. We obtained a data set from the caBIG repository which contains 338 cancer cell lines that have been profiled for gene expression and genotype. Principal component analysis of the gene expression data revealed a separation of hematopoietic cancer cell lines from those derived from solid tumors. Comparison of copy number calls generated by PennCNV and the recommended Affymetrix Tools indicates a greater sensitivity for CNV by the PennCNV algorithm. Integrated statistical analysis of the data revealed both cis and trans-associations of gene copy number with gene expression. CNV varied with the tissue of origin for the cell line. The majority of CNV does affect expression of genes that overlap with the locus of the CNV. The data revealed similarities in copy number of genes in signaling networks which are known to be involved with cell proliferation and cancer. In this study we have identified CNV with concomitant gene expression changes which are of interest for further investigation in the context of cancer cell signaling.

## 2777/F/Poster Board #485

**Effect of Copy Number Variation Inference in Association Study with eQTLs.** *K.-Y.A. Kim.* Dept Preventive Med, Northwestern Univ, Chicago, IL.

Recent technological developments in detecting copy number variants (CNVs), coupled with the advancements in statistical methods to identify their harboring regions, have improved our ability to understand their influence on various traits. A particular trait that CNVs are shown to influence is the gene expression levels; i.e. the CNVs act as expression QTLs (eQTLs). A study on malaria has shown that CNVs in regulatory hot spots control transcriptional variation. CNVs exhibit a distinct characteristic compared to other markers in that the underlying genotypes are integer based rather than a binary class such as the SNP markers. Similar to SNPs, the raw data of CNVs are the signal intensity values which is then inferred to integer-based copy numbers. Since the assignment of the integer-based copy number is based on a statistical model, we need to scrutinize whether the raw intensity values acting as proxies to the actual copy numbers or model-based copy numbers in integers provide increased accuracy and power. The raw intensity values along with several statistical methods that assign integer copy numbers are compared to evaluate their performance in identifying eQTLs .

## 2778/F/Poster Board #486

**Identification of translocation breakpoints and large deletions in brain cancer by whole genome paired-end sequencing.** *M. Clark[1], H. Homer[2], B. O'Connor[1], B. Merriman[1], Z. Chen[1], S. Nelson[1].* 1) Department of Human Genetics, Univ California, Los Angeles, Los Angeles, CA; 2) Deparment of Computer Science, Univ California, Los Angeles, Los Angeles, CA.

Whole genome resequencing strategies can be used to directly identify genomic variations including single nucleotide variants, copy number variants, and small insertions and deletions. Here we describe our strategy for using paired-end sequence data to identify larger structural variation breakpoints by searching for significant changes in paired-end insert size. We performed two-and-a-half runs of the ABI SOLiD sequencer to completely sequence the entire genome of the U87-MG brain cancer cell line, which is known to contain a large number of structural aberrations. The raw colorspace data was aligned using the BFAST algorithm. We extracted paired-end reads with highly aberrant insert sizes (insert sizes greater than 3000 bases) from the set of uniquely aligned reads and then clustered them in this subset based on their genomic position. A number of filtering strategies were implemented to reduce false positives from the subset. First, reads within the clusters were analyzed to ensure that they had a distribution that fit given our paired end insert size. Then, reads within 1Mb of centromeres and telomeres were removed, as these regions include densely packed repetitive regions. The filtered dataset includes interchromosomal events, large intrachromosomal events, and smaller regional deletion events. Most of these events occur at positions where changes in base coverage suggest a change in copy number. Some represented complete genomic losses where base coverage between the breakpoints was zero. A few of the more interesting candidates were confirmed by PCR and dideoxy sequencing. In a separate experiment, paired-end whole genome sequencing was performed on a tumor/normal sample pair. The same analysis was run again, but this time events present in the normal and tumor were masked in order to identify novel events present in the tumor. Here we demonstrate our ability to identify the precise genomic positions of translocation, insertion and deletion breakpoints in cancer samples using paired-end whole genome sequence data generated in a small number of SOLiD sequencer runs and aligned with the BFAST algorithm.

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## 2779/F/Poster Board #487

**Identify genomic imbalance by high density whole genome microarrays in Chinese patients with neurodevelopmental disorders.** *X. Gong[1,*], X. Chen[2,*], Y. Jiang[3,*], Y. Shen[4,*], S. Li[1], Y. An[1], Y. Wang[1], X. Zhang[1], Y. Sun[1], H. Fang[4], X. Sheng[4], H. Shao[4], H. Zhu[4], A. Cheng[4], L. Jin[1], X. Wu[3], T. Zhang[2,#], H. Wang[1,#], B-L. Wu[1,4,#].* 1) Institutes of Biomedical Science and School of Life Science, Fudan University, Shanghai, China; 2) Capital Institute of Pediatrics, Capital Medical University, Beijing, China; 3) Department of Pediatrics, Peking University First Hospital, Peking University, Beijing, China; 4) Children's Hospital Boston and Harvard Medical School, Harvard University, Boston MA, USA (*contributed equally; #senior authors for each group of this collaboration study).

Genomic imbalances/rearrangements are a common cause of developmental delay, mental retardation and autism spectrum disorders, which have been demonstrated by the studies with cohorts of the western countries. We employed two most current high density whole genome microarrays (Agilent 244K CGH array and Affymetrix SNP 6.0 array) and 4 independent analytic algorisms to evaluate/compare the deletions and duplications in a Chinese cohort consisting of 350 patients. During the process, we also cross-validated the findings between the two DNA chip platforms and the results showed very high concordance rate. We detected total of 7891 copy number variants (CNVs) in the patient cohort and 608 CNVs in matched controls. The size of CNVs range from 50 kb to 15 Mb and the detection ratio is 3:1 in patients and controls. We identified two types of genomic imbalances in the Chinese cohort: deletions or duplications at 15q11.2-12, 15q13.2-13.3, 16p11.2, 16p13.3 and 17q12 are the examples of recurrent reciprocal imbalance, all of these are recently recognized genomic disorders/syndromes; unrelated cases with overlapping deletions or duplication involving 2p12, 2q33.1, 3q12.3, 7q22, 7q31.1, 13q13.1-14.1, 14q13.2, 17p12, exemplify the imbalance occurred randomly or de novo. The most frequent recurrent reciprocal imbalances (del/dup) identified in Chinese patients are at 1q21.1, 3q11.2, 9p13.1, 9q11.2, 10q11.2, 15q11.2-12, 16p11.2, 19q12, 22q13.3, Xp22.3 and Xq28. The high density whole genome arrays also detect multiple imbalance events in each patient but many are novel CNVs with unknown clinical significance. Based on current database and literature, we identified clinically relevant genomic imbalances in about 20% of total cases. Our data demonstrated the utility of whole genome array for detecting genomic imbalance. The detection of recurrent genomic imbalance events among Chinese patient further confirmed shared etiologies among patients of different ethnicities. Since developmental disorders are a broad clinical diagnosis of heterogeneous genetic etiologies, extensive clinical phenotype evaluation and further dissection of genomic rearrangement are of great importance towards better understanding of the disorder and for eventual better patient care. In addition, to our knowledge this study is the first to reveal detailed CNVs (pathogenic and polymorphic) data of the patient cohort and matched controls both from Han Chinese population.

## 2780/F/Poster Board #488

**Development of a Custom Microarray for Inherited Vascular Diseases.** *S. Mitchell[1], T. Lewis[2], P. Bayrak Toydemir[2,3].* 1) Technology Transfer, ARUP Laboratories, Salt Lake City , UT; 2) ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT; 3) the Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT.

Oligo-comparative genomic hybridization (CGH) microarray technology has become an important tool in detecting large duplications and deletions of the human genome. Multiple formats are available with different resolutions that allow for many different applications. The development of custom oligo-CGH arrays can be useful to target specific regions of the genome that are associated with disease. This can be a useful tool when clinical symptoms of multiple diseases overlap. Samples analyzed by sequencing that show no significant changes could be further interrogated using a custom oligo-CGH array. The ability to strip used microarray slides and re-hybridize can help decrease costs when used on a research basis. We present our results with a custom oligo-CGH array we developed to detect deletions and duplications in patients with inherited vascular diseases. A custom oligo-CGH array was designed to analyze 13 genes for 7 different vascular diseases including Hereditary Hemorrhagic Telangectasia, RASA1-related disorders, Loris-Dietz Syndrome, and Hereditary Glomuvenous malformations. The format chosen was a 1 by 385K array. The density was 1 probe for every 10 base pairs of all exonic and intronic regions with a 2kb extension on the 5' end and 1kb extension on the 3' end of each gene. Five patients with known aberrations verified by Multiple Ligation-Dependent Probe Amplification (MLPA), and 3 unknown samples were studied. Self/self hybridization was performed to test the probe efficiency. Five microarray slides were used and stripped multiple times to test re-hybridization efficiency. Known aberrations were confirmed for the samples that worked on the microarray. The smallest change in our cohort was a 300bp deletion, which corresponds to a single exon deletion in ACVRL1 gene. One duplication greater than 380bp was found for one unknown sample but was not reproducible by MLPA. Discrepant results could be due to criteria set for calling a true aberration. Multiple re-hybridizations (up to 4 times) of slides showed a decrease in confidence in calling true aberrations. A custom oligo-CGH array for inherited vascular diseases is beneficial in cases where sequencing shows no significant changes. It provides information about large genomic rearrangements of multiple genes simultaneously and is a cost effective tool for research applications. Further sensitivity and specificity studies need to be done to reach single exon resolution for diagnostic purposes.

## 2781/F/Poster Board #521

**Gene dosage analysis of SMN1 gene for screening carriers of spinal muscular atrophy.** *J.Y. Wang[1], C.H. Lee[2], J.S. Lee[2].* 1) BK 21 Project Medical Sci, Graduate Sch Yonsei Univ, Seoul, Korea; 2) Dept Cilinical Genetics, Yonsei Univ Col Medicine, Seoul, Korea.

Spinal muscular atrophy (SMA) is an inherited autosomal recessive disorder characterized by progressive muscle weakness resulted from degeneration and loss of the anterior horn cells in the spinal cord and the brain stem nuclei. The two genes associated with SMA are survival motor neuron 1(SMN1) and survival motor neuron 2(SMN2) on chromosome 5q13. SMN1 and SMN2 are present in two highly homologous copies. SMN1 gene is the primary disease-causing gene and about 95-98% of individuals with SMA show homozygous deletion of exon 7 and 8 of SMN1. In SMA patients, SMN2 partially compensates for the lack of SMN1. The incidence of SMA is 1 in 10,000 livebirths and carrier frequency is 1 in the range of 35 to 50. In practice, there is no effective treatment for this disease. Therefore, precise quantification of SMN1 and SMN2 gene copy numbers is essential for diagnosis and genetic counseling. The aim of our study was to establish an efficient and rapid method for prenatal diagnosis of SMA and detection of carriers for proper counseling. We developed fragment analysis with amplified exon8 of SMA1 and SMN2 genes covering each of fluorescence labeled Locked Nucleic Acid (LNA) to quantify SMN1 gene copy number using the ABI 3100 genetic analyzer. DNA samples from a total of 175 individuals including 5 patients with SMA lacking the SMN1 gene, 70 patients with developing symptoms of SMA, and 100 control individuals from the general population were analyzed. By applying this technique, we could detect SMA patients efficiently just by recognizing a SMN2 gene peak. Furthermore, for the diagnosis of carriers, we used multiplex PCR by simultaneously amplification of the human albumin gene and examined SMN1/SMN2 gene dosages by fragment analysis. It was possible to differentiate carriers from normal controls. In this study, a simple, rapid, non-gel-based method for the diagnosis of patients and carriers carrying deletion(s) of SMN 1 gene was described and the method can be useful for the diagnosis of SMA in practice.

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## 2782/F/Poster Board #522

**Determination of parental origin by SNP array of *de novo* copy number mutations identified in offspring of atomic-bomb survivors.** *Y. Satoh[1], K. Sasaki[1], I. Fukuba[2], E. Hiyama[2], M. Imanaka[1], Y. Shimoichi[1], J. Kaneko[1], M. Kodaira[1], N. Takahashi[1].* 1) Dept. Genetics, Radiation Effects Research Foundation, Hiroshima, Japan; 2) N-BARD, Hiroshima Univ., Hiroshima, Japan.

It is widely known that there are many copy number polymorphisms in the human genome and several are related to certain kinds of disease or individual differences. While most are transmitted from parent to child, a few arise *de novo*. We have investigated whether atomic-bomb radiation causes *de novo* copy number mutations in offspring of atomic-bomb survivors. In case only one of the parents was exposed to radiation, it was necessary to discriminate in which one of homologous chromosome of offspring derived from the parents the mutation occurred. In order to examine the whole human genome, we made BAC-array CGH on which about 2,500 BAC clones were printed. The study used genomic DNA from 265 offspring of atomic-bomb survivors and 40 offspring of an unexposed control group. Three mutations were identified in three offspring, that were not in their parents. One mutation was a deletion type involving a 1.4 Mb length of chromosome 1q41 and two mutations were an amplification type involving 131,290 bp of 5p15.2 and 431,737 bp of 17p13.3, respectively. In the breakpoints of the deletion mutation, segmental duplications were involved in both termini. Precise breakpoints could not be determined. There were microhomologies in the breakpoints of both two amplification mutations. Because only fathers were the atomic-bomb survivors in all three families, we needed to determine parental origin of the *de novo* mutations. We used an Affymetrix SNP array 6.0 to examine mendelian inconsistency of SNP information in the genome region involving the mutations. We examined 422 SNPs in the genome region involving the deletion mutation, of which 43 SNPs were informative for discrimination of parental origin. There were 54 and 85 SNPs in the genome regions involving the two amplification mutations, of which 15 SNPs were informative for each. Informative SNPs for each case showed that all three mutations occurred on a chromosome derived from the father. The SNP array was valuable for determination of the parental origin of *de novo* copy number mutations. We will continue and expand our study to increase the statistical power.

## 2783/F/Poster Board #523

**Mobile Element Scanning (ME-Scan): complete ascertainment and genotyping of transposons in large population samples using targeted high-throughput sequencing.** *D.J. Witherspoon[1], J. Xing[1], Y. Zhang[1], M.A. Batzer[2], L.B. Jorde[1].* 1) Dept Human Genetics, Univ of Utah; 2) Louisiana State Univ.

**Background:** Mobile elements (MEs) are diverse, common and dynamic inhabitants of nearly all genomes. Their transposition generates a steady stream of polymorphic genetic markers, deleterious and adaptive mutations, and substrates for genomic rearrangements. Research on the impacts, population dynamics, and evolution of MEs is constrained by the difficulty of ascertaining new ME insertions and genotyping them in many individuals. **Methods:** We use ME-specific primers to construct DNA libraries that contain the junctions of all ME insertions, with their flanking genomic sequences, from many individuals. Up to 50 individual libraries are pooled and sequenced on an Illumina Genome Analyzer (36 bp paired-end reads) using a ME-specific sequencing primer. All ME insertion loci of the target family in the sample are uniquely identified by their junction sequence, and all insertion junctions can be linked to their source individuals by means of an individual-specific “barcode” sequence designed into the library oligonucleotide adapters. **Results:** The large number of *AluYb8* insertions in humans (~3,000 per haploid genome) and the high background of related *Alu* elements (> 1 million) makes this subfamily a suitable challenge for our method. Initial sequencing generated ~5.5 million read pairs from seven individual libraries that were combined at various concentrations. More than 95% of read pairs represent the desired *AluYb8*-junction products and are tagged by exact matches to one of our barcode sequences. Barcode counts show that the sequenced products reflect the intended individual library pooling proportions to within 1%. We are able to detect more than 98% of the ~1,400 *AluYb8* insertions that are believed to be fixed and contain exact matches to the amplification and sequencing primer binding sites. The few failures include some insertions that are in fact polymorphic and absent in our samples. Less-perfect *AluYb8* insertions and known polymorphic insertions are also very reliably detected, as are the expected several hundred novel insertions per individual. We are currently able to reliably ascertain - in all individuals in a sample - the presence or absence of at least 80% of *AluYb8* insertion loci.

## 2784/F/Poster Board #524

**Involvement of DNA repair-based mechanism in genomic rearrangements of Pelizaeus-Merzbacher disease.** *G. Hobson[1], K. McLean[2], L. Banser[1], D. Lavoie[1], K. Clark[1], T. Alberico[3], J. Garbern[4], K. Sperle[1].* 1) Nemours Biomedical Research, Alfred I. duPont Hospital for Children, Wilmington, DE; 2) Penn State Hershey Medical School, Hershey, PA; 3) Functional Genomics Unit, National Institutes of Health, Bethesda, MD; 4) Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI.

Pelizaeus-Merzbacher disease (PMD) is a leukodystrophy most frequently caused by duplication of the proteolipid protein 1 gene (*PLP1*) at Xq22.2. It has been proposed that these genomic duplications are a result of a coupled homologous and nonhomologous DNA repair-based mechanism (CHNR) involving a double-strand DNA break and nonhomologous end-joining (NHEJ). More recently it was reported that many rearrangements in the *PLP1* region are complex and refractory to junction analysis. A replication-based mechanism termed Fork Stalling and Template Switching (FoSTeS) requiring a single-strand DNA break and microhomology for the template switch was proposed for these rearrangements. We present our analyses of genomic rearrangements in 60 PMD patients by semiquantitative PCR, array CGH, interphase FISH, and junction analysis. We report evidence for complex rearrangements in 33% of patients, including those with two duplicated regions, and with both duplicated and higher copy number regions. We amplified and sequenced a total of 37 junctions. Templated filler insertions of 14 to 112 bases that are homologous to nearby regions create 2 sequence fusion points in 14% of these junctions; the remaining junctions each have one sequence fusion point. We report that 63% of sequence fusion points in our 37 junctions are microhomologies of 1 to 7 bases, 16% are inserts of 2 or 3 bases, 11% are blunt fusions, and 9% are chimeric repeat elements (Line or Alu). Based on similarity of our junction sequences with those found after repair of experimentally induced double-stranded breaks, we argue that a DNA repair-based mechanism is likely to be involved in the formation of simple and complex rearrangements in PMD.

## 2785/F/Poster Board #525

**Association Analysis of Kinesin family member 3 A (KIF3A) polymorphisms with aspirin intolerance in asthmatics.** *J. Cha[1], J. Park[1], A. Jang[1], S. Uh[2], Y. Lee[2], M. Kim[3], I. Choi[4], S. Cho[5], B. Park[6], H. Shin[6], C. Park[1].* 1) Genome Research Center for Allergy and Respiratory Disease, Soonchunhyang University Bucheon 1174 Jung-dong, Wonmi-gu, Bucheon, Gyeonggi-do, Republic of Korea 420-767; 2) Division of Allergy and Respiratory Medicine, Soonchunhyang University Hospital, 657, Hannam-Dong, Yongsan-Gu, Seoul, Republic of Korea,140-743; 3) Department of Internal Medicine, Chungbuk National University, College of Medicine, 62 Gaesin-dong, Heungduk-gu, Cheongju, Chungcheongbuk-do, Republic of Korea 361-711; 4) Department of Allergy, Chonnam National University Medical School and Research Institute of Medical Sciences, 8 Hakdong, Dong-gu, Gwangju, Republic of Korea 501-757; 5) Institute of Allergy and Clinical Immunology, Seoul National University Medical Research Center, 28 Yongon-Dong, Chongno-Ku, Seoul, Republic of Korea 110-744; 6) Department of Genetic Epidemiology, SNP Genetics, Inc.,1407 14th Floor, Woolimrall’ey B, Gasan-dong, Geumcheon-Gu, Seoul, Korea 153-803.

Background: The Kinesin family member 3A (KIF3A) gene on chromosome 5q31, is associated with granules and organelles within the proplatelets. Platelet activation has also been reported in both patients with allergic asthma and the experimental animal models of allergic inflammation. We investigated genetic polymorphisms of the KIF3A gene, and evaluated this locus as a potential candidate for aspirin-exacerbated respiratory diseases (AERD). Methods: The asthmatics (n=470) underwent the oral aspirin challenge (OAC). OAC reactions were categorized into 3 groups as follows: 20% or greater decreases in FEV1 or 15% to 19% decreases in FEV1 with extrarbronchial reactions (naso-ocular or cutaneous) (Y), 15% to 19% decreases in FEV1 or extrarbronchial reactions(naso-ocular or cutaneous) only (I), and 15% less decreases in FEV1(N). Results: The entire KIF3A gene was sequenced. DNA sequencing in 24 Koreans of the KIF3A gene, revealed 24 sequence variants: 23 in the intron region, one in the exon region (+38148C>T) and five haplotypes were constructed. The single nucleotide polymorphisms (SNPs) and haplotypes were significantly associated with AERD. The rare allele homozygotes on +22420 A>G showed higher in Y group than in N group (P=0.005). Asthmatics who had rare alleles for +22420A>G exhibited a more pronounced decrease in FEV1% after OAC than did those who carried the common allele (P=0.007). KIF3A gene ht3 and ht5 were associated with aspirin-induced bronchospasm, as reflected in the rates of decrease in FEV1% after OAC (P=0.004 and P=0.008). In the aspirin treated BEAS2B cell, mRNA and protein levels of KIF3A were higher than those of untreated BEAS2B cell. In the nasal polyp epithelium of AIA, KIF3A proteins seems higher expression than that of ATA. Conclusions: KIF3A SNPs +2779C>G and their haplotypes (ht3 and ht5) were associated with AERD. This work was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (A010249).

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## 2786/F/Poster Board #526

**Alternative transcription of human CHM gene including LTR12C element in colorectal cancer.** *Y. Jung[1], Y. Kim[1], J. Huh[2], D. Kim[3], H. Ha[1], K. Ahn[1], H. Kim[1].* 1) Department of Biological Science, Pusan National University, Busan, Korea; 2) National Primate Research Center, KRIBB, Ochang, Chungbuk, Korea; 3) Korea Bioinformation Center, KRIBB, Daejeon, Korea.

CHM (Choroideremia) gene located on human Xq21.2 is indispensable for the posttranslational activation of retina-specific rab protein. Various mutations such as missense, nonsense, rearrangement of the CHM gene are responsible for choroideremia. Long terminal repeat (LTR) elements have potential of transcription regulatory factors by providing of promoter activity and various transcription factor binding sites and cryptic splicing sites. The CHM has two reference transcript variants (NM000390.2 and NM001145414). Transcript variant 2 (V2) is derived from LTR12C providing a cryptic splicing site. Using bioinformatic tools, we could analyze transcript variant 2 sequences, indicating that transcript variant 2 has LTR12C sequences which results in new exon and different amino acids sequences. In order to identify effects of LTR12C, we performed RT-PCR analysis in the normal and adjacent cancer tissues. The V1 was ubiquitously expressed in various tissues, whereas the V2 expressed higher in colorectal cancer than normal tissue samples with similar expression patterns in other cancer tissues (liver, lung, testes). We also confirmed high expression of colorectal cancer in patient samples, suggesting that the CHM-V2 could be used as biomarker for colorectal cancer.

## 2787/F/Poster Board #527

**Evolutionary transcript diversification of DYX1C1 gene by HERV-H LTR integration and its application to cancer biomarker.** *Y. Kim, Y. Jung, H. Ha, K. Ahn, H. Kim.* Department of Biological Science, Pusan National University, Busan, Korea.

DYX1C1 is a candidate gene for developmental dyslexia. It has three alternatively spliced transcripts (V1, V2, V3). The V1 has HERV-H LTR element providing polyadenylation signal in last exon. The HERV-H LTR element on human DYX1C1 gene seems to have been integrated into our common ancestor genome after the divergence of Old World monkeys and New World monkeys by PCR amplification. From the sequencing analysis of various primate DNA samples, we found the lineage specific tandem repeated sequences of ORF region in Old World monkeys. We examined the effect of HERV-H element on DYX1C1 gene with various alternative transcripts. RT-PCR analysis is conducted in order to detect expression of the DYX1C1 gene in various human and rhesus monkey tissues, indicating that all of the alternative transcripts are expressed ubiquitously in the human and monkey tissues. We also identified four new alternative transcripts (V1-1, V1-2, V1-3, V1-4) related to HERV-H element in human and rhesus monkey. Those alternative transcripts are examined as a biomarker to detect specific cancer. All of transcripts including four new alternative transcripts appeared in various tumors, but detected differently between normal and adjacent tumor tissue. Semi-quantitative RT-PCR analysis and Image J program showed that the transcriptional activity of V3 and V2 was higher in tumor than in normal tissue samples, especially in the colorectal tissues. Our results indicated that integration event of the HERV-H LTR element contributed to the DYX1C1 gene transcription diversification during the primate evolution and alternatively spliced transcript variants could be used as cancer biomarkers to detect colorectal cancer.

## 2788/F/Poster Board #528

**Exon-level expression profiling using small quantities of whole blood total RNA.** *J.P. Sainsbury[1], P. Lacap[1], F.A. Plummer[1,2], M. Luo[1,2].* 1) HIV & Human Genetics, National Microbiology Laboratory, Winnipeg, Manitoba, Canada; 2) Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada.

Affymetrix® GeneChip® Human Exon 1.0 ST Arrays provide a unique opportunity to interrogate global gene expression at the exon level. However, the use of whole blood total RNA on Affymetrix® Exon Arrays is hampered by abundant globin mRNA which has been demonstrated to decrease percent present calls, decrease call concordance and increase signal variation during microarray hybridization. Moreover, the limited quantities of total RNA available from clinical research specimens is often insufficient for direct analysis with Affymetrix® Exon Arrays. The aim of this study is to present proof-of-concept data on the use of a sense RNA - specific linear amplification strategy in combination with globin mRNA hybridization capture technology to qualify 100ng of whole blood total RNA for use on Affymetrix® Exon Arrays. The efficiency of amplification was assessed using quantitative PCR (qPCR) of housekeeping genes and the efficiency of globin reduction was assessed using qPCR of globin mRNA. Microarray target hybridization of amplified, reduced samples was correlated with that of unamplified, reduced samples to assess faithful preservation of expression profiles during amplification. This study has assessed the feasibility of using a small quantity of total RNA, containing a high proportion of globin mRNA, to conduct high-resolution expression profiling with coverage of the entire transcriptome.

## 2789/F/Poster Board #529

**A novel strategy for the simultaneous detection of alternatively spliced MEF2C transcripts enables evaluation of splicing event co-variation.** *K. Sciabica[1], B. Ramachandran[2], Y. Wu[1], H. Yowanto[1], T. Gulick[2].* 1) Genetic Analysis, Beckman Coulter, Inc., Fullerton, CA; 2) Diabetes and Obesity Research Center, Burnham Institute for Medical Research, Lake Nona, FL.

Myocyte Enhancer Factor 2 (MEF2) proteins are transcription factors that exist in all metazoans and play pivotal roles in the development and differentiation of tissues. All MEF2 proteins have an amino-terminal MADS box and adjacent MEF2 signature domain that together confer sequence-specific DNA binding and dimerization activities. The carboxy-termini of MEF2 proteins are less well conserved and harbor transcriptional regulatory and nuclear localization functions. There are 4 vertebrate MEF2 genes, MEF2A, B, C and D, and these have different temporo-spacial expression patterns. MEF2A, C and D encode protein variants by virtue of alternative splicing of primary transcripts, and these genes have similar structures and alternative splicing patterns that are conserved across evolution. The alternative splicing involves mutually exclusive exons (alpha1 and alpha2), a cassette exon (beta), and alternative splice acceptors that flank a short region (gamma). The corresponding short polypeptide domains encoded by these alternative segments are nested within the MEF2 carboxy-termini and are structurally conserved across isotypes. These domains confer specific functions, including splice variant-specific functional interactions with co-activators; potent transactivation by an "acid blob" (beta); and transrepression that is mediated by SUMOylation (gamma)that is under control of various signaling events that modify MEF2 and act in cis to control steady-state MEF2 SUMOylation. As one aspect of an effort to elucidate the roles of MEF2 alternative splicing variants, we have developed an RT-PCR long fragment assay in which all 8 MEF2C mRNA isoforms can be simultaneously monitored and quantitated from cell and tissue samples. This assay is used to confirm and extend prior observations of regulated MEF2 alternative splicing among tissues, during development and during muscle differentiation. The technique is well suited for rapid quantitative evaluation of splicing variant expression, and could be effectively used for candidates with established splicing variants or for the validation of findings observed with "next generation" sequencing. In addition, our strategy uniquely allows for the evaluation of co-variations in multiple alternative splicing events for primary transcripts of a given gene. *For Research Use Only; not for use in diagnostic procedures.

## 2790/F/Poster Board #530

**Pilot study: Pathway analysis performed on formalin-fixed, paraffin-embedded pancreatic tissue.** *N.V. Michelsen[1,2], K. Brusgaard[1], M. Thomassen[1,3], T. Qihua[1], K. Hussain[4], H.T. Christesen[2].* 1) Dept of Clinical Biochemistry, Pharmacology and Genetics, Odense University Hospital, Odense, Denmark; 2) Dept of Paediatrics, Odense University Hospital, Odense, Denmark; 3) Human Microarray Centre, University of Southern Denmark, Odense, Denmark; 4) Great Ormond Street Children's Hospital NHS Trust and Institute of Child Health, London WC1N 1EH, United Kingdom.

**Aim.***Italic Text* The aim of this pilotstudy is to show that formalin-fixed, paraffin-embedded pancreatic tissue can be used in gene expression microarray studies for pathway analysis. We have previously shown that fixed pancreatic tissue from children with congenital hyperinsulinism (CHI) still contains disease-specific information that can be retrieved by cluster analysis. **Background.***Italic Text* We have used pancreatic tissue from children with CHI. Congenital hyperinsulinism (CHI) is a disease characterized by hyperinsulinemic hypoglycemia irrespective of blood glucose levels and is associated with a high risk of developing neurological handicaps. Most cases appear neonatal, but appearances in the late infancy and adulthood are seen. It is a heterogeneous disease with respect to phenotype and genetics. So far, six genes are known to be disease-causing. Depending on the underlying genetic cause and the severity of the disease, treatment includes conservative and medical management, and alternatively pancreasresection (resection of 60-95% of the organ). Based on these pancreasresected CHI biopsies, that were routinely formalin-fixed and paraffin-embedded, the overall purpose of this project is to detect new underlying genetic causes for CHI. **Samples.***Italic Text* In all, 20 pancreases were included in the study: 17 FFPE samples and three frozen samples. FFPE pancreatic samples consist in five CHI samples, 10 control samples, and two insulinoma samples; frozen samples consist in one insulinoma sample and two CHI samples. **Methods.***Italic Text* RNA extraction using ethanol/chloroform precipitation, RNA integrity measurement with Agilent 2100 bioanalyzer, Amplification and labeling of RNA using AminoAllyl MessageAmpII, Generation of a Genome wide oligonucleotide chip (828,830 genes in duplicates), Hybridization of sample and reference with Agilent Gene expression hybridization kit, Image Analysis, Normalization and data analysis. **Results.***Italic Text* Cluster analysis show that FFPE CHI samples cluster separately from control and insulinoma samples. Bioinformatic tools such as Gene set enrichment analysis (GSEA) and GenMAPP2.0 will be used for further data analysis. Based on the cluster analysis, we know that the disease-specific information is still present in stored tissue. Our results will show whether this information can also be extracted with pathway analysis.

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## 2791/F/Poster Board #531

**A WORKBENCH FOR MEASURING TRANSCRIPTION USING NEXT-GENERATION SEQUENCING.** *M. Busby, C. Stewart, D. Barnett, M. Stromberg, G. Marth.* Biol, Boston College, Chestnut Hill, MA.

High-throughput RNA sequencing has the potential to replace microarrays as the default technology for measuring transcription. It can be used for both discovery and quantification, and does not suffer from some of the problems seen with microarrays, such as uneven hybridization.

The complexity of analyzing high-throughput RNA reads currently puts this technology beyond the reach of laboratories which do not have the support of bioinformaticians experienced in high-throughput sequencing. Existing command-line applications that provide alignment and quantification of RNA reads are often technology-specific, or are daunting in their complexity. Statistical protocols for providing quality control and measuring differential expression between samples have not yet been firmly established. Even routine tasks such as file management can be time-consuming for labs that rely on the labor of biology graduate students, many of whom have never previously used a command-line application.

To solve this problem, we have developed a workbench for analysis of RNA-based sequences. The workbench integrates several components of the our own suite of tools including the Mosaik aligner and the Gambit visualization tool, as well as new tools developed for RNA analysis.

The goal of the workbench is to provide an easy to use and statistically robust method for analyzing high-throughput RNA data. It is designed so that, at the most basic level, the user can enter the locations of read, genome, and annotation files, and come back to a list of differentially expressed genes. More advanced options allow the user to visualize gene regions in the alignment and analyze differential splicing. Tools are also provided to measure the quality of the data (e.g. the portion of reads that align or whether samples cluster appropriately), to measure the depth of transcriptome coverage, and to analyze how unique the known coding regions in a given genome are for the purposes of read alignment.

In its initial incarnation the tool supports whole-transcriptome shotgun and 5' cap trap reads, and provides basic support for the analysis of miRNA. It currently supports data from 454, AB SOLID, and Illumina/Solexa technologies.

## 2792/F/Poster Board #532

**Disease Gene Characterization Through Large-Scale Co-expression Analysis.** *V. Funari[1,3], A. Day[2], J. Dong[2], B. Harry[2], D. Cohn[1,2,3], S. Nelson[2,4].* 1) Med Gen Inst, Cedars-Sinai Med Ctr, Los Angeles, CA; 2) Human Genetics, School of Med at UCLA, Los Angeles, CA; 3) Pediatrics, School of Medicine at UCLA, Los Angeles, CA; 4) Psychiatry, School of Medicine at UCLA, Los Angeles, CA.

**Background** In the post genome era, a major goal of biology is the identification of specific roles for individual genes. We report a new genomic tool for gene characterization, the UCLA Gene Expression Tool (UGET). **Results** Celsius, the largest co-normalized microarray dataset of Affymetrix based gene expression, was used to calculate the correlation between all possible gene pairs on all platforms, and generate stored indexes in a web searchable format. The size of Celsius makes UGET a powerful gene characterization tool. As an example, using only 14 known cartilage-selective genes as a seed list, UGET identified 78/100 genes identified previously in a large well annotated tissue survey of gene expression. In addition, UGET extended the list of known genes by identifying 32 new highly cartilage-selective genes. Of these, 7 of 10 tested were validated by qPCR including the novel cartilage-specific genes SDK2 and FLJ41170. Using only a few genes as an example, UGET easily identifies disease genes in disease-linked intervals. We retrospectively tested this first by identifying two recently published skeletal dysplasia disease genes in SEMD Mexican type and Autosomal Dominant Brachyolmia. We similarly tested UGET to identify disease-causing genes within known linkage intervals for genetically heterogeneous disorders in Joubert syndrome, microcephaly, and Type 2 limb girdle muscular dystrophy (LGMD2) and suggest novel candidate genes within a linkage region for Joubert Syndrome (JBTS1). Finally, we observed a significantly higher gene correlation shared between genes in disease networks associated with similar complex or Mendelian disorders. **Conclusion** One of the major challenges for the geneticist with the current genome is how to prioritize sequencing of genes (many of which are poorly annotated) for mutation analysis within genomic intervals linked to a disease. We report a new genomic tool for gene characterization which uses gene expression networks to help geneticist establish the candidacy of each gene in the linked interval. UGET is an invaluable resource for a geneticist that permits the rapid inclusion of expression criteria from one to hundreds of genes in genomic intervals linked to disease. As we demonstrate, this information can be critical in prioritization of candidate genes for sequence analysis.

## 2793/F/Poster Board #533

**Using Integrative Genomics to Develop Novel Diagnostic Phenotypes for Asthma Severity.** *J.A. Howrylak[1], S. Sharma[1], A.J. Murphy[1], B. Schuemann[1], S.T. Weiss[1], E. Xing[2].* 1) Pulm Crit Care Medicine, Brigham and Woman's Hospital, Boston, MA; 2) Department of Machine Learning, Carnegie Mellon University, Pittsburgh, PA.

Gene expression profiling (GEP) for disease classification in clinical practice is quickly becoming an important aspect of patient management for a variety of disorders, particularly cancer. Though several groups have studied gene expression in asthma, the potential utility of GEP for severity classification in asthma remains unknown. To address this question, GEPs were developed from peripheral blood CD4+ lymphocyte RNA in 200 young adults (ages 16-23 years) with asthma. Subjects were participants in the Childhood Asthma Management Program Continuation Study, and blood samples were drawn during a routine study visit, together with detailed phenotype information obtained via questionnaire. Responses to questions related to asthma symptoms and medication use over the prior week were used to classify asthma severity. GEPs were generated using the Illumina HumanRef8 v2 arrays, with background correction and quantile normalization in lumi. The expression data set was randomly divided into training (n=144) and validation (n=56) sets. For each of six asthma severity phenotypes, we developed asthma severity signatures in the training set using the Support Vector Machine (SVM) learning algorithm, and assessed the predictive ability of the resultant gene set to accurately classify severity in the training set (internal cross-validation) and in the independent validation data set (external validation). We found the developed GEPs had very strong predictive accuracy for asthma severity classification. For example, the predictive accuracy for the nocturnal asthma GEP was 100% in the training set, and 95% in the validation set. The nocturnal asthma GEP was enriched for genes involved in metal-ion binding, zinc-ion binding, and transition metal binding. GEPs for use of asthma controller medications also performed well (accuracy of 75-95% in validation sets) though not as reliably as for nocturnal asthma. These data suggest that the CD4+ lymphocyte transcriptome captures aspects of asthma severity. These profiles can be used to identify novel genes and pathways implicated in the pathobiology of asthma. These results should motivate prospective evaluation of these profiles in evaluating asthma control and severity in the clinical setting. Funding: NIH/NHLBI R01 HL086601A.

## 2794/F/Poster Board #534

**An integrated multivariate approach for identifying hypoxia-induced differentially expressed genes, adjusting for cell culture covariates and performing latent structure eQTL analysis.** *F. Pettersson[1], B. Mohr[1], S. Campino[1], P. Ellis[2], C. Langford[2], K. Rockett[1], C. Pugh[3], P.J Ratcliffe[3], D. Kwiatkowski[1].* 1) WTCHG, University of Oxford, UK; 2) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 3) The Henry Wellcome Building of Molecular Physiology, University of Oxford, UK.

The cellular response to changing oxygen levels plays an important role in many biological processes that are potentially relevant to human disease, such as malaria or cancer. We set out to identify genetic variants of importance for the transcriptional response to hypoxia (oxygen deficiency). Yoruba HapMap lymphoblastoid cell lines were grown under both hypoxia and normal oxygen levels (normoxia) (1% and 21% oxygen). For each of the cell lines global gene expression was measured using microarray technology. Cell culture-related covariates such as growth rate and Epstein-Barr viral transcription were also measured. Applying principal component analysis (PCA) to gene expression data that were normalised using standard protocols revealed that the main structure captured by the model related to microarray scan order and biological covariates, rather than to the hypoxia stimulus. Applying a supervised approach using partial least squares discriminant analysis (PLS-DA) rendered a strong model where the effect of hypoxia was captured in the first component and could be used to identify differentially expressed genes, both up- and down-regulated. We applied two strategies for finding association between genetic variants and gene expression, where the responses in a linear regression framework were i) expression levels for individual probes or ii) the latent structures from multivariate models (a meta-gene approach). The gene expression data and latent structures from models thereof contained systematic variation correlated with the scan order and the covariates and needed to be normalised. To remove this effect we used an approach (orthogonal PLS or OPLS) which has previously been successfully applied to normalize gene expression data and remove technical artifacts. The OPLS model separates information related to the stimulus and structure that is mathematically unrelated (orthogonal) to it. This ensures that variation related to the response is not removed, but also allows investigation of the removed variation. Four components were eliminated and these were seen to be correlated to measured covariates and scan order. Using the latent structure based approach is attractive compared to exhaustive trans-eQTL testing because it focuses on the relevant response with a smaller number of tests. The OPLS normalisation procedure can also be applied to remove and estimate unwanted biological variability in studies where covariates have not been directly measured.

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## 2795/F/Poster Board #535

**Effects of *NF1* haploinsufficiency on stochastic gene expression.** *D. Stewart[1], A. Pemov[1], G. Cohen[2], N. Oden[2].* 1) National Human Genome Research Institute, Bethesda, MD 20892; 2) Emmes Corp, Rockville, MD 20850.

**Background**: Using a computer model, Cook et al (*PNAS* 95: 15641-15646, 1998) predicted that haploinsufficiency syndromes may result in increased stochasticity ("noise") in gene expression. Studies of the effects of *NF1* haploinsufficiency on morphological phenotypes in keratinocytes and melanocytes were congruent with these predictions (Koivunen et al. *J Invest Dermatol* 114: 473-479, 2000 and Kemkemer et al. *PNAS* 99: 13783-13788, 2002). We investigated differences in gene expression stochasticity in NF1-haploinsufficient and control cells. **Data Set and Analyses**: Whole-genome transcriptional profiling of lymphoblastoid cell lines from 13 females affected with NF1 (A) and 10 unrelated female controls (U) was performed under similar conditions in February and June. Raw expression data was background-subtracted, quantile normalized and $\log_2$ transformed. To measure noise, we calculated the correlation between the 2 time points for each of the 10,757 expressed transcripts for both groups (A and U). We then calculated the mean of the correlation coefficients across all transcripts in A and U, as well as the difference in mean correlation between the 2 groups. We tested the latter using a permutation test. We also examined the variability of the change in expression between February and June for all 10,757 expressed transcripts in the 2 groups. **Results**: In our first analysis, the average correlation for all transcripts was significantly greater in the unaffected group than the affected group (U = 0.549; A = 0.428; difference = 0.121, P = 0.003, 1000 permutations). The differences were observed across all genes; no single transcript was identified with sufficient evidence of a difference in correlation to remain significant after multiplicity adjustment. In the analysis of variability of change over time, there were no statistically significant differences between the 2 groups. **Discussion**: In the unaffected cells, we observed a statistically significant higher average correlation between time points when compared to the *NF1*-haploinsufficient cells. To the extent that the comparison of mean correlation over 2 time points approximates expression noise, our observations are consistent with the predicted effect of haploinsufficiency. However, this effect was not observed in our analysis of variability of change in expression over time. The causes of this discrepancy and the ontology of the top transcripts from each analysis are subjects of investigation.

## 2796/F/Poster Board #536

**Identification of Brain Transcriptional Variability Reproduced in Peripheral Blood: an Approach for Mapping Brain Expression Traits.** *A.J. Jasinska[1], S. Service[1], O.-C. Choi[1], J. DeYoung[1], O. Grujic[1], S.-Y. Kong[1], M.J. Jorgensen[2], J. Bailey[1], S. Breidenthal[1], L.A. Fairbanks[1], R.P. Woods[3], J.D. Jentsch[4], N.B. Freimer[1].* 1) Center for Neurobehavioral Genetics, University of California, Los Angeles, CA; 2) Department of Pathology, Section on Comparative Medicine, Wake Forest University Health Sciences, Winston-Salem, NC; 3) Departments of Neurology and Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, University of California, Los Angeles, CA; 4) Department of Psychology, University of California, Los Angeles, CA.

Genome-wide gene expression studies may provide substantial insight into gene activities and biological pathways differing between tissues and individuals. We investigated such gene expression variation by analyzing expression profiles in brain tissues derived from eight different brain regions and from blood in 12 monkeys from a biomedically important non-human primate model, the vervet (*Chlorocebus aethiops sabaeus*). We characterized brain regional differences in gene expression, focusing on transcripts for which inter-individual variability of expression in brain correlates well with variability in blood from the same individuals. By applying very stringent transcript selection criteria concerning the similarities in expression between brain and blood tissues, the consistency of repeat measurements in blood samples from particular individuals, the detection of transcripts in all tissue samples, and the observation of higher inter-individual than intra-individual variability, we identified 32 transcripts whose expression is measurable, stable, replicable, variable between individuals, and of interest to brain functions. Twenty-nine of these expression traits showed heritability at a significance level of p <0.05 (91%) and 25 transcripts showed heritability at p <0.001 (78%). More than 60% of the selected transcripts displayed heritability estimates of >0.4. The high heritabilities observed for levels of these transcripts in a large vervet pedigree validated our approach of focusing on transcripts that showed higher inter-individual compared to intra-individual variability. The selected stable blood expression biomarkers of brain gene expression may be investigated in the future for expression Quantitative Trait Loci (eQTLs) differentially regulating transcript levels in the brain among individuals. Given the high degree of conservation of tissue expression profiles between vervets and humans, our findings may facilitate the understanding of regional and individual transcriptional variation and its genetic mechanisms in humans. The approach employed here - utilizing higher quality tissue and more precise dissection of brain regions than is usually possible in humans - may therefore provide a powerful means to investigate variation in gene expression relevant to complex brain related traits, including human neuropsychiatric diseases.

## 2797/F/Poster Board #569

**Genome-wide expression profiling of human blood reveals biomarkers for hemorrhage in brain arteriovenous malformation patients.** *S. Weinsheimer[1], H. Kim[1,2,3], L. Pawlikowska[1,2], C.E. McCulloch[3], H. Xu[4,5], F.R. Sharp[4,5], W.L. Young[1,6].* 1) Center For Cerebrovascular Research, Dept Anesthesia, Univ. California, San Francisco, San Francisco, CA; 2) Institute for Human Genetics, Univ. California, San Francisco, CA; 3) Dept Epidemiology and Biostatistics, Univ. California, San Francisco, CA; 4) Dept Neurology, Univ. California, Davis, CA; 5) Medical Investigation of Neurodevelopmental Disease Institute, Univ. California, Davis, CA; 6) Dept Neurology and Neurological Surgery; Univ. California, San Francisco, CA.

**Background:** Brain arteriovenous malformations (BAVMs) are a tangle of abnormal vessels directly shunting blood from the arterial to venous circulation, and are an important cause of intracranial hemorrhage (ICH) in young adults. Studies have shown that blood genomic profiling in stroke patients can identify stroke subtypes. Based on previous expression studies of AVM tissue and on genetic studies, we hypothesized that BAVM patients at high risk for ICH will display a distinct gene expression pattern in their blood that could be used to identify prognostic biomarkers to predict ICH risk. Our study compared blood genomic profiles in BAVM patients presenting with or without ICH. **Methods:** We performed Affymetrix Human Genome U133 Plus 2.0 microarray analysis on blood from 40 BAVM patients (20 ICH, 20 non-ICH). Data were preprocessed using the robust multi-array analysis (RMA) normalization method and analyzed using Genespring GX 10.0 (Agilent Technologies). Genes with a fold change >1.5 at the false discovery rate (FDR) corrected significance threshold P<0.05 were selected as differentially expressed in ICH compared to non-ICH patients. We used the WebGestalt program to evaluate whether genes differentially expressed in ICH were represented in pathways (KEGG and BioCarta) more than expected by chance, using all probes on the array as a reference (one-sided Fisher's exact P<0.05). **Results:** 125 mRNA probes representing 52 distinct genes were differentially expressed in ICH vs. non-ICH BAVM patients (>1.5 fold change, FDR P<0.05). Pathway analysis revealed an overrepresentation of differentially expressed genes in two KEGG pathways including Fc epsilon RI signaling (P=0.02) and Toll-like receptor signaling (P=0.025) and six BioCarta pathways including TNF/stress related signaling (P=0.001), IL12 and Stat4 dependent signaling in Th1 development (P= 0.001), and signal transduction through IL1R (P=0.005). The following genes in these pathways were differentially expressed in ICH vs. non-ICH BAVM patients: IL18R1 (fold change=2.03, P=0.04), TNF (fold change=1.51, P= 0.04), and MAP2K6 (fold change=1.50, P=0.04). **Conclusion:** The gene expression pattern in blood differed between BAVM patients presenting with and without ICH. Identification of differentially expressed genes in ICH may provide clues to the pathogenesis of BAVM hemorrhage and point to new pathways for investigation.

## 2798/F/Poster Board #570

**Integrative and comparative genomics analysis of early hepatocellular carcinoma differentiated from liver regeneration in young and old.** *D. Colak[1], M. Chishti[2], A. AlBakheet[3], A. Al-Qahtani[4], M. Shoukri[1], M. Goyns[5], P. Ozand[6], J. Quackenbush[7], B. Park[8], N. Kaya[3].* 1) BESC, KFSHRC, Riyadh, Saudi Arabia; 2) King Khaled University Hospital, King Saud University; 3) NeuroGenetics, KFSHRC, Riyadh, Saudi Arabia; 4) BMR, KFSHRC, Riyadh, Saudi Arabia; 5) Immorgene Concepts Ltd; 6) Duzen Labs, Istanbul, Turkey; 7) Department of Biostatistics and Computational Biology; Dana-Farber Cancer Institute, Boston, MA,; 8) The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD.

Hepatocellular carcinoma (HCC) is third-leading cause of deaths worldwide, and often diagnosed at an advanced stage, and hence typically has poor prognosis. Though much progress has been made in understanding HCC, there is currently no molecular marker to diagnose the early HCC. To identify distinct molecular mechanisms for early HCC we developed a rat model of liver regeneration post-hepatectomy, as well as liver cells undergoing malignant transformation and compared them to normal liver using a microarray approach. As ageing is a known confounding factor embedded in the gene expression profile data from cancer tissues, we included age as a factor in our multi-factor statistical analysis, and identified age-specific differences in early HCC. Subsequently, we performed cross-species comparative analysis by re-analyzing data from two independent human early HCC microarray studies. We then performed integrative analysis of DNA genomic copy number aberration (CNA) data with gene expression profiles. We identified 35 genes that are conserved across species for early HCC, 17 of which were mapped to human genomic CNA regions. Most significantly altered pathways include apoptosis signaling, p53, p38 MAPK, ERK/MAPK, PI3K/AKT signaling, and TGF-$\beta$ pathways. Conclusion: The integrative analysis of transcriptional deregulation with the genomic copy number aberrations data together with comparative cross species analysis bring new insights into the molecular portrait of early hepatoma formation differentiated from regenerating liver, and identify potential novel biomarkers for the early detection of early HCC.

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## 2799/F/Poster Board #571

**MicroRNA analysis of formalin fixed paraffin-embedded prostate cancer tissues with different stages.** *G. Xiao, Q. Xie, R. Recker.* Dept Med, ORC, Creighton Univ, Omaha, NE.

INTRODUCTION: Prostate cancer still has the highest incidence among men in the United States. Our understanding of its molecular and cellular mechanisms is limited. Recently, microRNAs have gained favorable status as upstream regulators of prostate cancer progression since then it can posttranscriptionally regulate sets of genes. It is now estimated that there are about 1000 miRNAs in the human genome, but only about 300 miRNAs have been identified in humans now. Much of miRNAs and their roles in cancer formation still await discovery. METHODS: Formalin fixed paraffin-embedded (FFPE) prostate tissues from different stages of the cancer were de-waxed before performing total RNA extraction. Three stages of prostate cancer tissue with 15 subjects on each stage (n=15) were analyzed, including benign prostate tissue, low grade, and high grade. The total RNA was extracted with acid-phenol: chloroform. MicroRNAs were isolated from total RNA. MicroRNA microarray profiling was performed using LC Sciences technology (LC Sciences, LLC). The Bioconductor implementation of Limma was used to analyze the data. RESULTS: MicroRNA profiling experiments have been performed from 45 FFPE prostate tissues of prostate cancer subjects. By comparing the endogenous miRNA level between benign to low grade, we found that numbers of miRNAs (e.g. mir-95) were significantly induced, while some of miRNAs (e.g. mir-1247) were suppressed, in low grade. In subjects with high grade, we observed some up-regulated miRNA species (e.g. mir-95) and more down-regulated miRNA species (e.g. mir-200b*), indicating that those miRNAs could be important candidate mediators that regulate progressive process of the prostate cancer. These findings were confirmed by real time microRNA RT-PCR using prostate cancer patient samples. Further functional studies are still under investigation.

## 2800/F/Poster Board #572

**Deep surveying of whole transcriptome under CNV effect.** *E. Ait Yahya Graison[1], C.N. Henrichsen[1], J. Thomas[2], S. Pradervand[2], K. Harshman[2], A. Reymond[1].* 1) Center for Integrative Genomic, University of Lausanne, Lausanne, Switzerland; 2) DAFL, Center for Integrative Genomic, Lausanne, Switzerland.

Copy number variation (CNV) of DNA segments has recently been identified as a major source of genetic diversity, but a comprehensive understanding of the phenotypic effect of these structural variations is only beginning to emerge. We have generated an extensive map of CNV in wild mice and inbred strains (Henrichsen, 2009). These variable regions cover ~11% of their autosomal genome. Tissue transcriptome data show that expression levels of genes within CNVs tend to correlate with copy number changes and that CNVs influence the expression of flanking genes. Genes within CNVs show lower expression levels and more specific spatial expression patterns than genes mapping elsewhere. These analyses reveal differential constraint on CNV genes expressed in different tissues. Dosage alterations of brain-expressed genes are less frequent than those of other genes. This study suggests that CNVs shape tissue transcriptomes on a global scale and thus represent a substantial source for within-species phenotypic variation. To unravel the effects of CNV on expression of both coding and non-coding RNA at the nucleotide rather than locus level we used RNA-seq to monitor expression changes of transcripts that map to CNV regions and their flanks in brain and liver of three mouse inbred strains (C57BL/6J, 129S2, DBA/2J). We also sequence transcriptome of F1 crosses between these three strains. This work should (i) give an unprecedent global and precise view of the mouse transcriptome; (ii) help gauge the influence of CNVs on the transcriptome and (iii) monitor allele specific expression from F1 crosses transcriptome profiling considering Copy number variants.

## 2801/F/Poster Board #573

**Genetic Effect of Single Nucleotide Polymorphisms of PPARGC1B Gene on Airway Hyperreactivity in Asthmatics.** *S. Lee[1], J. Park[1], H. Chang[1], A. Jang[1], S. Park[1], J. Park[1], Y. Kim[2], S. Uh[2], Y. Kim[3], I. Chung[4], B. Park[5], H. Shin[5,6], C. Park[1].* 1) Genome Research Center for Allergy and Respiratory Diseases, Soonchunhyang University Bucheon Hospital, 1174, Jung-Dong, Wonmi-Gu, Gyeonggi-Do,420-020, South Korea; 2) Division of Allergy and Respiratory Medicine, Soonchunhyang University Hospital, 657, Hannam-Dong,Yongsan-Gu,140-743, South Korea; 3) Divison of Allergy and Respiratory Medicine, Soonchunhyang University Cheonan Hospital, 23-20, Bongmyeong-Dong, Cheonan-Si, Chungcheongnam-DO, 330-721, South Korea; 4) Division of Molecular and Life Sciences, College of Science and Technology, Hanyang University, 1271 Sa-1-dong, Ansan, Gyeonggi-do 426-791, South Korea; 5) Department of Genetic Epidemiology, SNP-Genetics Inc., B-1407,WonLim Lion's Valley,371-28 Gasan-Dong, Geumcheon-Gu, Seoul, 153-803, South Korea; 6) Department of Life Science, Sogang University, Sinsu-Dong, Mapo-Gu, Seoul,121-742, South Korea.

Rationale; PPARGC1B, peroxisome proliferators-activated receptor-gamma coactivator 1 beta, is a co-activator for intracellular receptors such as estrogen receptor, peroxisome proliferators-activated receptor and glucocorticoid receptor which participate in asthma development. Objectives; Genetic association of PPARGC1B polymorphisms were investigated with the risk of asthma and airway hyperreactivity and the functional effect of these polymorphisms on the expression of PPARGC1B gene and protein. Methods; Direct sequencing of DNA from 24 Koreans was applied for discovery of PPARGC1B gene polymorphisms. A large-scale genotyping was done by single base extension methods in 264 normal controls and 949 asthmatics. Alternative splicing and amount of mRNA was measured using RT-PCR and Real time PCR. Luciferase assay and gel shift assay were done for functional assay of polymorphism on the promoter. Results; Eighteen single-nucleotide polymorphisms (SNPs) and one insertion/deletion polymorphism were identified and seven SNPs (-1381T>C, -427C>T, +90138T>C,+96626 G>A, +102525G>A,+102605C>A,+111883G>C) were genotyped. There was no significant difference in the distribution of each SNPs and haplotypes between asthmatics and normal control. However, the allele frequency of PPARGC1B -427C>T, +90138T>C (Exon 2 (L42L), +96626G>A (intron 3), +102525G>A (exon 5 (R265Q)) showed a significant association with log transformed PC20 methacholine values in the asthmatics (P = 0.010-0.0001). Two RT-PCR products of 602bp (wild type) and 485bp (117 bp of exon 4 deleted) were identified. Real time quantitative PCR showed higher messenger RNA levels of PPARGC1B in -427C>T CC homozygotes than those having -427C>T TT or CT allele (P = 0.028). The ratio of alternative PPARGC1B mRNA over the wild mRNA was similar between the B cell lines carrying the each allele of +102525G>A. On Luciferase reporter system, -427C caused higher promoter activity than -427T. Gel shift assay showed that -427C double-stranded oligonucleotides had stronger binding activity to a nuclear protein of 293T cells than did the -427T double-stranded oligonucleotides. Conclusions; The polymorphisms on promoter and exons of PPARGC1B gene may affect development of airway hyperreactivity via modulation of PPARGC1B gene products and the analysis for genotypes on PPARGC1B gene may be a genetic marker for airway hyperreactivity.

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## 2802/F/Poster Board #574

**Apoptosis of CD4+CD25high T Cells in Type 1 Diabetes is Partially Mediated by IL-2 Deprivation.** *P. Jailwala[1], J. Waukau[1], S. Glisic[1], S. Jana[1], S. Ehlenbach[1], M. Hessner[1], R. Alemzadeh[2], S. Matsuyama[3], P. Laud[4], X. Wang[5], S. Ghosh[1].* 1) The Max McGee National Research Center for Juvenile Diabetes and The Human and Molecular Genetics Center, Department of Pediatrics at the Medical College of Wisconsin and the Children's Research Institute of the Children's Hospital of Wisconsin, Milwaukee; 2) Children's Hospital of Wisconsin Diabetes Center, Pediatric Endocrinology and Metabolism, Medical College of Wisconsin, Milwaukee, WI, USA; 3) Department of Pharmacology, Case Western Reserve University, Cleveland, OH, USA; 4) Division of Biostatistics, Medical College of Wisconsin, Milwaukee, WI, USA; 5) Department of Physics & the Comprehensive Diabetes Center, University of Alabama at Birmingham Birmingham, AL, USA.

Type 1 diabetes (T1D) is a T-cell mediated autoimmune disease targeting the insulin-producing pancreatic β cells. Naturally occurring FOXP3+CD4+CD25high regulatory T cells ($T_{regs}$) play an important role in dominant tolerance, suppressing autoreactive CD4+ effector T cell activity. Previously, in both recent-onset T1D patients and β cell antibody-positive *at-risk* individuals, we observed increased apoptosis and decreased suppression of polyclonal $T_{regs}$ in the periphery. Our objective was to elucidate the genes and signaling pathways triggering apoptosis in $T_{regs}$ from T1D subjects. Microarray analysis was performed on unstimulated $T_{regs}$ from recent-onset T1D (n=12) and healthy control subjects (n=15). Gene expression analysis was performed using BGX, a novel bayesian approach integrating all steps of the analysis. Components of an activation profile, including cytokine/chemokine receptor genes, HLA genes, GIMAP family genes and cell adhesion genes were downregulated in $T_{regs}$ from T1D subjects, relative to control subjects. CD4+ effector T-cells from T1D subjects showed a marked reduction in IL-2 secretion. Several downstream target genes of the AKT and p53 pathways were also upregulated in T1D subjects, relative to controls. Further, expression signatures and increased apoptosis in $T_{regs}$ from T1D subjects mirrored the response of healthy $T_{regs}$ under conditions of IL-2 deprivation. This could indicate that prior to and during the onset of disease, $T_{regs}$ in T1D are caught up in a relatively deficient cytokine milieu. In summary, expression signatures in $T_{regs}$ from T1D subjects reflect a cellular response that leads to decreased activation and increased sensitivity to apoptosis, partially due to cytokine deprivation. Further characterization of these signaling cascades should enable the detection of genes that can be targeted for restoring $T_{reg}$ function in subjects predisposed to T1D.

## 2803/F/Poster Board #575

**Association of the Genetic Polymorphisms with the Susceptibility of the Membranous Glomerulonephritis in Taiwanese Population.** *W. Chen[1,2], S. Chen[3,4], W. Lo[5,6], C. Chen[7], F. TsAI[6,8].* 1) Department of Surgery, China Medical University Hospital, Taichung, Taiwan; 2) College of Medicine, China Medical University, Taichung, Taiwan; 3) Genetics Center, Department of Medical Research, China Medical University Hospital, Taichung, Taiwan; 4) Graduate Institute of Chinese Medical Science, College of Chinese Medicine, China Medical University, Taichung, Taiwan; 5) Graduate Institute of Integrated Medicine, China Medical University, Taichung, Taiwan; 6) Department of Medical Research, China Medical University Hospital, Taichung, Taiwan; 7) Institute of Clinical Medicine, China Medical University, Taichung, Taiwan; 8) Department of Biotechnology and Bioinformatics, Asia University, Taichung, Taiwan.

Objective: Membranous glomerulonephritis (MGN) is the most common primary cause of the nephritic syndrome in adult, and 25% of cases proceed to end-stage renal disease. It is characterized by basement membrane thickening and subepithelial immune deposits without cellular proliferation or infiltration. Previous studies suggested that genetic polymorphisms play an important role in nephropathy progression. The purpose of this study was to clarify the relationship between possible genetic variants and the pathogenesis of MGN in Taiwanese population. Methods: Individuals were divided into two groups: (1) normal controls; (2) MGN patients. Polymorphisms of the 45 genetic variants were genotyped in 106 MGN cases and 265 unrelated healthy adult in Taiwanese cohort which matched for sex and age with the study patients using PCR system with TaqMan® allelic discrimination assay. Genotypes/allelic frequencies and haplotypes for these polymorphisms in each group were compared. Results: Five single-nucleotide polymorphisms (SNPs) were associated with the occurrence of MGN. Genotype distribution and allele frequency of rs12749808, rs7523521, rs7532635, rs2070744, IL-6 -572 polymorphisms in both groups were significantly different. The frequency of C allele in the IL-6 -572 polymorphism was significantly higher in the patient group (88.7%) than in the control group (76.4%; p = 0.032, odds ratio [OR] = 0.68; 95% confidence interval [CI] = 0.47-0.97). Therefore, persons in the IL-6 -572 polymorphism with the C allele may have higher risk of developing MGN.

## 2804/F/Poster Board #576

**Gene Expression Profiling of Human Whole Blood Samples with the Illumina DASL Assay.** *M.E. Winn[1,2], M. Shaw[2], C. April[5], B. Klotzle[5], J.B. Fan[5], C.S. Bloss[2,3], S.S. Murray[2,3,4], E.J. Topol[2,3,4], N.J. Schork[2,3,4].* 1) University of California San Diego, La Jolla, CA; 2) Scripps Genomic Medicine, Scripps Translational Science Institute, La Jolla, CA; 3) Scripps Health, La Jolla, CA; 4) Dept of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA; 5) Illumina Inc., San Diego, CA.

Peripheral whole blood is quickly becoming a commonly assayed tissue for the development of clinically relevant biomarker panels for a variety of human diseases. Despite a number of studies focusing on technical factors surrounding the handling and processing of whole blood, there remain a number of important biological and technical issues to resolve before blood-based gene expression biomarker panels can reach their full potential. One issue is decreased sensitivity and reliability of whole blood gene expression profiles due to the effects of high hemoglobin levels. Globin reduction assays have been shown to overcome such effects when used in conjunction with Affymetrix microarrays and the standard Illumina direct hybridization assay but never with the new Illumina DASL® assay. We compared whole blood and globin-reduced blood from eight Parkinson's disease blood samples on three Illumina assays: the IVT-based direct hybridization assay and the new DASL assay with and without globin probes. We found that whole blood hybridized on the DASL globin-probe negative assay results in higher levels of gene expression sensitivity and reproducibility than the DASL globin-probe positive and direct hybridization assays with and without globin reduction. Transcript detection is significantly increased with the use of DASL compared to IVT-based direct hybridization, while globin reduction appears to have little to no positive effect on transcript detection and raw intensity correlations.

## 2805/F/Poster Board #577

**Alternative Splicing and Differential Gene expression in Human Hematopoiesis During In Vitro Lineage Specific Cell Differentiation.** *P. Liu[1], J. Barb[2], X. Xu[1], P. Munson[2], N. Raghavachari[1].* 1) Genomic Core, NHLBI/NIH, Bethesda, MD; 2) CIT/NIH, Bethesda, MD.

Alternative splicing is a prevalent mode of posttranscriptional regulation, and occurs in approximately one half of all mammalian genes. Hematopoietic differentiation is strictly regulated by several exogenous factors and the frequency and functional impact of alternative splicing in stem cell differentiation process are yet to be determined. Disruptions of the intricate sequences of transcriptional activation and suppression of multiple genes can cause hematological diseases, such as leukemias, myelodysplastic syndromes (MDS), or myeloproliferative syndromes (MPS). Elucidating the pattern and sequence of gene expression during normal hematopoietic cell development may help to unravel the disease-specific mechanisms in hematopoietic malignancies. In this study using an vitro hematopoietic model system, CD34+ human stems cells isolated from healthy individuals were selectively induced in vitro with the lineage-specific cytokines, such as erythropoietin (EPO), granulocyte colony-stimulating factors (G-CSF), granulocyte macrophage colony-stimulating factors (GM-CSF) and thrombopoietin (TPO). Cells from each of the lineages were harvested after 11 days of culture for total RNA isolation. Gene chip analysis was performed using affymetrix human U133 plus expression arrays and human genome 1.0 ST exon arrays to delineate the changes in gene expression and associated alternative splicing events during lineage-specific hematopoietic cell differentiation. Data Analysis on the U133 plus expression chips indicated that during cell differentiation 943 genes were significantly up-regulated and 381 genes were found to be down-regulated. Gene level analysis on the Human 1.0 ST exon arrays showed good correlation (0.7) with the expression chip analysis. Analysis of the exon arrays for alternatively spliced genes during erythropoiesis, granulopoiesis and megakaryopoiesis, identified genes such as CLMN3, FBN24, FCN1, PDE34D, PDE34DIP, RGS3, BAX, SF1, THBS, NT5C2, HDAC5, ITGA2B, PTPRA, RTN4 and SLC4A2 to be significantly spliced during cell differentiation. RT-PCR analysis corroborated with the gene chip data on few selected genes. Our data thereby provides several new insights into understanding differentiation and proliferation of human stem and progenitor cells. This knowledge on the nature of stem cells and the molecular process by which these cells acquire their specific cell fate shows great promise to the success of cell-based therapies.

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## 2806/F/Poster Board #578

**Single Molecule and Paired Read Sequencing of the Human Transcriptome: Exons, Splice Junctions and Fusions.** *D. Lipson, T. Raz, P. Kapranov, J. Reifenberger, J. Thompson, S. Letovsky, P. Milos.* Bioinformatics, Helicos BioSciences, Cambridge, MA.

Full transcriptome sequencing (RNA Seq) enables the study of variations in biological samples, providing new insights into basic biology. Deep sequencing now provides a means to carry out a complete comparison of gene expression across tissues, time, development, and tumor status. In addition to sequence variation, RNA Seq can be used for quantification of alternative splice variants and identification of novel DNA fusion events. Single molecule sequencing can be carried out for single reads or paired reads with the simple sample preparation methods required for either mode including only reverse transcription of RNA followed by tailing of the first strand cDNA with terminal transferase. No ligations or amplifications are necessary, avoiding artifacts introduced by those methods. Each HeliScope run can generate 50 channels (for up to 50 different samples) with more than 10 million usable reads in each channel. This provides exhaustive coverage of exons as well as splice junctions and fusion transcripts often found in tumors. Highly reproducible profiles for a variety of tissues and species will be shown. To augment the single read data, we have used short paired reads generated by a HeliScope single-molecule sequencer for de novo discovery of fusion transcripts. Generation of single-molecule paired-reads involves no additional sample preparation steps and therefore greatly reduces the risk of false positives. In addition, a single run generates paired reads of different lengths as well as a large number of single reads. The combination of these different data types allows de novo discovery of putative fusion events using the longer pairs (e.g. 25+25bp or longer), validation of these events using the shorter pairs (e.g. 20+10bp or longer), and mapping of the exact breakpoints using single reads. We demonstrate the application of this approach for confirmation of known fusion events in well-characterized cell lines as well as identification of novel fusion events. A similar analytical approach may be used to identify unannotated transcript isoforms and local fusion events such as gene-skipping, demonstrating the significant utility of single-molecule paired-reads for transcriptome analysis.

## 2807/F/Poster Board #579

**Genetic control of the transcriptional landscape in humans.** *J.K. Pickrell[1], A.A. Pai[1], J.C. Marioni[1], J.F. Degner[1], E. Nkadori[1], M. Stephens[1], Y. Gilad[1], J.K. Pritchard[1,2].* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Howard Hughes Medical Institute.

Understanding how genetic variation in human populations influences transcription is an important step towards understanding the mechanisms by which regulatory polymorphisms influence disease risk. Recently, studies of expression quantitative trait loci (eQTLs) have become an important tool for connecting variation in gene expression levels to individual genetic loci. Additionally, next-generation sequencing technologies now allow for rapid quantification of the RNA content of a tissue. To take advantage of these tools, we have performed RNA-sequencing on 76 lymphoblastoid cell lines derived from Nigerian individuals that have been extensively genotyped by the International HapMap Project. We identify thousands of unannotated transcribed regions, hundreds of which are predicted to be novel protein-coding exons. Using the genotypes from the HapMap, we confirm the majority of eQTLs previously identified using microarrays, as well as identify novel ones. Turning to transcriptional processing, we identify hundreds of loci that impact the inclusion of individual exons, likely through influences on splicing. We discuss these results both in the context of disease mapping efforts and the evolution of gene structure.

## 2808/F/Poster Board #580

**A Comparison of Next Generation Sequencing and Microarrays for Transcriptome Expression Profiling.** *P. Whitley, A. Lemire, C. San Jose, N. Hernandez, J. Gu, K. Bramlett, D. Ilsley, J. Schageman, J. Brockman, K. Lea, R. Setterquist, S. Kuersten, S. Heater, L. Qu.* Life Technologies, Molecular Biology Systems Division, R&D, Austin, TX.

Microarray based expression profiling has been remarkably successful at elucidating the spatio-temporal patterns of mRNA transcripts within cells and tissues, however there are a number of shortcomings to the existing technology. Both sensitivity and specificity can be low with microarrays. Accuracy can also be negatively affected by the low dynamic range of existing microarray technology. Perhaps more importantly, microarrays restrict the expression profiling data to specific annotations and content that are present. Digital expression profiling using RNAseq and next generation sequencing (NGS) promises to reduce or in some cases eliminate these weaknesses. In order to evaluate the merits of RNAseq for expression profiling, we have performed an extensive comparison of data generated with the ABI SOLiD NGS platform and the Affymetrix U133Plus 2.0 and Human Exon 1.0 ST GeneChip platforms. Using the Microarray Quality Control Consortium RNA control samples as a model system we have demonstrated increased sensitivity, specificity and accuracy of the SoliD RNAseq data relative to the microarray platforms. Both microarray platforms showed similar levels of concordance with the RNAseq data, but the exon arrays provided additional information about exon usage and transcript diversity that were verified by the NGS data. TaqMan PCR was used as a third platform technology to assess relative performance of the NGS and array data and validate the findings for both systems. We also discuss some of the potential weaknesses of NGS, including transcript length bias. We have also compared the results of RNAseq using either polyA RNA fractions or total RNA that has been depleted of rRNA. While the results are highly concordant, the two sample types offer unique advantages and disadvantages for RNAseq. Using total RNA for RNAseq gives a fuller picture of the transcriptome, including non-coding RNAs and non-polyadenylated transcripts, but may require more sequencing depth to attain the same level of sensitivity. RNAseq with polyA selected RNA results in high sensitivity and accuracy for expression profiling, but does not survey the entire transcriptome sequence space. Applications, such as, novel transcript discovery, splice variant discovery, allele specific expression and traditional gene expression profiling may require the use of one or both RNA sample types.

## 2809/F/Poster Board #581

**Testing the predictive power of regulatory annotations using expression quantitative trait loci.** *D. Gaffney, J-B. Veyrieras, M. Stephens, Y. Gilad, J. Pritchard.* Human Genetics, University of Chicago, Chicago, IL.

The vast majority of regulatory sites in the human genome remain undiscovered. Computational prediction of regulatory regions typically suffers from a high false-positive rate while experimental approaches, such as ChIP-seq, can only examine a limited subset of transcription factors, cells and tissue types. Furthermore, although previous annotation efforts have frequently predicted tens of thousands of functional regulatory regions, experimental validation of these predictions is typically laborious and low-throughput. We propose a novel approach to complement traditional experimental validation. Our method rests on the assumption that true regulatory regions will also be enriched in expression quantitative trait loci (eQTLs). Using the Bayesian regression framework outlined in Servin & Stephens (2007), we infer putative eQTLs using gene expression data from HapMap cell lines and SNP data from HapMap and the 1000 genomes project. We then use the hierarchical model introduced in Veyrieras et al (2008) which enables a formal test for eQTL enrichment in any annotation. Using this approach we can test multiple, genome-wide annotations simultaneously while accounting for known predictors of eQTLs such as distance from the transcription start site. We test the level of eQTL enrichment in a diverse of array of regulatory annotations, both experimentally and computationally-predicted. We find that evolutionarily-conserved transcription factor binding sites as well as sites associated with histone modification and polyadenylation, are significantly enriched in eQTLs. We also demonstrate that this approach can provide functional information on the link between changes in the DNA sequence and changes in transcription. Our method provides a reliable test bed for future regulatory annotation efforts and will gain in power as increasing quantities of large scale genotyping and high-throughput gene expression data become available.

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## 2810/F/Poster Board #582

**From gene evolution to gene function: characterization of a newly identified SERPINA2.** *Z. Ferreira[1, 2], J. Figueiredo[1], J. Correia[1], J. Rocha[1, 2], S. Seixas[1].* 1) Biological Sciences, IPATIMUP, Porto, Porto, Portugal; 2) Faculty of Sciences of the University of Porto, Porto, Portugal.

Serine protease inhibitors (SERPINs) are a superfamily of highly conserved proteins that play a key role in controlling the activity of proteases in diverse biological processes. Alfa-1-antitrypsin (*SERPINA1*), the most studied member of this family, is encoded by a gene located within the proximal 14q32.1 *SERPIN* subcluster, together with its highly homologous sequence, SERPINA2, which has been thought to be a pseudogene. Previously, we have found that a polymorphic 2 kb deletion in *SERPINA2* was associated with a signature of positive selection in Africa, possibly through a role in fertility or in host-pathogen interactions. To understand the selective forces driving *SERPINA2* evolution, we initiated the functional characterization of *SERPINA2* non-deleted alleles, possibly encoding functional protein isoforms with unknown inhibitory activity. To achieve this goal we first amplified *SERPINA2* gene from a human testis cDNA library and cloned it into a pLenti6/V5 vector. Then, we co-transfected 293FT cells with *SERPINA2* plasmids and ViraPower Packaging Mix and collected the cell media containing the corresponding lentivirus particles, 48 to 72 hours later. This media was used to transduce two mammalian cell lines (HeLa and CHO). Stable cell lines were obtained by Blasticidin selection of transduced cells and the evaluation of SERPINA2 expression was done by performing Real-Time PCR, western blotting and immunoflurescence assays. We efficiently transduced both cell lines with three SERPINA2 variants (L308-E320, P308-E320 and P308-K320) and with an empty vector. For each variant, we identified a single protein band of approximately 50 kDa which is the expected size for a regular SERPIN. SERPINA2 was localized inside the cells in contrast with its close homologue, SERPINA1, which is secreted and only found within the cell in cases of misfolded variants. However, the higher SERPINA2 content, and the absence of aggregates after treatment with proteasome inhibitors, rather suggest that SERPIN2 expression is differently regulated by a post-translational mechanism, dependent on the proteasome. In summary, we provide the first evidence of SERPINA2 expression in vitro and propose that its expression and activity probably differs from SERPINA1. Our preliminary work favors the hypothesis of a polymorphic gene/pseudogene whose loss of function might be advantageous in human evolution.

## 2811/F/Poster Board #583

**A high-resolution anatomical atlas of the murine transcriptome at embryonic stage E14.5.** *G. Diez-Roux[1], S. Banfi[1], M. Sultan[8], L. Geffers[7], L. Kumar[4], P. Sarmientos[2], A. Reymond[3], D.R. Davidson[4], P. Dolle[5], S.E. Antonarakis[6,10], M.L. Yaspo[8], S. Martinez[9], R.A. Baldock[4], G. Eichele[7], A. Ballabio[1,11], EURExpress consortium.* 1) Fondazione Telethon, Telethon Inst Gen & Medicine, Naples, Italy; 2) PRIMM srl, Milan, Italy; 3) Center for Integrative Genomics, Genopode building, University of Lausanne, Switzerland; 4) MRC Human Genetics Unit, Western General Hospital, Edinburgh, UK; 5) Institut de Génétique et de Biologie Moléculaire et Cellulaire, Inserm, U 964, CNRS, Faculté de Médecine, Université de Strasbourg, France; 6) Department of Genetic Medicine and Development University of Geneva Medical School, Switzerland; 7) Genes and Behavior Department, Max Planck Institute of Biophysical Chemistry, Goettingen, Germany; 8) Max Planck Institute for Molecular Genetics, Berlin, Germany; 9) Experimental Embryology Lab, Instituto de Neurociencias UMH-CSIC, Univ. Miguel Hernandez , San Juan de Alicante, Spain; 10) University Hospitals of Geneva, Geneva, Switzerland; 11) Medical Genetics, Department of Pediatrics, Federico II University, Naples, Italy.

Ascertaining when and where genes are expressed is of crucial importance for the understanding of their physiological role. RNA in situ hybridization (ISH) provides an accurate spatio-temporal description, panoramic view and cellular resolution of the expression of individual transcripts. Digital anatomical atlases of gene expression can be built based on large-scale ISH. In a previous large-scale ISH study we described the transcriptome atlas of all paralogs of human chromosome 21 genes in the mouse embryo. We have now generated the first genome-wide digital transcriptome atlas by RNA ISH of an entire mammalian organism, the developing mouse at embryonic stage 14.5 (E14.5). The expression patterns of approximately 18,000 genes were generated, curated manually and textually annotated in a highly informative database freely available to the scientific community (www.eurexpress.org), which contains over 1400 hierarchically organized anatomical terms. The global analysis of gene expression annotation determined that 39% of genes displayed a regional or restricted expression pattern at this stage. We found that about 950 genes, 16% of which are of unknown function, display exclusive expression to particular anatomical structures. These represent an important source of novel tissue-specific markers for 37 different anatomical structures, which can be used for high-resolution histological analyses of complex organs and for pathology studies. The ISH data generated and the accompanying textual annotation allowed detecting synexpression gene clusters. Finally, the quality and the resolution of the data allowed us to perform high-resolution molecular regionalization, revealing novel molecular domains for several developing structures. This genome-wide digital transcriptome atlas represents a unique resource to investigate gene function and to identify novel ontogenetic and functional associations between genes relevant to development and disease.

## 2812/F/Poster Board #584

**Geographical genetics of expression variation in southern Morocco.** *Y. Idaghdour[1], W. Czika[2], H. Martin[3], R. Wolfinger[2], G. Gibson[3,4].* 1) Department of Genetics, North Carolina State University, Raleigh, NC; 2) SAS Institute, Cary, NC; 3) School of Biological Science, University of Queensland, Brisbane, QLD, Australia; 4) Center for Integrative Genomics, Georgia Tech, Atlanta, GA.

We describe a genome-wide association study for gene expression variation in peripheral blood samples from 194 residents of southern Morocco in which we jointly estimate the effects of ethnicity, relatedness, geographical location, gender and genotype on the transcriptome. Arab and Amazigh villagers differ from one another and from residents of Agadir for up to half of all expressed transcripts, with significant enrichment of ribosomal biosynthesis and oxidative phosphorylation as well as differential expression of numerous genes involved in disease. We also provide evidence for regional differences in particular transcript classes within genes, though many of these are not obviously due to annotated alternate splice forms. Despite the global effects of the environment, genome-wide significant genotype-transcript associations are detected involving 371 and 12 unique cis and trans autosomal target transcripts after genomewide Bonferroni adjustment (P<10E-8 or <10E-12, respectively). There is no evidence for genotype by environment interactions influencing these strong associations, but a clear tendency for derived alleles to have increased frequency in Moroccan and European relative to Yoruban samples was observed. Furthermore, we confirmed numerous cases of SNPs reported in other gene expression GWAS association studies, several of which are also associated with diseases or traits, and discovered several novel notable instances of this. Finally, we document the genetic population structure in the Souss region of Morocco and discuss the effect of jointly controlling for geograohic and genetic influences on the partitioning of sources of transcriptional variation, concluding that cultural factors influence human peripheral blood gene expression.

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## 2813/F/Poster Board #617

**Post-transcriptional profile of human transcripts using polyribosomal fractionation.** *S. Nikolaev[1], S. Deutsch[1], R. Genolet[2], C. Borel[1], L. Parand[1], C. Ucla[1], F. Schütz[3], G. Duriaux Sail[1], Y. Dupré[1], P. Jaquier-Gubler[2], T. Araud[2], B. Conne[1], P. Descombes[4], J-D. Vassalli[1], J. Curran[2], SE. Antonarakis[1].* 1) Department of Genetic Medicine and Development, University of Geneva Medical School, 1 Rue Michel Servet, CH-1211, Geneva Switzerland; 2) Department of Microbiology and Molecular Medicine, University of Geneva Medical School, 1 Rue Michel Servet, CH-1211, Geneva Switzerland; 3) Swiss Bioinformatics Institute, Genopode Builiding, CH-1015, Lausanne; 4) Genomics Platform, University of Geneva Medical School, 1 Rue Michel Servet, CH-1211, Geneva Switzerland.

Recent studies have demonstrated extensive transcriptional activity across the human genome, a substantial fraction of which is not associated with any functional annotation. However, the post-transcriptional processes that operate within the different classes of RNA molecules are largely unknown. To characterize the post-transcriptional properties of expressed genomic sequences we separated RNA molecules from 3 cell lines (GM06990, HeLa S3 and SK-N-AS) according to their ribosome content by sucrose gradient fractionation. Polyribosomal associated RNA (a proxy for translation) and total RNA were subsequently hybridized to HSA21 genomic tiling arrays. We found that approximately 50% of the transcriptional signals were located outside annotated exons and were considered as TARs (Transcriptionally Active Regions). However, RT-PCR and RACE experiments revealed that approximately 40% of TARs were likely to represent non-specific cross-hybridization artifacts. Bioinformatic analysis of TARs according to conservation and sequence complexity identified a set of high confidence TARs. This set of TARs was significantly depleted in the polysomes suggesting that they are not likely to be translated. Analysis of polysome representation of Refseq exons showed that at least 15% of RefSeq transcripts undergo significant post-transcriptional regulation in at least 2 of the 3 cell lines tested. Among the regulated transcripts, enrichment analysis revealed an overrepresentation of genes involved in Alzheimer's disease (AD), including APP, and the BACE1 protease that cleaves APP to produce the pathogenic beta 42 peptide. High throughput sequencing of polyribosomal associated RNA is in progress to better estimate the genome-wide posttranscriptional regulation of transcripts. These studies provide a characterization of the intermediate step between transcription and protein levels, and establish the translation potential of numerous annotated genes and TARs.

## 2814/F/Poster Board #618

**A Machine-learning Approach to Predict Genes that are Related to Gemcitabine Cytotoxicity.** *K. Kalari[1], L. Li[2], C. High Seng[1], JP. Kocher[1], R. Weinshilboum[2], L. Wang[2].* 1) Division of Biostatistics and Informatics Department of Health Sciences Research Mayo Clinic 200 First Street SW Rochester, MN, 55905; 2) Health Sciences Research Division of Clinical Pharmacology Department of Molecular Pharmacology and Experimental Therapeutics Mayo Clinic 200 First Street SW Rochester, MN, 55905.

**Motivation:** The cytidine analogue, gemcitabine shows significant therapeutic effect in a variety of cancers. However, response to this drug varies widely. Despite recent improvements in high-throughput approaches, it still remains to be a challenge to identify an informative subset of genes that influence drug response. In this study, we investigated the ability of several machine learning methods to identify the most informative markers for gemcitabine cytotoxicity using lympoblastoid cell lines from normal and pancreatic cancer patients. **Methods:** Affymetrix U133 Plus 2.0 expression data and IC50 (dose at which 50% growth is inhibited) drug cytotoxicity phenotypes were obtained for 197 Human Variation Panel lymphoblastoid cell lines (Epstein-Barr virus transformed) and 60 Mayo clinic pancreatic patient lymphoblastoid samples. Genome wide expression correlation with gemcitabine IC50 value was computed for both datasets. Common significant probeset correlations in the Human Variation Panel cell lines and Mayo patient samples were selected as features for data mining. All informative probesets, together with other covariates, were trained using a wide variety of machine learning methods on the cell-based model system and were tested using the patient samples. **Results:** Machine learning algorithms such as decision trees, logistic model tree, linear regression, random forest, artificial neural networks, support vector machines, and logistic regression were explored to identify genes that might contribute to variation in gemcitabine cytotoxicity. Sensitivity of the models varied from 51% to 80% and specificity from 58% to 80%, depending on the machine learning model used. The logistic regression method yielded the best results; five candidate genes identified by this method were further considered to perform functional validation. Knock down experiments of one candidate gene, *GSTM3*, increased tumor cell sensitivity to gemcitabine, which is consistent with our *in silico* analysis. Functional studies of the remaining candidate genes are being performed. **Conclusions:** Our results suggest that the application of machine-learning methods with high-throughput datasets, when combined with complementary functional validation of candidate genes, may help to identify biomarkers in response to gemcitabine therapy.

## 2815/F/Poster Board #619

**Mapping regulators of the transcriptional response to hypoxia in human cells.** *A.J. Mohr[1], F. Pettersson[1], S. Campino[1,2], P. Ellis[2], Y.Y. Teo[1], H. Lockstone[1], R. Andrews[2], C. Langford[2], K. Rockett[1], C. Pugh[3], P. Ratcliffe[3], D. Kwiatkowski[1,2].* 1) Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom; 2) Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom; 3) The Henry Wellcome Building of Molecular Physiology, Oxford, United Kingdom.

To identify genetic regulators of the response to hypoxia (oxygen deficiency), we study whether variation in the hypoxic transcriptional response is associated with DNA variation. Lymphoblastoid cell lines of 56 unrelated individuals from the HapMap Yoruba (Nigeria) panel were subjected to standardized culture at 1% oxygen (hypoxia) vs. 21% oxygen (normoxia) for 16 hrs, and RNA extracted (6-800,000 cells/ml). WG-expression was measured on Illumina WG-6 v.3 beadchips. Expression intensities were scaled by variance-stabilizing transform, quantile-normalized across arrays, and linear regression-normalized across days of array processing. Expression of the Epstein-Barr viral transcript LMP1 was qPCR-quantified. Of 15,000 expressed genes, 25% are differentially expressed (40% up-regulated; 60% down-regulated under hypoxia; 1% FDR). Up-regulated processes include glycolysis, biosynthesis of cholesterol and steroids; those down-regulated include the proteasome, TCA cycle, basal transcription factors, RNA processing, splicing, and translation. Growth rate explains significant (>10%) variation in expression for 10% of genes; pH for 8%; viral activity for 6%. Genotype is known for 2.5 million SNPs. We test for association between each gene's expression and SNPs within 500kb of the gene (cis; additive linear models). Significance is permutation-based (5% FDR). We find significant associations in both normoxia and hypoxia for ~400 genes; in only normoxia for 200 genes; and in only hypoxia for 200 genes. Significant SNPs cluster close to the gene (median SNP-probe-distance, 13 kb). Analysis of covariate-corrected datasets (growth rate, pH, viral activity) shows 85% overlap with above. When mapping differential expression (DE; log-fold-change) as cis-phenotype, we find only 50 associations (median SNP-probe-dist, 150 kb), 25% of which are also detected in normoxia or hypoxia analyses, above. Less cis-eQTLs with the DE phenotype may be due to variation in the normoxic baseline, reducing the accuracy of quantifying hypoxic change; or could indicate a large fraction of false-positives in normoxia and hypoxia datasets due to SNPs under probes, as DE analysis is internally controlled. To uncover master-regulators of the global hypoxic response, we derive a meta-variable of hypoxia-responsiveness per cell line, using principal components analysis of expression values over DE genes, and perform GW-association for this phenotype. Results from this approach will be presented.

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## 2816/F/Poster Board #620

**Global gene expression in human primary leukocytes - effect of stimulations and potential for eQTL mapping.** *P. Saavalainen[1], E. Dukes[1], M. Zucchelli[2,3], J. Vendelin[1,4], E. Einarsdottir[1], J. Kere[1,2,3,4].* 1) Dept Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Dept Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden; 3) Clinical Research Centre, Karolinska University Hospital, Huddinge, Sweden; 4) Folkhalsan Institute of Genetics, Helsinki, Finland.

BACKGROUND: Expression quantitative trait loci (eQTLs) are genetic variants that can control the expression level of genes - either by proximal (cis) or by distal (trans) effects. eQTLs are of increasing interest in the context of complex genetic diseases which are associated with a number of intronic or intergenic risk variants common in populations and with relatively low risk effects. Studies on the eQTL nature of these variants and their role in disease pathogenesis are needed. Several systematic genome wide eQTL studies have been recently carried out using lymphoblastoid cell lines. These studies are, however, limited by the cell type, as EBV-transformed B cells cannot truly reflect the transcriptome of other cell populations and tissues, nor unmanipulated cells. MATERIALS AND METHODS: We have developed an expression tool to identify eQTLs potentially relevant in immune mediated diseases and other complex traits. We created Agilent whole human genome (44K) expression data from unmanipulated primary human peripheral blood mononuclear cells (PBMCs) from 41 blood donors. Since PBMCs are a heterogeneous mix of various leukocyte populations, we further studied a single cell type, CD4+ T cells, from the same donors. In addition to unmanipulated cells, we studied also cells exposed in vitro to inflammatory stimuli (LPS for PBMCs and anti-CD3/CD28 for CD4+ T cells). To replicate previously identified eQTL effects, we genotyped SNPs in IRF5, ERAP2 and ORMDL3 genes. RESULTS AND CONCLUSIONS: Principal components analysis showed specific expression signatures of the two types of stimulations. PBMC stimulation with LPS was characterized by 607 unique upregulated and 408 downregulated probes, whereas T-cell receptor stimulation showed instead 762 unique upregulated and 283 downregulated probes. In addition, 300 probes were upregulated and 45 downregulated in both cell types. 15 probes were downregulated in PBMCs but upregulated in CD4+ T cells. The three well known eQTLs showed clear genotype vs expression association in both cell types, demonstrating the power of our sample size to detect common eQTLs and the usefulness of even the heterogeneous PBMC population in eQTL studies. For functional studies of any target gene, our database provides useful information on general expression levels of any gene in PBMCs and CD4+T cells, on the stability or variability of their expression between individuals and effects of immunological stimuli on them.

## 2817/F/Poster Board #621

**Genetic identification, replication, and functional fine-mapping of expression quantitative trait loci in primary human liver tissue.** *G.M. Cooper[1,2], C.D. Brown[1,3], S. Mirkov[4], J. Ramirez[4], J.D. Smith[2], I.B. Stanaway[2], E. Heinz[3], M.H. Domanus[3], N.D. Trinklein[5], D.A. Nickerson[2], A.E. Rettie[6], M.J. Rieder[2], M.J. Ratain[4,7,8], K.P. White[3], F. Innocenti[4,7,8].* 1) equal contributors; 2) Dept of Genome Sciences, University of Washington, Seattle, WA; 3) Institute for Genomics and Systems Biology and the Depts of Human Genetics and Ecology and Evolution, University of Chicago, Chicago, IL; 4) Dept of Medicine, University of Chicago, Chicago, IL; 5) SwitchGear Genomics, Menlo Park, CA; 6) Dept of Medicinal Chemistry, University of Washington, Seattle, WA; 7) Committee on Clinical Pharmacology and Pharmacogenomics, University of Chicago, Chicago, IL; 8) Cancer Research Center, University of Chicago, Chicago, IL.

Most loci identified in genome wide association studies of complex traits reside in non-coding DNA and may contribute to phenotype via changes in gene regulation. The discovery of expression quantitative trait loci ('eQTLs') can thus be used to more precisely identify modest but real genetic associations and provide insights into the underlying molecular mechanisms. This is particularly true for expression studies in non-transformed cells from tissues relevant to the complex traits of interest. We have conducted two independent studies to identify genetic, including both SNPs and CNVs, and environmental determinants of human liver gene expression variation. We analyzed two sets of primary livers (n=220 and n=60) using Agilent and Illumina expression arrays, respectively, and Illumina SNP genotyping (550K). At least 30% of genetic and non-genetic factors that are significant ($p < 1 \times 10^{-9}$) in one study fail to replicate in the second study, suggesting that artifacts, like unknown SNPs that affect RNA-probe hybridization or hidden confounding variables, often result in statistically significant but biologically irrelevant correlations. This illustrates the value of independent replication to enrich for truly predictive eQTLs, and given our study design we identify hundreds of reproducible correlations. We show that these data can provide insights into disease-relevant phenotypes, with examples including eQTLs related to cardiovascular disease (e.g. LDL cholesterol), immune system function, and drug response (e.g. warfarin). Furthermore, we hypothesized that promoters and 3'UTRs are enriched for causal eQTL variants. Therefore, we re-sequenced the promoters and 3'UTRs of 25 eQTL genes, cloned each discovered haplotype, and quantified their effect on transcription using a luciferase-based assay. We find multiple examples of robust, haplotype-specific in vitro functional differences that correlate directly with in vivo expression levels. This suggests that many eQTLs can be fine-mapped to one or a few single-nucleotide variants and mechanistically characterized using such assays. To our knowledge, these are the first analyses of primary liver eQTLs to include both independent statistical replication and functional characterization. Our data suggest that integration of functional assays with eQTL discovery, and eQTLs with complex trait associations, is a powerful means to improve biological interpretability of genotype-phenotype correlations.

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## 2818/F/Poster Board #622

**Cis-association mapping of expression trait loci underlying the transcriptional response induced by TLR7 activation in lymphoblastoid cell lines.** *S. Biswas[1], J. Strout[2], J.D. Smith[1], J.M. Akey[1], M.M. Wurfel[2].* 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Division of Pulmonary and Critical Care Medicine, Harborview Medical Center, University of Washington, Seattle, WA.

Recognition of single-stranded RNA present in viruses such as influenza and HIV is mediated by Toll-like receptors 7 and 8 (TLR7/8), and leads to activation of an innate immune response. Genetic variation that modulates this response could play a role in host susceptibility to infection. We hypothesized that the transcriptional response to a TLR7-specific agonist in B-lymphoblastoid cell lines (B-LCLs) is heritable and that loci controlling these responses can be identified. We used B-lymphoblastoid cell lines from the HapMap Project and publicly available SNP genotype and copy number polymorphism data to map cis-eQTLs that are associated with variation in the innate immune response. Gene expression profiles of ~24000 transcripts in 82 and 87 individuals with CEPH and Yoruban ancestry, respectively, were determined in resting and CL097-treated cells using Illumina Human Ref-8 arrays. At a conservative FDR threshold (FDR=0.01), 2470 transcripts were differentially expressed between the two conditions. The most significantly differentially expressed gene ZC3H12A ($p=1.4x10^{-47}$) confirms a recent finding describing its role as an IL6 regulator. In addition, the result includes several genes like SLC43A3 ($p=1.1x10^{-16}$), INSIG1 ($p=1.6x10^{-16}$) that are not known to play a role in TLR7 mediated response. Using a more stringent estimate of differential expression, 15% of the transcripts were found to have significantly heritable transcriptional variation ($p < 0.05$, $h^2$= 0.3) including PHF11, ALOX5AP, and HVCN1. We performed population-specific tests of association at cis-acting variants (SNPs and CNV clones within 1Mb of transcript) to map expression trait loci. At a bonferroni-corrected p-value of 0.05, significant cis-SNP associations were obtained for 19 and 38 transcripts in the CEPH and Yoruban samples respectively, while 9 and 11 significant ($p < 0.001$) cis-CNV associations were found in the respective samples. We also performed family-based tests of association using SNPs to confirm our findings. These analyses demonstrate that TLR7-mediated activation of B-LCLs induces a widespread transcriptional response, that a portion of this transcriptional response is heritable, and identifies sequence polymorphisms that underlie this heritability component.

## 2819/F/Poster Board #623

**Reference genes in LPS-stimulated monocytes.** *R.M. Grimholt, R. Øvstebø, M.K. Kringen, J.P. Berg, A.P. Piehler.* Department of Medical Biochemistry and Clinical Pharmacology, Oslo University Hospital, Ullevål, Oslo, Norway.

**Background:** Stimulation of monocytic cells with bacterial lipopolysaccharide (LPS) is a frequently used model system to study pathophysiological processes of inflammation and infection. For the investigation of gene expression, quantitative real-time reverse transcription PCR (RT-qPCR) is the method of choice. Internal reference genes, previously called housekeeping genes, are often used for normalization of mRNA transcription levels between different samples. The selection of suitable reference genes is essential as several studies have shown that expression of traditional reference genes may vary substantially under certain conditions. In this study, we sought to identify and validate stably expressed reference genes in the frequently used model system of LPS-stimulated monocytes. **Methods:** Elutriated, purified human monocytes from six consenting, healthy donors were stimulated with LPS from E. coli and N. meningitidis, respectively, for three hours. Expression of twelve potential reference genes were assessed in stimulated and control monocytes using RT-qPCR. The most stably expressed genes and the recommended number of reference genes required for RT-qPCR normalization were calculated using the software package geNorm. **Results:** In monocytes stimulated with LPS from E. coli, the genes B2M (Beta-2-microglobulin), TMBIM4 (Transmembrane BAX inhibitor motif containing 4), PPIB (Cyclophilin B), PPIA (Cyclophilin A) and TBP (TATA-box binding protein) were the most stably expressed genes. Stimulation of monocytes with LPS from N. meningitidis exhibited least variation of the genes B2M, TMBIM4, PPIB, PPIA and UBC (Ubiquitin C). In both models, the three most stable reference genes (B2M, TMBIM4 and PPIB) were calculated to be sufficient to obtain satisfactory normalization of mRNA expression levels. Notably, the genes ACTB (beta-actin) and GAPDH (Glyceraldehyde-3-phosphate dehydrogenase), which are frequently used for gene expression normalization, were among the genes with the highest variability in monocytes stimulated with LPS from E. coli and N. meningitidis, respectively. **Conclusion:** Validation of stable reference gene expression is mandatory for normalization of RTqPCR results as genes traditionally used for normalization may vary significantly depending on experimental conditions. Employment of the reference genes B2M, TMBIM4 and PPIB is suitable to normalize gene expression in monocytes stimulated with bacterial LPS.

## 2820/F/Poster Board #624

**Multiplexed Single Cells mRNA-Seq: Expression Lineage of Blastomeres during Early Mouse Development.** *C. Barbacioru[1], F. Tang[2], E. Nordman[1], J. Bodeau[1], K. Lao[1], A. Surani[2].* 1) Life Technologies, Foster City, CA; 2) Wellcome Trust/Cancer Research UK Gurdon Institute of Cancer and Developmental Biology, University of Cambridge, Cambridge,.

We developed a barcode-based multiplexed single cells mRNA-Seq method to obtain whole transcriptome landscape for 32 single mouse blastomeres at their different development stages on the next generation sequencing technique, SOLiD System [1]. While the expression difference between two blastomeres at the 2-cell embryo stage is not significant, there are more than one thousand genes up/down regulated with a more than 4-fold difference between two blastomeres at 4-cell and 8-cell embryo stages. The expression profiles between two blastomeres within the same 2-cell embryo are similar to each other but they are not identical. We have observed differences in coverage obtained from different blastomeres suggesting variation of the transcribed regions. Comparing the differentially expressed genes between blastomeres from the same 2-cell embryo to the oocytes, we found that the differentially expressed genes between blastomeres from a 2-cell embryo were mainly associated with the up/down regulated genes from oocytes to 2-cell embryos. The newly expressed genes of the 2-cell embryo are clearly associated with differentially expressed genes between two blastomeres. A similar result is obtained when comparing 2-cell to 4-cell embryos. We also compared the differentially expressed genes between blastomeres in the same 2-cell embryo with E3.5 TE and ICM and we found that the differentially expressed genes between blastomeres in the same 2-cell embryo match to the differentially expressed genes between E3.5 TE and ICM. These genes may lead to the final lineage diversification. We also found that the differentially expressed genes between blastomeres in the same embryos match to the differentially expressed genes between E3.5 TE and ICM. For this study, changes in gene expression are complemented with structural changes. We used de novo assembly tool [2] for each individual cell stage development, to generate transcript assemblies. Although the vast majority of the contigs represent known transcripts, which prove the accuracy of our de novo transcript assembly method, we were able to identify alternative splicing events and new fusion transcripts.

References [1] Tang F et al, mRNA-Seq whole-transcriptome analysis of a single cell, Nat Methods, 6(5), 377-382, 2009 [2] Zerbino D, Birney E, Velvet: Algorithms for de novo short read assembly using de Bruijn graphs, Genome Res, 18, 821-829, 2008.

## 2821/F/Poster Board #625

**Use of biological and technical replicates for expression analysis of spotted microarrays.** *C.T. Ekstrøm[1], S. Bak[2], M. Rudemo[1].* 1) Dept. Basic Sciences and Environment, University of Copenhagen, Frederiksberg, Denmark; 2) Dept. Plant Biology, University of Copenhagen, Frederiksberg, Denmark.

Dye swap designs and duplicate or triplicate printing are often used for spotted microarrays. The combination of dye swaps and multiple prints makes it possible to partition the variance in both within-array variation (due to multiple printing) and within biological sample variation (due to dye swaps) as well as the normal biological variation. However, the number of biological and technical replicates are often small for microarray experiments so the precision of these variance estimates will be low for a single gene. We extend the idea from the LIMMA package of having a single common within-array correlation for all genes to accommodate both multiple sources of technical replicates and biological replicates. The extended method either assumes that only the within-array variation is identical for all genes or alternatively that also the within biological sample variation is shared between all genes. The proposed method is applied to a dataset from a study of *Arabidopsis* and the results are compared to the traditional method of analysis. We conclude that substantial differences in gene expression levels can be found when variation from several sources is taken into account.

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## 2822/F/Poster Board #626

**Role of Trichothiodystrophy DNA Repair and Transcription Genes in Human Placental and Fetal Development.** *A.H. Dzutsev[1], A. Morgun[2], C. Signore[3], J.L. Mills[3], J. Troendle[3], R. Moslehi[1,4].* 1) National Cancer Institute (NCI), NIH, Bethesda, MD; 2) National Institute of Allergy and Infectious Diseases (NIAID), NIH, Bethesda, MD; 3) National Institute of Child Health and Human Development (NICHD), NIH, Bethesda, MD; 4) Epidemiology and Biostatistics, School of Public Health, and Center for Excellence in Cancer Genomics, State University of New York (SUNY) at Albany, Rensselaer, NY.

**Background** Effects of abnormalities in DNA repair and transcription genes in human prenatal life have never been studied. Trichothiodystrophy (TTD) is a rare (affected frequency of $10^{-6}$) recessive disorder caused by mutations in genes involved in the nucleotide excision repair (NER) pathway and in transcription. Mutations in *XPD* (*ERCC2*), *XPB* (*ERCC3*) and *TTDA* (*GFT2H5*) which code for subunits of transcription factor TFIIH can cause TTD. Mutations in *TTDN1* (*C7ORF11*), a gene of unknown function, have also been associated with TTD. **Methods** Based on clinical observations, we conducted a genetic epidemiologic investigation of the role of TTD genes in human fetal development. We compared pregnancies resulting in TTD-affected offspring (N=24) with respect to gestational complications to population reference values. **Results** Highly significant increased risk of hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome (RR=35.7,P=0.0002), elevated mid-trimester maternal serum human chorionic gonadotropin (hCG) levels (RR=14.3,P<0.0001), Small for gestational age (SGA<3rd percentile) (RR=13.9,P<0.0001), preterm delivery (<32 weeks) (RR=12.0,P<0.0001), pre-eclampsia (RR=4.0,P=0.006) and decreased fetal movement (RR=3.3,P=0.0018) were noted in TTD-affected pregnancies. Abnormal placental development may explain constellation of observed complications. Thus, we hypothesize that TTD genes play an important role in normal human placental development. We investigated this hypothesis by analyzing the expression patterns of the four TTD genes identified to date. Using EST Profile Viewer (NCBI), we determined expression of all four genes in human placenta. Using microarray dataset GDS596 containing expression data for *XPD*, *XPB* and *TTDA* in different human tissues/organs, we found high expression of all three genes in placenta, above the mean of their expression in all organs. All three genes appeared to be in the higher range of their expression in placenta than in skin. Analyzing dataset GDS2528 containing normal placentas collected at different periods during gestation from 14 to 40 weeks indicated expression of all four genes throughout this period. Expression of *TTDA* was strongly negatively correlated (r=-0.7,P<0.0001) with gestational age, while *XPD*, *XPB* and *TTDN1* were consistently expressed throughout this period. **Conclusion** Our results indicate an important role for *XPD*, *XPB*, *TTDA* and *TTDN1* gene products during normal human placental and fetal development.

## 2823/F/Poster Board #627

**Characterization of Transformed Lymphoblastoid Cell Lines for use in Gene Expression and CNV Studies: An Alternative to Nucleic Acid from Whole Blood.** *G.A. Heiman[1], D. Fugman[1], P. Van Hummelen[2], J. Sypula[2], K. Arora[2], J.A. Tischfield[1], A.I. Brooks[1,2].* 1) Rutgers University Cell and DNA Repository, Human Genetics Institute of New Jersey, Department of Genetics, Rutgers University, Piscataway, NJ; 2) Environmental and Occupational Health Science Center, Bionomics Research and Technology Center, RWJMS-UMDNJ, Rutgers University, Piscataway, NJ.

Epstein Barr virus (EBV)-transformed lymphoblastoid cell lines (LCLs) made from blood lymphoblasts are the major renewable resource for both DNA and RNA in genetic studies. We compared LCLs, whole blood and fresh small lymphocytes for both gene expression and copy number to better understand their utility in constructing models of gene expression. Samples from 20 subjects were subjected to the following processing: A) PaxGene tube RNA isolation from whole blood, B) RNA isolation from Ficol-fractionated fresh lymphocytes, C) RNA isolation from EBV-transformed lymphocytes cultured for 30 days. In parallel, DNA was isolated from each subject for CNV analysis. Total RNA was processed in parallel for all samples and hybridized to Affymetrix U133 2.0 Plus expression arrays with labeled cDNA prepared with the NuGEN Whole Transcriptome Ovation protocol, which yields cDNA suitable for array analysis and QPCR with no directional bias from reverse transcription. Preliminary analyses of gene expression demonstrate significant differences between whole blood, fractionated fresh lymphocytes and LCLs. Cluster analysis revealed that lymphocytes from different subjects are relatively similar to each other but distinct from EBV-transformed cell lines. Differences in expression of specific genes between whole blood, fresh lymphocytes and LCLs will be reported in depth with regard to known cellular functions. It may be possible to identify and statistically “isolate” those parts of the transcriptome that are significantly affected by EBV transformation, thereby allowing a correction in LCL gene expression studies leading to the enhanced effectiveness of LCLs as models for gene expression. Our goal is to evaluate the utility of LCLs vs. fresh blood or lymphocytes for the interrogation of differences in gene function that arise through epigenomic or DNA sequence variation. Ongoing studies will generate CNV profiles of different cellular ontogenies for all subjects. LCLs from genetics research subjects are the most available surrogate tissues for gene expression and CNV profiling, but their utility revolves around specific gene expression similarity between whole blood, fresh lymphocytes, LCLs and specific tissues of interest in disease (e.g., brain or liver).

## 2824/F/Poster Board #628

**Myostatin inhibition gene expression profiling.** *F. Rahimov[1], O.D. King[3], L.C. Warsing[4], K.R. Wagner[4,5], L.M. Kunkel[1,2].* 1) Program in Genomics, Children’s Hospital Boston and Harvard Medical School, Boston, MA; 2) Howard Hughes Medical Institute; 3) Boston Biomedical Research Institute, Watertown, MA; 4) The Johns Hopkins University, School of Medicine, Baltimore, MD; 5) Kennedy Krieger Institute, Baltimore, MD.

Myostatin is a potent negative regulator of skeletal muscle mass and consequently it has widely been considered as a potential therapeutic target for neuromuscular disorders. The putative myostatin receptor is activin receptor type IIB (ActRIIB). In order to evaluate the molecular effects of a biotherapeutic that mimics the myostatin binding site of the receptor and identify potential downstream target genes of the myostatin signaling pathway, we compared gene expression patterns in two different muscle types from mice treated with a soluble ActRIIB with those deficient for the myostatin gene. We assessed global gene expression profiling in quadriceps and soleus muscles obtained from mice treated with ActRIIB.Fc over one and two weeks, from $Mstn^{-/-}$ and control mice using the Affymetrix GeneChip® 1.0 ST arrays. There was a significant overlap in up and downregulated genes in both muscle types between mice treated for two weeks with ActRIIB.Fc and $Mstn^{-/-}$ mice ($P$<0.0001). Consistent with previous reports, we observed an overrepresentation of Type IIa muscle fiber genes of the slow-twitch muscles among the downregulated genes in both the knock-out and inhibitor treated mice, although we did not detect a concomitant upregulation of Type IIb fiber genes encoding fast-twitch muscle fibers. Furthermore, we validated expression of 30 genes that showed the most significant up and downregulation on arrays with quantitative real-time PCR using TaqMan gene expression assays on Fluidigm’s BioMark microfluidic dynamic arrays. There was a strong correlation between microarray results and quantitative real-time PCR with a correlation coefficient of over 0.9. Our results demonstrate that intervening in the myostatin signaling pathway using exogenous drugs gives gene expression signatures that are comparable to those of myostatin knock-out mice.

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## 2825/F/Poster Board #629

**The impact of racial differences in epidemiologic studies of gene expression.** *S. Sharma[1, 2], A. Murphy[1], B. Himes[1,3,4], J. Chu[1], M. Cho[1,2], B. Klandermann[1], J. Sylvia[1], V. Carey[1], S. Weiss[1,4], B. Raby[1,2].* 1) Dept Respiratory Epidemiology, Channing Lab, Boston, MA; 2) Division of Pulmonary and Critical Care, Brigham and Women's Hospital, Boston, MA; 3) Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA; 4) Partners Center for Personalized Genetic Medicine, Boston, MA.

Although population differences in gene expression have been established, the impact of these differences on epidemiologic studies of gene expression is not well understood. We describe the effect of race on a gene expression study of impaired lung function in asthma. We generated Illumina HumanRef8(v2)gene expression profiles for 254 young adults(205 non-Hispanic whites; 49 African American)with mild- to moderate persistent asthma participating in the Childhood Asthma Management Program (CAMP) on whom concurrent total RNA derived from peripheral blood CD4+ lymphocytes and spirometry was obtained. After quantile normalization and log2 transformation of the raw expression data was performed, 18,373 autosomal probes were analyzed for association with lung function measures. Principal component analysis (PCA) as implemented in the *pcaMethods* package in Bioconductor was performed on all subjects to identify smaller clusters of genes that accounted for overall differences in gene expression. PCA identified three principal components that explained 57% of variance in gene expression. Using regularized t-tests with multiple comparison adjustments as implemented in the Bioconductor *siggenes* package, we identified 9,460 genes that were differentially expressed between self-identified non-Hispanic whites and African Americans. The effect of race was highly correlated with the first PCA principal component, which explains approximately 30% of the variance in gene expression across subjects. The impact of these racial differences in gene expression was also observed when attempting to model the relationship between gene expression and lung function. Using linear models with and without adjustment for race, we tested whether gene expression was associated with post-bronchodilator FEV1 percent predicted, a spirometric value that incorporates an adjustment for race. Although unadjusted linear models of post-bronchodilator FEV1 percent predicted identified genes that were correlated with lung function, after adjusting for race this correlation was no longer statistically significant, indicating confounding of gene expression by race. These results suggest that race is a critical covariate in epidemiologic studies of gene expression and that as in genetic studies, consideration of race in gene expression profiling studies is needed to avoid spurious association. Funding: NIH K12 HL089990 and R01 HL086601.

## 2826/F/Poster Board #630

**Global analysis of adipose tissue gene expression in response to insulin among insulin-resistant and insulin-sensitive women.** *J.M. Soronen[1,2], P-P. Laurila[1,2], J. Naukkarinen[1,2], J. Westerbacka[3], M. Jauhiainen[2], V. Olkkonen[2], H. Yki-Järvinen[3], L. Peltonen[1,2,4,5].* 1) Institution for Molecular Medicine Finland (FIMM), Helsinki, Finland; 2) National Institute for Health and Welfare/ Public Health Genomic Unit, Helsinki, Finland; 3) Department od Medicine, Division of Diabetes, Helsinki, Finland; 4) The Broad Institut, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA; 5) Wellcome Trust Sanger Institute Wellcome Trust Genome Campus, Hinxton, Cambridge, UK.

The development of insulin resistance is tightly connected to the metabolic status of adipose tissue and its endocrine functions. To gain a better understanding of the transcriptional changes in fat that develop along with increasing insulin resistance, we compared transcriptional profiles of 5 insulin-resistant and 5 insulin-sensitive women before and after a hyperinsulinemic euglycemic clamp. Subcutaneous adipose tissue biopsies were obtained after overnight fasting and after 3 hours of intravenously maintained euglycaemic hyperinsulinaemia and they were analyzed for transcript profiles using Affymetrix HG U133 Plus 2 microarrays. Data analysis and pathway analysis were performed with GeneSpring 10 and by using an in-house developed nonparametric pathway analysis. We observed extensive up-regulation of inflammatory pathways in the insulin-resistant subjects including antigen processing (GO:0002504), complement activation (GO:0050778) and chemotaxis (GO:0006935). The most prominent difference in gene expression during the hyperinsulinemic euglycemic clamp between insulin-resistant and insulin-sensitive group was observed in lipid biosynthesis pathways associated with steroid, sterol and cholesterol biosynthesis (GO: 0016126, GO:0006694, GO:0006695) as well as in several mitochondrial pathways. This study demonstrates that insulin resistance alters markedly the insulin reactivity of several lipid biosynthesis pathways and pathways associated with mitochondrial function in human adipose tissue.

## 2827/F/Poster Board #631

**Genome-wide microRNA profiles in peripheral blood as potential biomarkers for schizophrenia.** *C.Y. Lai[1,6], H.Y. Chen[2,6], Y.H. Huang[1], S.L. Yu[3,6], P.C. Hsiao[6], C.C. Wen[4], C.K Hsiao[1], C.M. Liu[4], P.C. Yang[5,6], H.G. Hwu[1,4], W.J. Chen[1,4,6].* 1) Department of Public Health, Institute of Epidemiology, National Taiwan University, Taipei, Taiwan; 2) Institute of Statistical Science, Academia Sinica, Taipei, Taiwan; 3) Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, National Taiwan University, Taipei, Taiwan; 4) Department of Psychiatry, College of Medicine and National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan; 5) Department of Internal Medicine, College of Medicine and National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan; 6) Division of Genomic Medicine Research Center for Medical Excellence Taipei, Taiwan.

Several recent studies have demonstrated the potential utility of the blood-based gene expression profiling as a diagnostic tool for schizophrenia, though these studies were limited to the expression of protein-coding genes. The expressions of non-coding genes such as microRNAs (miRNAs) are now considered to play a significant role for the regulation of gene expression by means of inhibiting the translation of messenger RNAs (mRNAs), indicating that the miRNAs profiling in the peripheral blood might be potential biomarkers for schizophrenia. This study aimed to identify blood-based miRNA signature and evaluate its potential as biomarkers for schizophrenia. This study enrolled 60 schizophrenia patients at National Taiwan University Hospital and 60 age- and gender-matched normal controls, and the paired sample was randomly separated into the learning sample and the validation sample. In the learning sample (n = 30 pairs), the expression levels of genome-wide 368 human miRNAs from peripheral blood were measured using ABI PRISM 7900 Real Time PCR system. Supervised classification with internal cross-validation method was used to identify miRNAs that might be useful as biomarkers for schizophrenia. Possible biological mechanisms implicated in the target genes involved by the miRNAs were explored using bioinformatics methods. We identified a blood-based nine-miRNA signature (miR-34a, miR-449, miR-564, miR-548d, miR-652, miR-659, miR-185, miR-4095p, and miR-501) that could discriminate schizophrenia patients from normal controls with an accuracy ranging from 78.5% to 90%. Bioinformatic analyses using the software Ingenuity Pathway Analysis indicated that the top networks associated with dysregulation of these miRNAs were those involved in neurological diseases, lipid metabolism, and respiratory system development and function. These nine candidate miRNAs are being evaluated in the validation sample (n = 30 pairs) using real-time RT-PCR. These results, although preliminary, indicate that genome-wide miRNA profiling is a feasible way for the identification of biomarkers for schizophrenia and the nine-miRNA signature identified in this study warrants further investigation.

## 2828/F/Poster Board #632

**From miRNomics to pathway analysis in Parkinson's Disease (PD).** *S.A. Oliveira[1,2], M. Martins[1,2], A. Rosa[3,2], B.V. Fonseca[1,2], S. Violante[2], L.C. Guedes[1], T. Mestre[1], M. Coelho[1], M.M. Rosa[1], J.M. Vance[4], J.J. Ferreira[1].* 1) Unidade Neurológica de Investigação Clínica, Instituto de Medicina Molecular, Lisboa, Portugal; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 3) Departamento de Ciências da Saúde, Universidade da Madeira, Portugal; 4) Miami Institute for Human Genomics, University of Miami, USA.

PD is the second most prevalent neurodegenerative disorder after Alzheimer disease. The main pathological hallmark of PD is the loss of dopaminergic neurons within the substantia nigra, leading to insufficient formation and action of dopamine in the basal ganglia circuitry. The cardinal clinical signs are muscle rigidity, resting tremor, bradykinesia and, in more advanced cases, postural instability. Although much has been learned in recent years about the genetic aetiology of Mendelian-inherited PD, far less is known about the molecular mechanisms underlying the vast majority of cases. MicroRNAs (miRNAs) are short, endogenous non-protein coding RNAs that post-transcriptionaly modulate gene expression by base-pairing to target mRNAs. miRNAs are abundant in the brain, are essential for efficient brain function, and increasing evidence implicates miRNA dysfunction in PD pathogenesis. To further investigate the role of miRNAs in PD etiology, we conducted miRNA expression profiling in peripheral blood mononuclear cells (PBMCs) of 19 PD patients and 13 controls, using Exiqon microarrays spotted with probes for 733 human miRNAs. Out of the 275 miRNAs expressed in PBMCs, 24 miRNAs were differentially expressed. All of these miRNAs are under-expressed in patients relative to controls. The chip results were validated by quantitative RT-PCR for selected miRNAs. 745 genes are predicted by at least six programs using different algorithms in miRecords to be target genes of the differentially expressed miRNAs, and pathway analysis using Ingenuity revealed that the top canonical pathways in which these genes are involved are novel pathways including hypoxia signaling in the cardiovascular system and retinoic acid receptor activation, and the previously implicated protein ubiquitination pathway. These findings are currently being further explored.

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## 2829/F/Poster Board #697

**Discovering biomarkers in dystrophinopathies.** *A. Ferlini, M. Bovolenta, P. Rimessi, C. Scotton, S. Brioschi, L. Merlini, F. Gualandi.* Dept Med Gen, Univ Ferrara, Ferrara, FE, Italy.

The DMD gene is the largest in the human genome, it spans 2.4 Mb and is made of 79 exons and 7 isoforms, all finely regulated and expressed in specific tissues. Mutations in this gene lead to three distinct phenotypes depending on the genomic variation: Duchenne Muscular Dystrophy (DMD), Becker Muscular Dystrophy and X-linked dilated cardiomyopathy. Therapeutic approaches are now becoming reality in DMD, nevertheless, clinical outcome measures may not always be sensitive enough to detect small changes in disease progression/regression and after short treatment periods. It is therefore imperative to identify enrichment endpoints, as biomarkers, able to document benefits of the treatment at the individual level. Recently, genome-wide approaches to monitor transcription have revealed a notable number of non coding RNAs (ncRNAs) with many regulatory functions. We designed a new gene-specific Gene Expression tiling array focused on the DMD gene and used it to detect non-coding transcripts in brain, heart, skeletal muscle and skin normal human tissues. We identified 35 sense transcripts from total RNA and 15 sense and 2 antisense transcripts from polyA+ RNA preferentially associated with heart and muscle tissues. The 35 transcribed units, clustered preferentially within the introns surrounding the isoforms first exons and ranging in size from 120 to 1205 nucleotides, were identified in the DMD gene introns and in its same sense of transcription after total RNA hybridization using the 90th percentile analysis. By aligning the transcribed units (TUs) with all the ESTs in UCSC Genome Browser we found that about 35% correspond to previously annotated ESTs; the remaining are transcribed sequences never identified before. We validated through Northern blotting and Real-time PCR all 15 polyA+ sense transcripts and fully characterised by RACE 3 polyA+ transcripts close to 7 isoforms' promoter regions. We are monitoring the transcription behaviour of the polyA+ transcripts in DMD patients myogenic cells before and after treatment with antisense oligoribonucleotides in order to identify if any of these ncRNAs may change its profile as well its effect on the cell phenotype (in terms of dystrophin isoforms representation). The aim is to evaluate if these ncRNAs might be employed as biomarkers of dystrophinopathic phenotype progression or regression.

## 2830/F/Poster Board #698

**Non-coding RNA: Using TaqMan® Assays to Quantitate the Expression Level of Long Non-coding RNAs.** *K. Lee, Y. Wang, B. Kong, P. Brzoska, F. Hu, C. Chen, M. Augustine.* Applied Biosystems, Foster City, CA. 94404.

It has been shown that the vast majority of the eukaryotic genome is transcribed and that a large proportion of the transcriptome includes non-protein coding RNAs (ncRNA). These noncoding transcripts are derived from intronic, intergenic and overlapping coding gene sequences that are arranged in complex networks where expression may come from the sense or antisense strand. The study of these sequences has emerged as an important area of investigation and recent studies have shown that ncRNAs play a role in chromatin modification, transcription and post transcriptional gene regulation. Deep sequencing has identified tens of thousands of short ncRNAs as well as long ncRNAs (>200 nts). In addition microarrays and genomic tiling arrays have been used to identify and study the expression pattern of long ncRNAs. For accurate and reproducible quantitation, we have developed human, mouse, and rat TaqMan® Assays that target specific long ncRNA sequences. Using Applied Biosystems genomic assay design pipeline and an enhanced set of selection criteria, we have designed TaqMan® Assays to the majority of the annotated noncoding RNA sequences listed in NCBI (RefSeq, NR_) as well as from the literature curated noncoding RNA database, RNAdb (http://research.imb.uq.edu.au/rnadb/) which contains a large collection of biologically relevant ncRNAs. The performance of the TaqMan® Assays was evaluated using assays designed to target >150 known ncRNAs. For high expressing transcripts, linearity across 4 logs was >0.96. In addition we found that there was no significant detection of gDNA for assays designed to cross known exon-exon junctions, indicating that these multi-exon assays are transcript specific.

## 2831/F/Poster Board #699

**Expression pattern of ABC transporter pseudogenes in 44 human tissues.** *A.P. Piehler, R.M. Grimholt, J.P. Berg.* Clinical chemistry, Oslo University Hospital, Ulleval, Oslo, Norway.

Background: Recently, our group has identified all ABC transporter pseudogenes in the human genome. We found that 45% of these pseudogenes are transcribed and accumulated evidence that a regulatory interdependency exists between a transcribed pseudogene (ABCC6P1) and its parental, protein coding gene (ABCC6). In this study, we sought to assess the expression pattern of other transcribed ABC transporter pseudogenes in a broad variety of human tissues. Methods: RNA pools (at least 3 donors) from 44 different healthy human tissues were reverse transcribed. Expression stability of twelve potential reference genes in these tissues and the sufficient number of reference genes for normalization were assessed by RT-qPCR and the program geNorm. Based on the five most stable reference genes, RNA levels of five transcribed ABC transporter pseudogenes and their parental, protein coding genes were determined. Results: Except for the pseudogene homologous to the rodent ABC transporter ABCA15, which is predominantly expressed in testis and blood vessels, all transcribed pseudogenes are expressed in a wide variety of healthy human tissues. The pseudogene ABCC6P1 shows a similar expression pattern like its parental gene ABCC6 with highest expression in kidney and adult and fetal liver. The other transcribed pseudogenes do not follow the expression pattern of their parental genes. For the pseudogene ABCA11P, highest RNA levels can be found in whole adult and fetal brain and in artery blood vessels, whereas the parental gene, ABCA10, is mainly expressed in ovary, heart and bladder tissue. The surfactant deficiency causing gene, ABCA3, exhibits highest mRNA levels in lung and adult and fetal brain, whereas its pseudogene, ABCA17P, is predominantly expressed in the salivary gland, fetal brain and testis. The gene encoding the multidrug resistance protein 2 (MRP2, ABCC2) is mainly expressed in the kidney and the adult and fetal liver. Its homologous pseudogene ABCC13 shows highest RNA levels in peripheral blood leukocytes, bone marrow and fetal liver. Conclusions: Transcription of pseudogenes in the human ABC transporter superfamily is a common phenomenon. Together with the expression of pseudogenes into several alternatively spliced variants and the evidence of gene-pseudogene interference, differential expression of ABC transporter pseudogenes in a broad variety of human tissues suggests distinct physiological functions of these genes.

## 2832/F/Poster Board #700

**Association of ALOX5 gene polymorphisms with asthma and functional validation.** *S. Park[1], H. Chang[1], H. Song[1], J. Park[2], A. Jang[2], Y. Lee[3], S. Uh[3], M. Kim[4], I. Choi[5], S. Cho[6], C. Hong[6], Y. Lee[6], J. Lee[7], B. Park[8], H. Cheong[8], H. Shin[8,9], C. Park[1,2].* 1) Genome Research Center, Soonchunhyang Univ. Hosp., Bucheon, Korea; 2) Division of Allergy and Respiratory Medicine, Department of Internal Medicine, Soonchunhyang University Hosp., Bucheon; 3) Division of Allergy and Respiratory Medicine, Department of Internal Medicine, Soonchunhyang University Hosp.,Seoul; 4) Department of Internal Medicine, Chungbuk National University; 5) Department of Allergy, Chonnam National University Medical School and Research Institute of Medical Sciences; 6) Department of Internal Medicine and Institute of Allergy, Yonsei Univ; 7) Department of Interanal Medicine, College of Medicine, Hallym Univ; 8) Department of Genetic Epidemiology, SNP Genetics, Inc; 9) Department of Life Sciences, Sogang Univ.

Background: CysLTs exert important roles in airway inflammation of asthma. ALOX5 is the first committed enzyme in the biosynthetic pathway leading to the production of the LTs. Methods: We genotyped five SNPs of ALOX5 gene in a Korean asthma cohort (n=1189) and association analysis was done using logistic regression analysis. Promoter activity was measured by luciferase reporter assays. mRNA levels of B cell lines were measured by real-time PCR. Urinary concentrations of lipoxin A4 (LXA4) and LTE4 were measured by EIA. Results: The ratio of major allele homozygote of -754C>G on promoter region and +21757G>A on intron 3 were higher in asthmatics than those in normal subjects (dominant model, -754CC; 89.7 vs 83.1%, +21757GG; 39.4 vs 29.3%, p = 0.001 - 0.041). The quantitative mRNA expression levels and promoter activity were significantly higher in B cell lines having G allele on -754 than that possessing C allele (p=0.007, 0.003). RT-PCR demonstrated an alternatively-spliced variant of ALOX5. This variant lacked an exon 3 of the previously-reported full length sequence, resulting that produces a pre-mature stop codon. A variant form lack the lipoxygenase domain. The ratio of quantitative mRNA levels for alternatively spliced variant to wild form were significantly lower in B cell lines having G allele on +21757 than that possessing A allele. The basal urinary concentration of LTE4 and LXA4 was significantly higher in asthmatic patients with the -754 C allele than those who were homozygous for the G allele (p=0.031). The +21757 G>A was not associated with the basal urinary concentration of LTE4 and LXA4. Conclusion: The ALOX5 gene polymorphisms may be associated with the development of asthma via modulation of the ALOX5-gene expression and/or alternative splicing.

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## 2833/F/Poster Board #701

**Extensive cell type specificity of common regulatory variation.** *A.S. Dimas[1], S. Deutsch[2], B.E. Stranger[3], S.B. Montgomery[1], C. Borel[2], H. Attar-Cohen[2], C. Ingle[1], C. Beazley[1], M. Gutierrez-Arcelus[1], M. Sekowska[1], M. Gagnebin[2], J. Nisbett[1], P. Deloukas[1], E.T. Dermitzakis[1,2], S.E. Antonarakis[2].* 1) Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 2) Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland; 3) Division of Genetics, Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA.

Studies correlating genetic variation to gene expression provide information for the interpretation of common human phenotypes and disease. To date most studies have examined the genetic basis of gene expression variation either in large collections of EBV-transformed B-cells (lymphoblastoid cell lines or LCLs) or in single cell types. However, the cell type-dependence of genetic variants associated with gene expression (eQTLs) still remains largely unexplored. To address this we performed gene expression profiling and association with genetic variants (SNPs) on three cell types (LCLs, primary fibroblasts and T-cells) for 75 individuals. eQTLs in LCLs are well-replicated between previous studies and the present analysis, with over 80% of identical SNP-probe pairs passing stringent significance thresholds. This highlights the value of large collections of LCLs from different cohorts for studies of gene expression. We detected extensive cell type-specific genetic effects with only 10% of gene associations shared in all three tissues and 69 - 80% of regulatory variants operating in a cell type-specific manner. After controlling for the correlation structure between SNPs in the genome, we found that at least 7% of genes had more than one independent eQTL and the abundance of these eQTLs was positively correlated with the number of transcripts per gene. Cell type-specific eQTLs were found at larger distances from genes and had lower effect size similar to known enhancers. Furthermore, a number of eQTLs were found to overlap with well-documented variants from genome-wide association studies including SNPs associated with Crohn's disease, bipolar disorder, triglycerides, HDL cholesterol, BMI and height, thus suggesting a regulatory role for these variants. Overall, our study suggests that the complete regulatory and functional repertoire of variants can only be uncovered in the context of tissue and cell type specificity.

## 2834/F/Poster Board #702

**Enhancing the Power of eQTL Mapping: A Catalog of 6,000 cis eQTLs in Lymphocytes.** *L. Liang[1], A.L. Dixon[2,3], M.F. Moffatt[2], W. Chen[1], S. Heath[4], G.M. Lathrop[4], W.O.C. Cookson[2], G.R. Abecasis[1].* 1) Center for Statistical Genetics, Dept. of Biostatistics, SPH II, Ann Arbor, MI 48109, USA; 2) National Heart and Lung Institute, Imperial College London, SW3 6LY, England; 3) Wellcome Trust Centre for Human Genetics, University of Oxford, OX3 7BN, England; 4) Centre National de Genotypage, 91057 Evry Cedex, France.

Genome-wide expression quantitative loci (eQTL) mapping has been widely used in recent years to provide biological insights about the regulation of gene expression, to further understanding of the differences in gene expression profiles and regulation among populations and tissues, and to aid in the interpretation of disease associated loci from many genome-wide association studies. Appropriately powered analyses of mRNA transcript levels have the power to detect hundreds of genome-wide significant cis association signals, but in previous reports only ~14% transcripts with high heritability have been mapped with genome-wide significance. Here, we reanalyze previously published eQTL datasets with newly available statistical methods and show that the number of eQTLs mapped in cis increases from 2,175 to 6,162 when (a) better statistical models are used to describe shared correlation between different gene expression measurements and (b) data from the 1000 Genomes Project is used to drive genotype imputation and augment the list of genotyped SNPs. Specifically, we show that principal component analysis (PCA) can be used to capture non-genetic contributions to many different gene expression levels and that the resulting information can be used to adjusted observed expression values generating a phenotype that is more mappable (because it results in a greater number of cis eQTL that reach genomewide significance, without increasing background false positive rates). Similarly, we show that genotype imputation using the 1000 Genomes haplotypes as templates can increase the number of markers available for testing to >8 million and increases the number of cis eQTL, even after adjusting statistical significance thresholds to account for the greater number of markers tested.

## 2835/F/Poster Board #703

**Measurement of Allelic Expression in postmortem brain samples using Illumina Goldengate assay.** *K.K. Mantripragada, L.C. Carroll, M.C. O'Donovan, M.J. Owen, N.M. Williams.* Psychological Medicine, Cardiff University, Cardiff, United Kingdom.

Differential allelic expression has been implicated in many human diseases including complex disorders. The importance of cis-acting regulatory polmorphisms on gene expression has been well documented. However, currently there are no high throughput methods that allow assessment of differential allelic expression under cis-acting influences. We utilised Illumina's Goldengate methodology and the Cancer SNP panel for validating the allelic expression in 14 postmortem brain samples obtained from the Stanley foundation. We also spiked in varying proportions of Human and Chimpanzee DNA samples in order to validate the performance of Goldengate protocol in differential copy number measurement. Preliminary results demonstrate that the approach can detect quantitative differences in allelic expression and further work is required to optimise the protocol for robust performance.

## 2836/F/Poster Board #704

**Causal regulatory variants for complex trait associations.** *A.C. NICA[1,2], S.B. MONTGOMERY[1,2], B.E. STRANGER[1,3], A.S. DIMAS[1,2], C. BEAZLEY[1], I. BARROSO[1], E.T. DERMITZAKIS[1,2].* 1) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1HH, UK; 2) Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland; 3) Harvard Medical School/Brigham and Women's Hospital, Boston MA, USA.

The recent success of genome-wide association studies (GWAS) is now followed by the challenge to determine how the reported susceptibility variants mediate the manifestation of complex traits and diseases. Expression quantitative trait loci (eQTLs) have been implicated in disease associations through overlaps between eQTLs and GWAS signals. However, the high abundance of eQTLs and the strong correlation structure of variants in the genome make it likely that some of these overlaps are coincidental and not driven by the same functional variants. In order to distinguish accidental colocalizations from causal regulatory variants, we propose an empirical methodology that directly combines eQTL and GWAS data while accounting for the correlation structure (linkage disequilibrium - LD) of the region harbouring the GWAS SNP. By assessing the impact on the expression phenotype of the removal of the GWAS SNP effect, we discover causal regulatory variants and their affected candidate genes in *cis* and *trans*. To test this, we use gene expression measured in lymphoblastoid cell lines derived from 109 Phase 3 HapMap CEU individuals and focus on the subset of 976 GWAS single nucleotide polymorphisms (SNPs) catalogued by NHGRI and genotyped in the HapMap 3 CEU samples. We observe a significant abundance of regulatory signals for the analyzed GWAS loci, prominently in *cis*. We further dissect this signal and detect potential disease causing regulatory effects for a number of traits, with a strong enrichment for immunity-related conditions, consistent with the nature of the cell lines tested. Furthermore, we present an extension of the method in *trans*, where the primary biological effect of the disease variant is not necessarily *cis*-regulatory. As such, we discover multiple genes throughout the genome with equally convincing evidence for disease relevance and we indicate that these are likely to act downstream of the causal variant. Finally, we also show that the correlation strength ($R^2$) between the GWAS SNP and the eQTL is not, as generally assumed, a sufficient predictor of regulatory mediated disease effects.

## 2837/F/Poster Board #705

**Direct RNA Sequencing.** *P. Milos, A. Platt, D. Jones, J. Reifenberger, P. McInerney, J. Thompson, J. Bowers, M. Jarosz, F. Ozsolak.* Helicos BioSciences Corp, Cambridge, MA.

Methods for in-depth characterization of transcriptomes and quantification of transcript levels have emerged as valuable tools for understanding cellular physiology and human disease biology. Current methods, however, typically require RNA to be converted to complementary DNA (cDNA) prior to measurements and sequencing. cDNA synthesis has been shown to introduce biases and artifacts. We have developed a single molecule RNA sequencing technology, in which RNA is sequenced directly without prior conversion to cDNA. As a proof-of-concept experiment, we applied this technology to sequence attomole quantities of poly-A+ Saccharomyces cerevisiae RNA and human small RNAs utilizing a surface coated with poly(dT) oligonucleotides to capture the RNA and serve as primers for sequencing by synthesis. We observed 3' end RNA heterogeneity and poly-adenylated short RNA species. This novel technology paves the way towards high-throughput and low-cost direct RNA sequencing, with the ultimate goal of understanding transcriptomes in a comprehensive and bias-free manner.

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## 2838/F/Poster Board #706

**Integrating large-scale genetic and monocyte expression data reveals major *trans* regulators of biological processes.** *M. Rotival[1,2], T. Zeller[3], P. Wild[3], S. Szymczak[4], A. Schillert[4], T. Munzel[3], F. Cambien[1,2], A. Ziegler[4], L. Tiret[1,2], S. Blankenberg[3].* 1) INSERM UMRS 937, Paris, France; 2) Université Pierre et Marie Curie, Paris, France; 3) II. Medizinische Klinik und Poliklinik, Johannes-Gutenberg Universität Mainz, Mainz, Germany; 4) Institut für Medizinische Biometrie und Statistik, Universität zu Lübeck, Lübeck, Germany.

The simultaneous assessment of gene expression and genetic variation on a genome-wide basis provides a powerful tool for unravelling the mechanisms by which genes might contribute to disease. While *cis* expression SNPs (eSNPs) have ubiquitous effects that can be easily mapped with high-throughput technologies, identification of *trans* eSNPs is more challenging because they are more context- and tissue-dependent and prone to spurious findings. Yet, *trans* eSNPs are of considerable interest because they constitute the ground of systems genetics by their capacity to control transcriptional modules, ie sets of highly co-regulated genes. Since *trans* eSNPs are expected to have pleiotropic effects on a large number of genes, each being modestly affected, mapping these SNPs may be facilitated by prior recognition of subsets of co-regulated genes. In a large population-based cohort (n=1,490), we have analyzed 12,808 well characterized genes expressed in circulating monocytes - a key player in the development of atherosclerosis and the immune response - in relation to genome-wide variability (675,350 SNPs). We applied first a method of extraction of expression patterns - Independent Component Analysis - allowing us to identify 91 transcriptional modules of co-regulated genes, several of them being significantly enriched in functional pathways. These patterns were then related to SNPs. At a study-wise significance threshold of $8.14 \times 10^{-10}$, we found 8 blocks of SNPs associated with expression patterns. One block mapped to the *ERBB3* gene previously identified as a susceptibility gene for type 1 diabetes (T1D). As the associated pattern did not contain *ERBB3*, an effect via *ERBB3* expression could be excluded. The associated pattern suggested *MADCAM1*, an endothelial adhesion cell molecule, as the most plausible mediator linking *ERBB3* to T1D. Another block mapped to the *ARHGEF3* gene which is involved in RhoGTPase activation. The associated pattern was strongly enriched in genes involved in cell adhesion and platelet activation, including several integrins. *ARHGEF3* is known to undergo alternative splicing and one explanation for the association might be that the splicing leads to a less efficient interaction of *ARHGEF3* with RhoGTPases, with subsequent repercussions on the activation of the Rho signaling pathway. This study demonstrates how coupling genome-wide expression and genetic variability in large cohorts may help to identify major *trans* regulators.

## 2839/F/Poster Board #707

**Genome-wide prediction and expression analysis of common SNPs affecting mRNA splicing.** *P.K Rogan[1,2], T. Whitehead[3], E.J. Mucaki[1].* 1) Biochemistry; 2) Computer Science; 3) SHARCNET, University of Western Ontario, London, ON, Canada.

Non-coding, incompletely penetrant mutations can be difficult to detect, but are of significant interest due to their likely contributions to complex genetic disorders. Genome-wide prediction of common SNPs with subtle effects on mRNA splicing was undertaken followed by targeted assessment of differences in gene expression between the major and minor alleles. Software was developed in the Haskell language to compute information changes of constitutive splice sites ($\Delta R_i$, in bits) for all validated SNPs across the genome. Genome-wide, 963 natural donor and 2134 natural acceptor sites exhibited significant alterations (≥1 bit or ≥2 fold) in strength. ($\Delta R_i$ values were compared with allele-specific differences in expression based on exon microarray data from Phase II HapMap individuals (GEO Accession GSE7851). Splicing indices for each exon (the ratio of the exon to core probeset intensities; SI) were determined for each SNP genotype. 9997 SNPs were predicted to alter splice site strength of natural or cryptic splice sites within the sequences defining splice site intervals (-26 to +2 and -3 to +7). Of 987 SNPs which are associated with significant changes in SI values, 573 SNPs have minor alleles with reduced $R_i$ values and SI's < 1. Natural acceptor splice sites were weakened or abolished for ~20% (n=2008) of minor SNP alleles; most were associated with exons with significant allele-specific differences in SI (83%). Minor alleles weakened or abolished donor sites in ~5% (n=453) of SNPs, and most were associated with allele-specific differences in SI (75%). Many minor alleles were predicted to activate cryptic acceptor (838 of 2065) or donor (161 of 405) sites and 40% had altered SI values. The exon boundaries of published expressed sequence tags corroborated predictions of strengthened SNP-induced cryptic splice forms for 60 different acceptor sites and 43 different donor sites. SNPs predicted to result in leaky splicing mutations are much less likely to result in exon skipping. This class of mutations ($\Delta R_i < 4$ bits for donors and <7 bits for acceptors, $R_i \geq R_{i,minimum}$) comprise 40-60% of the total and also exhibits reduced SI values. At least 16 of these SNPs are found in 15 genes (*LILRA2*, *EGF*, *FGF1*, *IGFBP3*, *COL2A1*, *DOCK9*, *CALCA*, *MAD2L2*, *CSMD1*, *TRPV1*, *GNAL*, *PDE11A*, *LAMA2*, *CRYZ*, and *ABCA1*) previously documented in genome-wide association studies. Several of these are in LD with SNPs known to exhibit significant associations.

## 2840/F/Poster Board #708

**Gene expression profile of human multipotent mesenchymal stromal cells derived from bone marrow during osteoblast differentiation.** *C. Kaneto[1], P. Lima[2], W. Silva-Jr.[1].* 1) Dept Genetics, Faculdade de Medicina de Ribeirão Preto, Sao Paulo, Brazil; 2) Natural Science Dept, Universidade Estadual do Sudoeste da Bahia, Vitória da Conquista, BA, Brasil.

Mesenchymal stem cells (MSCs) are multipotent precursors present in adult bone marrow that differentiate into osteoblasts, adipocytes and myoblasts. However, their low frequency in bone marrow requires ex vivo expansion for clinical application. We examined gene expression of these cells by serial analysis of gene expression during differentiation into osteoblasts. SAGE analysis of MSC during osteogenic differentiation identified three candidate genes for further examination and functional analysis: CRYAB, TNFRSF11B and ID3. These genes showed differential expression in the early phases of osteoblastic differentiation, providing evidence that they exhibit specific roles during this process. Additionally, we were able to identify cancer-related pathways represented in our data, suggesting that mesenchymal stem cell and cancer can share some particularities. This study reports the profile of gene expression in MSCs during differentiation and identifies the important contribution of extracellular protein products, adhesion molecules and growth factor molecules as part of their transcriptome. Finally, we were able to illustrate the different phases of osteogenic development in a human model and could associate gene clusters to four distinct phases of the development of osteoblast phenotype.

## 2841/F/Poster Board #709

**Genetic genomics of Parkinson Disease: an association-based method for detecting modules of co-expressing genes in disease.** *E. Kenny[1,2], R. Melchiotti[2], O. Litvin[3], M. Kedmi[4], A. Bar-Shira[4], N. Giladi[5,6], D. Pe'er[3], I. Pe'er[2], A. Orr-Urtreger[4,6].* 1) Rockefeller Univ, New York, NY; 2) Department of Computer Science, Columbia University, New York, USA; 3) Department of Biological Sciences, Columbia University, New York, USA; 4) Genetic Institute Unit, Parkinson Center, Department of Neurology, Tel-Aviv Sourasky Medical Center, Israel; 5) Movement Disorders Unit, Parkinson Center, Department of Neurology, Tel-Aviv Sourasky Medical Center, Israel; 6) Sackler Faculty of Medicine, Tel-Aviv University, Israel.

Gene expression phenotypes have proven uniquely useful for association mapping. Not only are genetic effects stronger and more isolated at the molecular level, but also the inherent modularity in expression space and the functional interpretation of such associations is more informative. We consider modules in transcription profiles as the link between genotypes and disease. We have adapted a framework, originally presented in yeast genomics, to search for association between SNPs and transcription modules. We apply our method to a unique dataset including genomewide SNP and transcription data in Parkinson disease patients and matched controls from an Ashkenazi population. This data is enriched for carriers of disease mutations, providing a useful genetic handle on disease mechanisms. The scale of this problem, involving association mapping of a million SNPs by 20,000 transcripts poses a challenge for traditional methods. Furthermore, mapping is complicated by the multiple correlations due to linkage disequilibrium in the SNP data on the one hand and co-expression on the other. We thus implement a rapid and extensive permutation system to select statistically significant associations in a computationally feasible manner - 30,000 hours or 12.5 days on a 100 CPU core (3.0Ghz) cluster. Our methodology exposes 2,142 SNPs that map to modules of 5 or more co-expressed transcripts, where the nominal p-value for each transcript in the module exceeds the permutation threshold. The modules range in size from 5 to over 1,000 co-expressed transcripts and are enriched for functional annotations. Motivated by these initial findings, we next seek to assess whether any of these associated modules influence Parkinson disease. Current work seeks to implement a likelihood based scoring function for disease status given both genotype and expression data.

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## 2842/F/Poster Board #710

**Investigation of gene expression levels and XCI patterns in female carries of *MECP2* microduplications.** *K. Ravn[1], RS. Møller[2], B. Bertelsen[1], W. Reardon[3], Z. Tümer[1], LB. Møller[1].* 1) Kennedy Center, Glostrup, Denmark; 2) Danish Epilepsy Centre, Dianalund, Denmark; 3) Our Lady's Hospital for Sick Children, Crumlin, Dublin 12, Ireland.

Microduplications at Xq28, which include the dosage-sensitive methyl-CPG binding protein gene (*MECP2*), cause severe X-linked mental retardation in males. Other phenotypic characteristics are absent or limited speech, progressive neurological problems (such as spasticity and seizures), axial and facial hypotonia, mild and non-specific facial dysmorphism, and severe recurrent respiratory infections. The typical size of these microduplications varies between 0.2 to 2 Mb. The male patients often inherit the duplication from their phenotypically normal mothers. X chromosome inactivation (XCI) studies using peripheral blood lymphocytes from the phenotypically normal female carriers show a complete skewing of one of the X chromosomes, probably favouring the normal X chromosome. Here we report two families with overlapping Xq28 duplications, involving *MECP2*. In both families the duplications segregate for three generations involving three affected males and five female carriers. One of the female carriers displays neurological symptoms. In this study we investigated the gene expression levels of *MECP2*, *IRAK1* and *L1CAM*, which all are located within the duplicated regions, in cultured fibroblasts of the involved affected males and female carriers. Furthermore we determined the local inactivation pattern of the female carriers using several differentially methylated polymorphic loci scattered on the long arm of the X chromosome. This study may give clues to the phenotype-genotype correlation of the duplications observed in this region.

## 2843/F/Poster Board #711

**Establishment of a whole transcriptome data analysis workflow and cognate modules for quality control and quantitation using the SOLiD 3 system.** *J.J. Schageman[1], J. Brockman[1], C. Mueller[1], D. Ilsley[1], P. Whitley[1], A. Lemire[1], K. Bramlett[1], S. Heater[1], J. Gu[1], S. Kuersten[1], C. Barbacioru[2], A. Siddiqui[2], B. Tuch[2], M. Muller[2], R. Setterquist[1].* 1) Research and Development, Life Technologies Corp., Austin, TX; 2) Life Technologies Corp., Foster City, CA.

Ultra high-throughput sequencing of RNA (RNA-Seq) has shown great promise for the measurement of transcriptional activity at a single nucleotide resolution. The tradeoff for such a powerful technology is management of the voluminous resultant data and what analysis workflows are appropriate for answering specific biological questions. We will present an analysis workflow for assessing quantitation and characterization of millions of RNA sequence reads generated by the SOLiD 3 system. We have established logical bioinformatics workflow consisting of a combination of read mapping to a reference genome after specific filtering of overrepresented RNA species combined with several summarization modules. Included are modules that effectively report QC information in the form of reproducibility measures, filter mapping statistics as well as quantitation and per base coverage of well known spike in controls and housekeeping genes. Downstream modules report a per nucleotide transcriptional index across each chromosome which may be used for comparing run to run reproducibility and serve as an estimator of differential expression for annotated segments of the genome. In addition, saturation reports describe the fraction of annotated RNAs that are detected per sample per million reads in common reference RNA sequence databases including RefSeq and the more comprehensive AceView. This workflow has allowed for facile interpretation and faster turnaround when evaluating transcriptional activity and QC information from a given sample RNA.

## 2844/F/Poster Board #712

**Three individual human genome sequences.** *J. van Velkinburgh[1], J. Kim[2], Y. Ju[2], H. Park[2], S. Kim[3], S. Lee[3], J. Yi[2], J. Mudge[1], N. Miller[1], D. Hong[2], R. Kim[1], H. Kim[2], G. Schroth[6], G. Church[5], C. Lee[4], J. Oksenberg[7], S. Baranzini[7], S. Hauser[7], S. Kingsmore[1], J. Seo[2].* 1) NCGR, Santa Fe, NM; 2) ILCHUN Genomic Medicine Institute, Medical Research Center, Seoul National University, Seoul 110-799, Korea; 3) Macrogen Inc., Seoul 153-023, Korea; 4) Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, 02115, USA; 5) Department of Genetics, Harvard Medical School, Boston, MA 02115; 6) Illumina Inc., Hayward, CA 94545, USA; 7) Department of Neurology, School of Medicine, University of California San Francisco, 513 Parnassus Ave. Room S-256, San Francisco, CA 94143-0435, USA.

Recent advances in sequencing technologies have initiated an era of personal genome sequences. To date, human genome sequences have been reported for male individuals with ancestry in four distinct geographical regions: a Yoruba African, two individuals of Northwest European origin, a person from China and a Korean. Here, we provide highly annotated, whole genome sequences for a Korean male (AK1) and two Northwest European females. The genome of AK1 was determined by a combined approach that included whole genome shotgun sequencing (27.8x coverage), targeted bacterial artificial chromosome (BAC) sequencing, and high-resolution comparative genomic hybridization (CGH) using custom microarrays featuring over 24 million probes to provide the most detailed analysis of an individual human genome to date. The two female genomes were determined by whole genome shotgun sequencing (approximately 18x coverage of each). Alignment to the NCBI reference disclosed approximately 3.5 million single nucleotide polymorphisms (SNPs) and approximately 200,000 deletion or insertion polymorphisms (indels) in each genome. SNP and indel densities were strongly correlated genome-wide. Applying very conservative criteria yielded highly reliable copy number variants (CNVs) for clinical considerations. Potential medical phenotypes were annotated for nsSNPs, coding domain indels, and structural variants. Integration of human whole genome sequences, particularly from groups of individuals with minimal ethnic admixture, will assist in understanding genetic ancestry, migration patterns, and population bottlenecks.

## 2845/F/Poster Board #745

**Fine mapping of association signals with haplotype association analysis in CGEMS when a functional variant is known.** *J. Gonzalez Bosquet[1], M. Yeager[1], L. Amundadottir[1], P. Kraft[1], K.B. Jacobs[1,3], S. Wacholder[1], R.N. Hoover[1], G. Thomas[1], D.J. Hunter[1,2], S.J. Chanock[1].* 1) Division of Cancer Epidemiology and Genetics, NCI, Bethesda, MD; 2) Department of Epidemiology, Harvard School of Public Health, Boston, MA; 3) SAIC-Frederick, NCI-Frederick Cancer Research and Development Center, Frederick, MD.

The NCI Cancer Genetic Markers of Susceptibility (CGEMS) initiative has conducted multi-stage GWAS in both breast and prostate cancer with the discovery of new loci highly associated with both cancer risks. In an attempt to fine map these associations, we performed haplotype association analyses of regions based on the selection of tagged SNPs genotyped in two multi-stage GWAS of breast cancer and prostate cancer in the CGEMS study. The initial breast GWAS genotyped 528,173 SNPs using the Human Hap500 Infinium Assay in the Nurses' Health Study (NHS), and in a second stage over 30,000 markers with a custom Illumina iSelect panel in the additional studies, totaling 5,723 women with breast cancer and 5,598 controls (total of 11,321). The region 10q26 around locus rs1219648, within intron 2 of FGFR2, reached genome-wide significance (p-value$\leq 10^{-10}$), and functional studies were available in the same region. The initial prostate GWAS and follow up scans reported by the CGEMS initiative, followed the same study design as that in breast cancer. The locus rs10993994 in the 10q11 region, located in the promoter region of the MSMB gene, was genome-wide significant (p-value$\leq 10^{-10}$). A total of 12,223 men (6,118 cases and 6,105 controls) were genotyped for 14 tagSNPs surrounding the MSMB gene. Also, over 2,500 bp of the coding gene and its 5' region were sequenced. Functional data also was available for this region. We reconstructed haplotypes based on the regional LD structure, and tested for association with breast and prostate cancer risk using 3 different haplotype methods: 2 haplotype association analyses, the variable-sized sliding-window regularized regression approach, and the sequential haplotype scan, and a location estimation analysis, based in evolutionary clustering using Bayesian partition modeling to cluster haplotypes with similar disease risks. The most significant haplotypes for each included the most significant loci in both areas and delimited a genomic region where significant functional variation has been described. We then compared the utility of tagged SNPs based on HapMap between two regions in which a putative functional variant has been identified. We conclude that fine mapping analysis is useful to narrow the region but still requires additional genotyping based on a comprehensive resequence analysis of the region to effectively test common variants.

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## 2846/F/Poster Board #746

**ERBB3 variants at 12q13 confer genetic risk for type 1 diabetes.** *K. Keene, S. Onengut-Gumuscu, P. Concannon.* Center for Public Health Genomics, University of Virginia, Charlottesville, VA.

Type 1 diabetes (T1D) is a complex autoimmune disease that involves both genetic and environmental risk factors. Genome wide association studies (GWAS) have recently provided a new list of potential T1D predisposing loci including the 12q13 locus containing the ERBB3 gene. GWAS for T1D provide evidence for association with two SNPs across the 12q13 region, rs2292239 (ERBB3, intron 7; P= $1.89 \times 10^{-14}$) and rs11171739 (intergenic RPS26-ERBB3; genotypic P= $9.71 \times 10^{-11}$). In order to fully determine a functional role for variants in the 12q13 region, in relation to T1D susceptibility, it is imperative that the causal variants are identified and characterized. Using sequencing and fine-mapping approaches, we aimed to identify and subsequently perform tests of association for variants within the 12q13 region. Using HapMap CEPH data, we identified 25 common SNPs (MAF >5%) that span a five gene region of 12q13 that included ERBB3. Seven tagging SNPs were genotyped, using the Eclipse genotyping platform, in 382 multiplex T1D families from the Type 1 Diabetes Genetics Consortium. Single SNP association was performed using Family Based Association Tests (FBAT). Four tagging SNPs (all located in ERBB3) provided evidence for association (P< 0.05) with T1D. The most significant single SNP associations were observed with rs2271189 (Exon 27; P= 0.000182) and rs705708 (Intron 15; P= 0.00113), while rs2292239, one of the significantly associated SNPs from GWAS, provided somewhat less evidence of association (P= 0.013) in this population. There are relatively few reported SNPs in this region. To further explore the associations observed in ERBB3, we re-sequenced all 28 exons and intron/exon boundaries of ERBB3 in 48 individuals, confirming nine previously identified SNPs and five novel SNPs. Three of the additional SNPs identified by sequencing were genotyped, of which two were significantly associated with T1D. Of the ten total SNPs genotyped across 12q13, six SNPs showed evidence for association with T1D. Three SNPs provided stronger evidence for association than the previously associated GWAS SNP rs2292239, suggesting that variants within the ERBB3 gene are responsible for the genetic risk for T1D observed on 12q13.

## 2847/F/Poster Board #747

**Locus heterogeneity in Hereditary Hemorrhagic Telangiectasia (HHT): Linkage and candidate gene analysis of four large HHT families.** *W. Wooderchak[1], C.J. Bukjiok[1], K. Damjanovich[1], T. Lewis[1], F. Gedge[1], J. McDonald[1,2], P. Bayrak-Toydemir[1,3].* 1) ARUP Institute for Clinical and Experimental Pathology; 2) Department of Radiology, University of Utah; 3) Department of Pathology, University of Utah.

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder characterized by aberrant vascular development. All known genetic defects that cause HHT are found to be within the transforming growth factor-beta (TGF-$\beta$) signaling pathway. Endoglin (ENG, chromosome 9q34), activin A receptor type II-like 1 (ACVRL1, chromosome 12q13), and SMAD4 (chromosome 18q21), are known to cause HHT1, HHT2, and Juvenile Polyposis/HHT syndrome, respectively. Mutations in two unidentified genes on chromosome 5q31 and chromosome 7p14 cause HHT3 and HHT4, respectively. Four types of HHT have been identified, yet over 85 percent of patients diagnosed with HHT have mutations in ENG or ACVRL1.

We have identified four large families with HHT that do not have ENG or ACVRL1 coding region mutations. Mutation analysis of SMAD4 and other SMAD genes (SMAD1-3 and 5) revealed no mutation. In order to determine the genetic cause of HHT for each family, we performed locus specific linkage analysis using short tandem repeat markers located in the 5 known HHT loci. Interestingly, one family linked to chromosome 9q34 where ENG is located, while two families linked to chromosome 12q13 where ACVRL1 is found. These results suggest that genetic aberrations in the noncoding or regulatory regions of ENG and ACVRL1 may also cause HHT. One of the families linked to chromosome 7p14 where the causative gene has yet to be determined. A custom designed oligo-CGH array was performed on the 7p region for a 7p14-linked family, and no large deletions were detected. Several candidate genes from this 7 Mb region were sequenced including BMPER, a lead candidate gene that encodes an endothelial cell regulatory protein expressed in the earliest stage of endothelial cell lineage. No mutations have been found. Studies are underway to narrow the critical region on chromosome 7p and determine the causative gene. Our findings further support the locus heterogeneity observed in HHT.

## 2848/F/Poster Board #748

**Agilent Technologies SureSelect$^{TM}$ All Human Exon Target Enrichment Kit Development.** *C. Pabón-Peña[1], A. Giuffre[2], B. Novak[1], M. Visitacion[1], S. Joshi[2], A. Wong[1], J. Eberle[1], W. Rasmussen[2], S. Hunt[1], J. Ong[2], R. Kanemoto[1], S. Happe[2], E. LeProust[1], D. Roberts[1].* 1) Agilent Technologies, Inc., Santa Clara, CA; 2) Agilent Technolgies, Inc., Cedar Creek, TX.

Next-generation sequencing technology has dramatically increased the ability to sequence DNA in a massively parallel manner. This improvement in throughput can be further augmented by targeting only the specific regions of the genome under study. Agilent Technologies has leveraged its ability to efficiently manufacture high fidelity long oligonucleotides to develop the SureSelectTM Target Enrichment System platform, a portfolio of sample-preparation products to enable next-generation sequencing users to sequence only their regions of interest. Here we describe development and validation of the SureSelect$^{TM}$ Target Enrichment System. The design covers human exonic regions corresponding to the ~37Mb of the NCBI Consensus CDS database (CCDS) in addition to over 1000 human non-coding RNAs. We discuss the performance of the system with respect to the efficiency, uniformity, and reproducibility of enrichment.

## 2849/F/Poster Board #749

**Functional interactions of conserved non-coding (CNC) sequences with other CNC using circular chromosome conformation capture (4C).** *D. Robyr[1], M. Friedli[1], C. Gehrig[1], M. Arcangeli[1], M. Marin[1], L. Farinelli[3], A. Quazzola[2], S. Verp[2], D. Trono[2], S.E. Antonarakis[1].* 1) Genetic Medicine/ Development, Univ Geneva Medical Sch, Geneva, Switzerland; 2) Global Health Institute, School of Life Sciences, EPFL, Lausanne Switzerland; 3) FASTERIS SA, Ch. du Pont-du-Centenaire 109, P.O. Box 28, CH-1228 Plan-les-Ouates, Switzerland.

The comparison of human chromosome 21 (Hsa21) sequences with the mouse syntenic regions led to the identification of roughly 3500 regions displaying an identity of >70% over a length of a least 100 nucleotides of ungapped alignment. About 65% (~ 2300) of these are conserved non-coding sequences (CNCs). Very little is known about the function of most CNCs, although some act as enhancers. We speculated that a functional CNC might interact with its genomic target (i.e. an enhancer would bind to its cognate gene promoter). Thus, the identification of any part of the genome that interacts directly with a CNC could provide clues on the function of the latter. We have generated libraries of CNC-interacting DpnII fragments by chromosome conformation capture (4C) whose identity is determined by subsequent high-throughput sequencing. We have identified genomic loci that interact physically with a selection of 10 CNCs from human chromosome 21. All CNCs are capable of interacting with loci in trans albeit at different levels, ranging from 0.1% to 95.9% of all sequence tags. Remarkably, there is a statistical significant enrichment of other CNCs among the interacting fragments. Moreover, we have identified two evolutionary conserved DNA elements (separated by 89kb) that have the ability to interact with the oligodendrocyte gene OLIG2 and that drive the expression of a reporter gene in the mouse embryo in regions corresponding to its native expression. This study also demonstrates the power of 4C technology to identify interactions of functional DNA elements.

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## 2850/F/Poster Board #750

**Human Sequencing and Project Management in the Broad Institute Genome Sequencing Platform.** *L. Ambrogio[1], L. Aird[1], K. Ardlie[2], J. Baldwin[1], T. Bloom[1], J. Blye[1], T. Fennell[1], S. Fisher[1], R. LeVine[1], K. Ross[1], C. Sougnez[3], H. Spurling[1], J. Wilkinson[1].* 1) Genome Sequencing Platform, The Broad Institute, Cambridge MA; 2) Biological Samples Platform, The Broad Institute, Cambridge MA; 3) Genetic Analysis Platform, The Broad Institute, Cambridge MA.

One of the biggest challenges of increasing throughput across multiple sequencing platforms is managing sample receipt, project tracking and data submission. In the first five months of 2009 the Sequencing Platform at the Broad Institute received approximately 1,300 human DNA samples for sequencing and generated over 4.9 terabases of data, a significant increase from comparable time frames in prior years. Data was generated across multiple initiatives, including The Cancer Genome Atlas, The 1000 Genomes Project, and The Human Microbiome Project. To meet this challenge, we have formed a cross-platform team of Project Managers who work closely with internal and external collaborators to develop project designs, timetables and goals. In addition, we have a suite of tools for monitoring project status beginning with sample procurement and concluding with data submission. The Genome Sequencing Sample Repository (GSSR) ensures the fidelity and accuracy of all sample information entered into the project-tracking database. Work Requests link samples to sequencing workflows via the LIMs console. The Analysis pipeline interacts with Project Workbench to verify the data submission protocol. As new sequencing technologies increase the velocity at which a sequencing project can be completed, there is a continuous need to improve current project management methods and build new tools where necessary. The Broad Institute Project Management Team has worked to improve and streamline communication across multiple platforms, develop flexible tracking and reporting methodologies, and has also implemented standard procedures for managing a project through its life cycle. Combined, these efforts allow us to better manage and balance the needs of all project stakeholders.

## 2851/F/Poster Board #751

**Third-Generation Complete Human Genome Sequencing of Multiple Individuals.** *R. Drmanac.* Complete Genomics, Mountain View, CA.

We sequenced the complete human genomes of four individuals, including two parents-offspring trios, using Complete Genomics' third-generation technology to yield the first series of high quality human genome sequences at a medically relevant cost and scale. The technology employs high density nanoarrays, an unchained read chemistry, and high performance imaging to reduce overall cost and improve time to results. Our latest human genome sequencing data includes individual coverage depths ranging from 50- to 89-fold (150-270 Gb per genome), and average discordance of 0.63% in 85% of the highest quality bases. An average of over 3,000,000 SNPs were found in each of the individual sequences, of which an average of 10.7% were novel SNPs. An average of over 353,000 INDELs were identified, of which over 45% were novel INDELs. We will also discuss some implications of this rapidly expanding sequencing capability for use in other studies.

## 2852/F/Poster Board #752

**Resequencing All Unique X Choromosome Exons to Identify Genetic Variants Contributing to Autism Susceptibility.** *K. Mondal[1], D. Okou[1], K. Meltz-Steinberg[1], V. Patel[1], A.C. Shetty[1], M.E. Zwick[1,2,3].* 1) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Graduate Program in Population Biology, Ecology and Evolution, Emory University School of Medicine, Atlanta, GA; 3) Graduate Program in Genetics and Molecular Biology, Emory University, Atlanta, GA.

Our goal is to assess the impact of X-linked genetic variants as contributing factors to autism susceptibility. We are focused on comprehensively sequencing all unique exons on the X chromosome in 300 males with autism from the Simons Simplex Collection. Recently, we have shown that microarray-based genomic selection (MGS) using custom microarrays manufactured by Roche NimbleGen Systems, Inc. can be combined with the Illumina Genome Analyzer (IGA) to successfully capture and sequence diploid genotypes in large discontiguous genomic regions. Here we report the data obtained from an 8-fold scale-up targeted towards all the exons on the X chromosome. To do this, an initial custom-design MGS array (385K oligos) was used to enrich for 7417 exons (~2.4MB) on the human X-Chromosome in 10 Male Hapmap samples. Our results suggest that MGS is a feasible strategy for resequencing most of the exons on the X chromosome, although some technical challenges remain in obtaining high quality data at all targeted sequences. A univariate analysis of the results showed that longer exons gave higher basecalling rate because they had more MGS probes. Also, MGS probes with lower GC content and Tm (melting temperature) gave higher basecalling rates. Our results suggest that MGS performance can be enhanced by both improving algorithms for probe selection and further optimizing experimental protocols. Finally, our data suggest that enrichment approaches will be prone to false positive SNP identification when probes capture paralogous sequences from other genomic regions.

## 2853/F/Poster Board #753

**The human reference genome, v 2.0.** *V.A. Schneider[1], D. Grafham[2], T. Graves[3], M. Caccamo[4], Genome Reference Consortium (GRC).* 1) NIH/NCBI, Bethesda, MD, USA; 2) The Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 3) The Genome Center at Washington University, St. Louis, MO, USA; 4) The European Bioinformatics Institute, Hinxton, Cambridge, UK.

The description of the essentially complete human genome sequence described by the International Human Genome Sequencing Consortium in 2004 marked a scientific milestone[1]. At the time, it was the only mammalian assembly of such high quality and coverage. While the assembly described in this publication has been the basis of much scientific discovery, such discovery has also made it clear that additional work is needed to allow the genome representation to grow as our understanding of the human genome grows. To this end, the Genome Reference Consortium (GRC) was created. Over the past two years, we have built systems and tools to facilitate the assembly and curation of clone based assemblies such as the reference human and mouse genomes. The culmination of this effort has resulted in the release of an updated human genome assembly, referred to as Genome Reference Consortium Human Build 37 (GRCh37). We will review the significant improvements in this new human reference assembly. The initial focus of the group has been to fix assembly and sequence errors identified in human Build 36. Notably, the availability of new clone resources in non-BAC vectors[2] has also allowed us to re-examine gaps previously thought to be inaccessible to cloning. We have been able to add sequence to or completely close approximately one third of the unspanned gaps in Build 36. Additionally, much of the sequence that had been considered part of the 'bottom-drawer' has now either been localized or determined to be redundant and removed from the assembly. To accommodate our growing awareness of genetic diversity, this assembly also provides alternate representations of highly divergent loci for a limited number of regions. Additionally, the GRC has increased access to the data underlying the genome assembly. Tools to review component overlaps, as well as curation decisions, are publicly available. Regions that are currently under review can be seen at both the GRC portal and Ensembl Browser. Users can also report problems and ask questions through the GRC website (http://genome-reference.org). [1]Nature (2004) 431:931-45, [2]Nature (2008) 453:56-64.

## 2854/F/Poster Board #754

**LuCAMP: Genome-wide association study of patients with the combined at-risk metabolic phenotypes through whole-exome sequencing of 2,000 individuals.** *J. Wang.* Genomics & Bioinformatics, Beijing Genomics Inst, Shenzhe, Shenzhen, China.

Genome-wide association studies (GWAS) provided evidence that common genetic variation contribute to metabolic disorders including obesity and type II diabetes. About 19 genes were identified to be associated with type II diabetes. However, these loci only explain ~10% of the genetic variation of type II diabetes, since these studies all used a genotyping approach investigating between 100~500K different known genetic variants that covered approximately 80% of the common variation in HapMap with a minor allele frequency (MAF) >5%. To identify novel variations, especially rare variations, that increase the risk of type II diabetes, we used the combination of recently developed DNA capture and next-generation sequencing technologies. Through sequencing the whole coding part of the genome (including 5'- and 3'-UTR) and highly conserved regions, we studied 1,000 Danish Caucasian patients with gender-defined visceral obesity who in addition suffer from type II diabetes and essential hypertension and 1,000 matched control individuals. The novel genetic variations with MAF as low as 0.1% were identified; the difference of allele frequency of novel and known variation between patients and controls were identified. The project also contributes with the sequence of thousands of human genomes for studying the population structure of the Danish population. In general, the method we developed and the study we performed demonstrated the feasibility and advantages of large-scale genome sequencing approach for population genetics and genome-wide association studies.

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## 2855/F/Poster Board #755

**Identification and resequencing of the cis-regulatory elements surrounding Tbx5.** *S. Smemo, M.A. Nobrega.* Department of Human Genetics, University of Chicago, Chicago, IL.

Congenital heart malformations are the most common form of birth defect, affecting nearly 1% of all children. Such a high prevalence is partly due to the inherent difficulty in maintaining the complex interplay of many genes whose expression is finely-tuned spatially and temporally. A critical gene in this dynamic system is T-box 5 (Tbx5), and coding mutations in Tbx5 are known to cause Holt-Oram Syndrome (HOS), which is characterized by congenital heart and limb malformations. Interestingly, 30-70% of patients with HOS lack any such coding mutations. One hypothesis, explored here, is that the cis-regulatory elements that normally confer Tbx5 with its precise spatiotemporal expression pattern have been disrupted by mutation, resulting in defects in the heart developmental program and ultimately in heart malformations. We have used a combination of bioinformatics, comparative genomics and mouse transgenic assays to identify many of the individual elements responsible for controlling Tbx5's tissue and temporal expression specificity in the developing heart. Preliminary sequencing of a few of these short, highly conserved elements in human patients with congenital heart defects has revealed a number of mutations, the functional significance of which is being determined. This study not only has implications for identifying variation underlying congenital heart disease, but the strategy is broadly applicable to other human diseases as well.

## 2856/F/Poster Board #756

**Taybi-Linder syndrome (MOPD I/III) maps to a 3Mb interval on chromosome 2q.** *P. Edery[1,2], A. Labalme[1], R. Touraine[3], P.-S. Jouk[4], D. Sanlaville[1,2], F. Clerget-Darpoux[5,6], A.-L. Leutenegger[5,6].* 1) CHU de Lyon, Service de Cytogénétique Constitutionnelle, Bron, F-69677, France; 2) Université Lyon 1, Lyon F-69003, France; 3) CHU de Saint-Etienne, Service de Génétique, Saint-Etienne, France; 4) CHU de Grenoble, Département de Génétique et Procréation, Grenoble, France; 5) Inserm, U535, Villejuif, France; 6) UMR-S 535, Univ Paris-Sud, Villejuif, France.

Taybi-Linder syndrome, also named microcephalic osteodysplastic primordial dwarfism (MOPD) type I/III (OMIM 210710), is a rare autosomal recessive condition characterized by intrauterine growth retardation, low birth weight, dwarfism, bone dysplasia, facial anomalies, microcephaly and severe brain malformations. In 2006, we mapped the Taybi-Linder locus to a 13 cM interval on chromosome 2q14.2-q14.3, by performing homozygosity mapping in one inbred sibship (2 affected and 3 unaffected sibs) and in two unrelated inbred patients, using inbreeding coefficients estimates from the patients' genomic information (Leutenegger AL et al, Am J Hum Genet 2006). Here, we present a fine mapping study of the Taybi-Linder syndrome locus together with the genotyping of an additional Taybi-Linder family, which confirms that this syndrome maps to chromosome 2q. Altogether, our data show that the Taybi-Linder syndrome locus maps to a 3.19 Mb chromosome 2q interval, flanked by the RALB gene (centromeric boundary) and by a polyAC microsatellite marker in close vicinity to D2S347 (telomeric boundary). We also performed direct sequencing of the coding exons and flanking introns of several candidate genes, including TFCP2L1, CLASP1, MKI37IP and TSN, but no pathogenic base change was found. We are currently performing high throughput sequencing of the 3.19 Mb genomic region (capture by Agilent's Oligo Library Synthesis Technology, and, sequence by Illumina Genome Analyzer II) likely to contain the pathogenic mutations in order to identify the Taybi-Linder causative gene.

## 2857/F/Poster Board #757

**Linkage analysis and comprehensive resequencing of the whole exon-captured genomic DNAs toward identification of causative genes for hereditary neurodegenerative diseases.** *Y. Fukuda[1], K. Reginaldo[2], W. Qu[2], Y. Nakahara[1], H. Ishiura[1], K. Hara[3], Y. Suzuki[4], S. Sugano[4], S. Morishita[2], S. Tsuji[1].* 1) Dept Neurology, Univ Tokyo, Tokyo, Japan; 2) Dept Computational Biology, Univ Tokyo, Kashiwa, Japan; 3) Dept Neurology, Brain Research Institute, Niigata Univ, Niigata, Japan; 4) Dept Medical Genome Science, Univ Tokyo, Kashiwa, Japan.

Objective: Identification of causative genes for diseases with Mendelian inheritance still remains a challenging project when the size and the number of pedigrees are limited. To overcome the difficulty, we conducted comprehensive resequencing of the whole exon-captured genomic DNAs from a patient with familial multiple system atrophy (fMSA), a recently identified rare hereditary neurodegenerative disease. To facilitate the search for the causative genes, we simultaneously conducted a high-throughput linkage analysis using SNP microarrays to define the candidate regions. Methods: A genome-wide linkage analysis of a pedigree of fMSA was conducted employing a recently developed SNP microarray-based high-throughput linkage analysis system (BMC Bioinfo., 2009, 10:121). We have performed linkage analysis of a consanguineous MSA pedigree where autosomal recessive inheritance is suggested. Whole exons were enriched using NimbleGen Sequence Capture 2.1M Human Exome Array, and selective, high-throughput DNA resequencing was conducted using next generation sequencers (Solexa/Illumina). Results and Discussion: From 50 million reads, total of approximately 80K candidates of single nucleotide variations (SNVs) were detected after our fist filtering. Of those approximately 15K SNVs were located in primary target regions designed on capturing array (average read depth = ~15x). Based on the SNP array-based high-throughput linkage analysis, the candidate region was defined to be ~70Mb regions with the homozygosity shared by the affected patients. Application of these SNVs identified in the candidate regions for the search for the causative genes will be discussed.

## 2858/F/Poster Board #758

**Profiling of small ncRNAs in psoriatic skin with microarrays and Next Gen sequencing.** *C. Joyce, A. Bowcock.* Dept Gen, Washington Univ, Saint Louis, MO.

Psoriasis is a complex, chronic inflammatory disease of the skin affecting 2-3% of the European population. While the majority of changes in mRNAs associated with protein coding genes are known, and genetic risk factors are beginning to be identified, many of the underlying mechanisms of gene expression changes in the disease are not clear. Several classes of non-coding RNAs (ncRNAs), such as microRNAs (miRNAs) and naturally occurring antisense transcripts (natRNAs), are endogenous regulators of gene expression and may account for some of these alterations. To begin to determine whether changes in ncRNA expression might contribute to altered gene expression in psoriasis, we examined global changes in miRNA levels in normal and psoriatic skin. We first used Exiqon miRCURY arrays to interrogate expression levels of miRNAs in normal versus psoriatic skin samples. Potentially differentially expressed miRNAs were then followed up with qRT-PCR analyses of eight normal, ten involved and ten uninvolved skin samples. This lead to the identification of three miRNAs- miR21, miR31, and miR133- that are misexpressed in psoriatic involved versus normal skin ($P = 0.0003$; 0.0001; 0.0001, respectively). Since the Exiqon arrays only harbor probes for 561 miRNAs, and many more are likely to exist, we are also interested in identifying additional miRNAs and other small ncRNAs. Hence, we are generating small ncRNA libraries from normal and involved psoriatic skin and sequencing them with Next Gen technologies. A first pass analysis of 4,193,899 sequences from a library derived from involved skin included known differentially expressed miRNAs, such as miR21 and miR31, and many novel sequences. Hence, this study will lead to the identification of novel ncRNAs in both normal and psoriatic skin and provide additional insights into the pathogenesis of this inflammatory skin disease.

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## 2859/F/Poster Board #759

**Whole Genome Amplification and screening methods of Formalin-fixed Paraffin-embedded Tissues for Selective Hybridization Sequencing on Illumina's Genome Analyzer II Sequencer.** *M. DeFelice[1], B. Blumenstiel[1], W. Winckler[1], M. Freedman[1,2], L. Ziaugra[1], S. Gabriel[1].* 1) Broad Inst, Cambridge, MA; 2) Dana-Farber Cancer Institute, Boston, MA.

There are an estimated 300-500 million paraffin tissue blocks in the United States today and that number is growing by roughly 20 million samples per year. Formalin fixed tissue is more abundant than fresh frozen tumor tissue but presents a multitude of technical challenges for molecular profiling. Next generation sequencing technologies coupled with hybrid selection techniques are quickly making large tumor sequencing studies an affordable reality. DNA degradation, formalin modification, and total yield of extracted DNA all pose challenges to obtaining high quality sequence for mutation discovery. Using a hybrid selection panel targeting 4.4 megabases across ~20,000 exons, we observe the performance of extracted FFPE samples sequenced on the Illumina Genome AnalyzerII. With solution and array based hybrid selection methods requiring upwards of 3 and 20 ug of input DNA respectively, we tested three whole genome amplification methods to assess their suitability for amplifying FFPE DNA for library construction, hybrid selection, and sequencing. WGA methods tested include: Genomeplex, REPLI-g FFPE, and Restriction and Circularization-Aided Rolling Circle Amplification. Because of the broad spectrum of sample variation in FFPE collections there is a critical need to understand the quality of a sample for any type of genetic analysis before valuable time and money is expended. We report on the development of an up-front screening assay for predicting hybrid selection sequencing success on any given FFPE sample on the Illumina sequencing platform. Since concentration and size distribution of genomic DNA isolated from FFPE tissue are often inadequate predictors for future genomic analysis success, we used a multi-plex PCR assay coupled with the Sequenom Homologous Mass Extension (hME) assay to quantify the extent of degradation/ modification and ultimately select for FFPE extractions with optimal sequencing potential. The ability to screen for sequence-quality FFPE samples will greatly expand the available collection of tumor DNA samples, enabling well-powered studies of the cancer genome.

## 2860/F/Poster Board #760

**Genomic Approaches to Understanding PGC-1a Regulated Metabolism.** *A. Charos[1], B. Reed[1], D. Raha[1], S. Weissman[2], M. Snyder[1].* 1) MCDB, Yale University, New Haven, CT; 2) Genetics, Yale University, New Haven, CT.

PGC-1a is a transcriptional coactivator that functions as a master regulator of energy homeostasis. It becomes activated in response to a variety of metabolic stressors including physical activity, dietary conditions and temperature and mediates the proper transcriptional response through its interactions with a number of transcription factors. Although PGC-1a mediates transcriptional outputs across a range of tissue types, its role in the liver is of key interest to this study. In the liver, PGC-1a has been shown to control the fasting response by upregulating genes involved in gluconeogenesis and the oxidation of fatty acids. Polymorphisms in PGC-1a have been identified in patients with type II diabetes amongst different ethnic populations. The expression of PGC-1a is aberrantly upregulated in the livers of both type I and type II diabetics, contributing to hyperglycemia. Despite its critical role in metabolism, few PGC-1a target genes have been identified to date. Recent advances in ultra high-throughput DNA sequencing technology have made it possible to identify transcription factor binding sites across the entire genome at very high resolution compared to other technologies such as tiling array platforms. In order to identify the complete set of genomic binding sites of PGC-1a and its associated factors, we performed chromatin immunoprecipitation followed by DNA sequencing (ChIP-seq) in human hepatocarcinoma (HepG2) cells that were subjected to treatment with forskolin to mimic glucagon signaling in the fasted liver. The immunoprecipitated DNA was subjected to massively parallel DNA sequencing on the Illumina Genome Analyzer platform. Several PGC-1a binding sites were located in the promoters of known PGC-1a target genes. In addition, several novel binding sites were found. Enriched motifs were identified for each of eight different factors. An analysis of the data revealed two clusters of transcription factors with overlapping binding sites, providing evidence for new functional relationships between different factors. ChIP-Seq data were compared with Affymetrix expression data to correlate binding sites with changes in gene expression. Finally, a network determining the relationships between the factors revealed an interesting hierarchy in which some factors bind to the promoters of each other.

## 2861/F/Poster Board #793

**Human Variome Project - Pilot projects and progress.** *R.G.H. Cotton[1,2], F. Macrae[3], Collaborators.* 1) Genomic Disorders Research Centre, & Convenor, Human Variome Project, Melbourne, Australia; 2) Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville, Australia; 3) Colorectal Medicine and Genetics, The Royal Melbourne Hospital, Parkville, Australia.

The Human Variome Project (Axton 2007) (www.humanvariomeproject.-org) was initiated in June 2006 (Ring, Kwok et al. 2006) drawing attention to the importance of collection of variation and its phenotype and to develop programs to put this into effect. The project builds on work and concepts of the HGVS over many years (www.hgvs.org) to focus on all variation associated with disease. HVP collaborators include those discovering mutations, their effects and then collect the data making it instantly available for those who need it to inform clinical decisions and research. The HVP and InSiGHT (www.insight-group.org) has developed a major pilot study to develop procedures and systems to allow effortless flow of de-identified data from the patient/clinic/diagnostic laboratory via curated locus or gene specific databases to central databases/genome browsers such as NCBI, UCSC and EBI. The system will be easily adaptable to other genes and to multiple laboratories, states and countries worldwide. A country specific collection pilot is underway in Australia and an International Confederation of these countries has been initiated with Korea and China in the application process. Such collection will aid genetic healthcare in a country and be a second failsafe collection mechanism. Other pilot studies developed include specific ethical studies related to mutation collection, loading of LSDB content to dbSNP, funding of curation of LSDBs, a system of Microattribution/ reward for mutation submission. A high level meeting in Spain (Kaput, Cotton et al. 2009) developed plans to implement the recommendations of the HVP Melbourne meeting (Cotton, Appelbe et al. 2007). The HVP was featured recently in relation to Neurogenetic databases (Cotton, Auerbach et al. 2008). References: Axton, M. (2007). "What is the Human Variome Project?" Nat Genet 39(4): 423. Cotton, R. G., W. Appelbe, et al. (2007). "Recommendations of the 2006 Human Variome Project meeting." Nat Genet 39(4): 433-6. Cotton, R. G., A. D. Auerbach, et al. (2008). "GENETICS. The Human Variome Project." Science 322(5903): 861-2. Kaput, J., R. G. Cotton, et al. (2009). "Planning the Human Variome Project: The Spain report." Hum Mutat 30(4): 496-510. Ring, H. Z., P. Y. Kwok, et al. (2006). "Human Variome Project: an international collaboration to catalogue human genetic variation." Pharmacogenomics 7(7): 969-72.

## 2862/F/Poster Board #794

**A novel deletion in HSL causes a frameshift mutation associated with high triglycerides and low HDL in the Amish.** *J.S. Albert, L.J. Reinhart, B.D. Mitchell, A.R. Shuldiner, T.I. Pollin, C.M. Damcott.* University of Maryland School of Medicine, Baltimore, MD.

Lipases are enzymes responsible for the catabolism and mobilization of stored lipids. Hormone sensitive lipase (HSL) is responsible for most of the catabolic activity in lipolysis. Regulated by norepinephrine, adrenaline, and glucagon, HSL breaks down triacylglycerol, diacylglycerol, and monoacylglycerol for energy. In the HSL knockout mouse, adipocytes are significantly enlarged, indicating dysregulation of lipid catabolism. Thus, we hypothesize that polymorphisms in HSL disrupt the lipase activity of the enzyme leading to abnormal lipid metabolism. To test this hypothesis, we sequenced the 10 exons in HSL in 12 Amish subjects with extremely high and 12 with extremely low fasting triglycerides. In our sequencing set, there was very little variation across the gene; however, we found a novel 19 base pair deletion in the coding region of exon 10. This mutation abolishes the stop codon, and lengthens the protein by 92 amino acids. We genotyped the deletion in 1,635 Amish subjects who are well-characterized for traits related to cardiovascular disease, and compared mean trait levels of fasting lipids across genotype groups adjusting for age, sex and family structure. We found that the deletion had an allele frequency of 0.02 and was significantly associated with higher fasting triglycerides (p-value = 3.9 x 10-5) and lower HDL-cholesterol (2.6 x 10-6). Median TG levels and interquartile ranges were 81 (46 - 98) in II, 111 (52 - 149) in ID and 204 (145 - 264) mg/dl in DD. Mean HDL levels were 56 +/- 15 in II, 49 +/- 14 in ID, and 36 +/- 15 mg/dl in DD (mean +/- SD). These data provide evidence that the novel 19 base pair deletion found in HSL is associated with increased fasting triglycerides and low HDL-cholesterol, suggesting a role for HSL variation in dysfunctional lipid metabolism in humans.

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## 2863/F/Poster Board #795

**Sensitivity and Specificity of High Throughput Melting Curve Analysis for the Detection of Common Genome Variants and Rare Mutations in PCR Amplicons.** *H. Prucha[1], M.F. Sinner[2], S. Lindhof[1], M. Vieracker[1], B.M. Beckmann[1], D. Sibbing[3], N. Von Beckerath[3], B. Lorenz-Deperieux[1], H. Prokisch[1], S. Kääb[2], T. Meitinger[1], A. Pfeufer[1].* 1) Institut für Humangenetik, TU München und Helmholtz Zentrum München; 2) Klinikum Grosshadern, 1. Med. Klinik, LMU München; 3) Klinikum rechts der Isar, 1. Med. Klinik, TU München.

High resolution melting curve analysis (HRM or MCA) is an innovative screening method for unknown genome variants in PCR amplicons. MCA has shown convincing potential as a cost efficient high throughput method (≤10 cents per PCR product screened in 384 well plates) beyond medium throughput methods like chemical or enzymatic cleavage, SSCP, TGGE and DHPLC or more expensive methods like Sanger- or next generation resequencing. We have evaluated MCA in a series of 362 patients with autosomal dominant dilative cardiomyopathy. In a discovery set of 96 patients who had undergone complete Sanger resequencing of 18 exons we discovered overall 44 sequence variants. 17 of the variants had an allele frequency below 1%, i.e. were detected only once in the discovery set, while 27 variants were more common i.e. observed more than once in the set. Sensitivity of MCA to detect sequence variants was on average 96.5% (range: 74.2-100%) if variants were common (i.e. occurred more than once) and PCR amplicons were no longer than 350 bp. This was the case even if several common and rare variants were present within the same amplicon. Sensitivity was decreased for rare variants, for longer amplicons and for amplicons containing several variants. At the cutoffs that we used to select a PCR product for further analysis the specificity of MCA was higher (78% average) than with previously used methods in our hands, avoiding unnecessary resequencing expenses. Overall the current implementations of MCA in a 384 well format offer a good detection of private mutations and other rare variants in PCR-amplifiable short genomic regions, applicable in high throughput with a reasonable cost-efficiency ratio and bridges the technology gap between conventional mutation screening methods and next generation resequencing.

## 2864/F/Poster Board #796

**A census of LINE-1 and Alu elements expressed in human embryonic stem cells.** *J. Garcia-Perez, A. Macia, J. Cortes, S. Morell, M. Munoz, G. Lucena.* Andalusian Stem Cell Bank, Univeristy Granada, Armilla, Granada, Granada, Spain.

Almost half of the human genome has been generated by the activity of mobile DNA elements, and their activity continues to shape the genome. Long Interspersed Element-1 (LINE-1 or L1) is a family of active non-LTR retrotransposons that comprise 17% of the human genome. An active LINE-1 element code for the proteins required to mediate their mobility (known as retrotransposition), and these proteins are also used by Short Interspersed Elements (SINEs) like Alu to mediate their mobility in trans. The mobility of L1 and Alu has resulted in a diverse range of diseases and it has been estimated that 1/10 newborn may contain a de novo L1/Alu retrotransposition event. As a type of selfish DNA, L1 and Alu might ensures its evolutionary transmission to new generations trough the accumulation of new insertions in germ cells or during early embryonic development. Human Embryonic Stem Cells (hESCs) represent an excellent model to study the accumulation of L1/Alu in early human development, and we recently described that L1 is expressed in these cells and that hESCs can accommodate a low level of L1 mobilization using a cell cultured based assay. In the present study, we adapted a previously characterized L1 antisense promoter to precisely map expressed L1s in undifferentiated hESCs and a straight cloning approach to determine the bulk of Alus expressed in hESCs. We found that several subfamilies of Alu are expressed in hESCs, and that the core from these elements is active in a cell culture based assay. Surprisingly, we found that the antisense promoter is conserved trough LINE-1 evolution, and that almost half of the expressed L1s are not present in the Reference Human Genome (RHG). Furthermore, we also described several locus shared by all hESCs lines analyzed that are characterized by active L1 expression, which suggest that L1 expression in the developing embryo might be linked to defined and discretes places of the genome.

## 2865/F/Poster Board #797

**Detection of small indels from next generation sequencing data by Bayesian evaluation of alternative haplotypes.** *C. Albers[1,2], W. Ouwehand[1,2], R. Durbin[2].* 1) Dept Haematology, University of Cambridge and National Health Service Blood and Transplant, Cambridge, United Kingdom; 2) Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

Small insertions and deletions are the second most abundant type of variation in the genome and play a substantial role in genetic disorders. We present a probabilistic method for inferring small indels from next generation sequencing data.

In essence the method performs a local diploid reassembly of reads around a candidate indel site. For every candidate indel identified by gapped alignment of reads to the reference we first generate a set of 16 potential haplotypes incorporating combinatorially up to 4 local variants. Next we compute the likelihood of every read given each potential haplotype using a probabilistic alignment model that takes into account base qualities, mapping qualities and indels due to sequencing errors. From these likelihoods and additional priors we then infer the pair of haplotypes with the highest a posteriori probability.

We evaluated the method on 10 replicates of 46 bp paired-end reads simulated for a 5 Mb diploid region containing ~6000 SNPs and ~600 indels. Respectively 77.3 %, 96.5 % and 97.2 % of the simulated indels at 4x, 20x, and 40x were recovered as candidate indels after alignment with BWA [Li and Durbin, Bioinformatics 2009]. Using 16 potential haplotypes of 120 bp the false-positive rate was 0.06 %, 0.14 % and 0.17 % with sensitivities (of all simulated indels) of 50.0 %, 92.4 % and 94.6 % at respectively 4x, 20x and 40x. The SAMtools program [Li et al, Bioinformatics; in press] produced indel likelihoods an order of magnitude faster, but was less accurate with false-positive rates of 0.18 %, 2.7 % and 5.9 % at sensitivities of 9.1 %, 64.9 % and 91.4 % at respectively 4x, 20x, and 40x. Considering haplotypes allows us to call linked SNPs: at 40x our method identified 93 % of the SNPs within 35 bp of the candidate indels at a false-positive rate of 0.9 %. Our analyses suggest that considering additional local variants improves accuracy of the indel calls.

We applied the new method to the ~36x Illumina data for HapMap individual NA19240 sequenced as part of the 1000 Genomes project. We inferred 531770 indels of less than 11 bp, of which 299016 had a match in dbSNP within 10 bp. We show that discordance between our SNP genotype calls and the HapMap SNP genotype calls was significantly increased in the vicinity of indels.

## 2866/F/Poster Board #798

**A Variation Database for Amyotrophic Lateral Sclerosis.** *M. Yoshida[1], Y. Takahashi[2], A. Koike[1], Y. Fukuda[2], J. Goto[2], S. Tsuji[2].* 1) Central Research Laboratory, Hitachi, Ltd., Tokyo, Japan; 2) Graduate School of Medicine, University of Tokyo.

A variation database for amyotrophic lateral sclerosis (ALS) has been constructed as a publicly accessible online resource for recording all nucleotide and amino-acid variants identified in genes associated with ALS along with corresponding clinical conditions. ALS is a common neurodegenerative disorder worldwide, which is considered as a complex disease involving many genetic factors in the pathogenesis and pathophysiology. The database currently consists of more than 600 entries, including about 200 unique variants found in 33 disease-causative or disease-related genes. All sorts of variations including mutations, variants of uncertain pathogenic significance, and polymorphisms are accumulated. In addition to published data exhaustively collected from literature, novel variants identified by microarray resequencing in our laboratory are also incorporated in the database. Every reported gene has a respective page that provides information on its variation positions with various statistics, resulting clinical characteristics, and primary references as well as gene-sequence and protein-structure information which will assist in assessing the variation effects on gene and protein functions. Through a homology search function, users are able to find variations in arbitrary sequences of interest and check if they have already been described in the database. This database is expected to fulfill the essential need for integrating comprehensive information on genetic and clinical data regarding ALS. It will become a valuable tool for epidemiological and pathophysiological research through correlating genotypes with phenotypes in patients and evaluating the clinical significance of detected variations. In addition, it is intended to be a useful resource in actual clinical practice, especially as an aid to predicting disease course and prognosis based on clinical information of patients with the same mutations. The database is designed for inclusion of new variants when discovered, both from published data and from direct submission. Data submission is open to all researchers and highly encouraged. The database is accessible at the following URL: https://reseq.lifesciencedb.jp/resequence/SearchDisease.do?targetId=1. Acknowledgements: This study was supported by the integrated database project of MEXT.

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## 2867/F/Poster Board #799

**Genetic variation in the *TCF7L2* gene in Mexican Amerindian populations.** *L. Del Bosque-Plata, S.N. Cazañas-Padilla, C. Acosta-Correa, H. Miranda, S. Hernandez, G. Jimenez-Sanchez.* National Institute of Genomic Medicine (INMEGEN), Mexico City, Mexico.

Variants in the *TCF7L2* gene confer high risk to type-2 diabetes (T2D), it encodes a transcription factor in the WNT signaling pathway and has also been associated to cancer. Several links between the WNT signaling, insulin secretion, and proliferation of human beta cells have been reported. Association of this gene and T2D, have been universally replicated, but risk alleles in the *TCF7L2* vary in the populations analyzed thus far. Given the particular demographic history and the high prevalence of T2D in the Mexican population, we re-sequenced its 17 exons, and ~300 bp flanking each exon in 30 Mexican Mestizos and the following Amerindian populations: 30 Zapotecan, 60 Mazatecan, and 32 Nahua. We identified complex patterns of variation in microsatellite length in introns 7 and 14. In exons 9 and 17 we found four non previously reported SNPs. There are frequency differences of these sequence variants between analyzed Amerindian groups. Pro490His was found in 20% of the Mazatecas (Oaxaca) and 21.9% in Nahuas (Veracruz), in contrast with 3.3% in Zapotecas from Oaxaca. In Nahuas, Pro502Thr and Pro502Pro were found in 9.4% for each polymorphism. In Zapotecas Pro490His (3.3%), Pro502Thr (6.7%), Pro502Pro (6.7%) have a similar frequency that in Mestizos Pro490His (3.3%), Pro502Thr (10%), Pro502Pro (10%). We have found novel SNPs in the *TCF7L2* gene, a gene that has been associated with DT2 in most of the populations of the world, some of which show particularly high frequency in some Amerindian populations in Mexico.

## 2868/F/Poster Board #800

**High-throughput variation scanning system of responsible genes for X-linked autistic disorder spectrum, *NLGN3* and *NLGN4*, by a PCR coupled high-resolution melting curve analysis.** *K. Tanaka[1,2], T. Kaname[2,3,4], H. Maehara[1], Y. Fukushima[4,5], K. Naritomi[2,4], K. Yanagi[2].* 1) Med , Orthopedic, Ryukyu Univ, Nishihara town, Japan; 2) Dept Med Genet, Univ Ryukyus, Nishihara, Japan; 3) Okinawa Cutting-Edge Genome Project; 4) SORST, JST, Tokyo, Japan; 5) Dept Med Genet, Shinshu Univ, Matsumoto, Japan.

It is important to establish an easy and reliable system to detect mutations or variations for gene examination and genetic association study. High-resolution melting curve analysis (HRM analysis) is a method, which allows simple and rapid detection of gene variations. We constructed a sensitive system for detecting gene variations in the *NLGN3* gene and *NLGN4* gene. In those genes, disease-associated mutations were found in patients with autism or Asperger syndrome. Some reports are pointed possibilities that the gene variations are associated with autistic disorder spectrum (ADS). Since it is, however, estimated that small number of patients will be affected by variations in those genes, a scanning system is needed to investigate correlations between the gene variations and ADS patients, which allows rapid detection of variations in large scale. We set up PCR/HRM system for all exons of *NLGN3* and *NLGN4* using combination with SYTO9 fluorescent dye (Invitrogen), Ex-Taq polymerase (TAKARA), and LightCycler 480 Instrument (Roche). Then we evaluated the PCR/HRM system in the screening known SNPs of both genes in 48 controls by confirming direct sequencing of amplicons. The PCR/HRM system could detect all the known and novel variations in 48 control individuals. Besides, the PCR/HRM discriminated not only each haplotypes in the same exon, but also between heterozygous and hemizygous or homozygous of those haplotypes. The system is a valuable method for rapid and reliable scanning of the *NLGN3* or *NLGN4* gene variations to survey variations in ADS patients.

## 2869/F/Poster Board #801

**Genome-wide genotyping of pooled samples for ancestry assessment of populations of unknown origin.** *Z.K.Z. Gajdos[1,2,3], C.W.C. Chiang[1,2,3], J.M. Korn[3], F. Kuruvilla[3], J.L. Butler[1,3], R. Hackett[3], C. Guiducci[3], T. Nguyen[1,3], H.N. Lyon[1,3], K.D. Henderson[4], C. Haiman[5], L. Le Marchand[6], B. Henderson[5], M.R. Palmert[7], R. Wilks[8], T. Forrester[8], C.A. McKenzie[8], R.S. Cooper[9], X. Zhu[10], J.N. Hirschhorn[1,2,3].* 1) Children's Hospital Boston, Boston, MA; 2) Harvard Medical School Department of Genetics, Boston, MA; 3) Broad Institute, Cambridge, MA; 4) City of Hope National Medical Center, Duarte, CA; 5) University of Southern California, Los Angeles, CA; 6) University of Hawaii, Honolulu, HI; 7) Hospital for Sick Children, Toronto, Canada; 8) University of the West Indies at Mona, Mona, Jamaica; 9) Stritch School of Medicine, Loyola University of Chicago, Chicago, IL; 10) Case Western Reserve University, Cleveland, OH.

Genetic ancestry is an important covariate to include when conducting genetic association studies to reduce the possibility of spurious associations. There are two main methods currently used to estimate global genetic ancestry: genotyping a set of known ancestry informative markers (AIMs) or assessing individual-level genome-wide genotype data. However, AIMs are only useful if the ancestral populations are known with confidence and there are known AIMs, and individual-level genome-wide data are expensive to obtain for large numbers of individuals. Here we present admixture proportion estimation data from pooled genome-wide genotype data to demonstrate that pooling can be an effective method to estimate genetic ancestry when neither AIMs nor individual-level genome-wide data are available. We genotyped pooled DNA samples from four cohorts (one African-American, two Jamaican, and one Native Hawaiian) using the Affymetrix 6.0 array. After removing poorly performing SNPs, we estimated allele frequencies in the pooled samples. We used these allele frequencies and the HapMap populations as reference panels in a regression-based analysis for estimating admixture proportions relative to the HapMap references in these cohorts. The admixture proportions in the African-American cohort were estimated at ~82% YRI (West African) and ~18% CEU (European) ancestry, and the Native Hawaiian cohort was estimated at ~60% CHB+JPT (East Asian), ~32% CEU, and ~6% YRI. These estimates are very similar to those obtained using genotype data from known AIM loci that distinguish the HapMap populations. One of the Jamaican cohorts was estimated to be ~86% YRI and ~12% CEU and the other Jamaican cohort was estimated to be ~82% YRI and ~10% CEU. There appears to be a missing component of ancestry in the 2nd Jamaican cohort that remains unknown. To identify any additional components of ancestry and additional AIMs for each of our samples, we constructed corresponding "pseudopopulations" using the HapMap reference panels, weighted according to our ancestry estimates. We identified putative population-specific AIMs as those with large allele frequency differences between pooled estimates and that expected based on the pseudopopuation. A subset of putative AIMs was validated by individual genotyping in the samples that comprise the pools, demonstrating that a pooling approach can be effective for identifying markers that are highly informative for genetic ancestry.

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## 2870/F/Poster Board #802

**Personal genome interpretation with the Genome Commons and the Genome Commons Navigator.** *S.E. Brenner.* University of California, Berkeley, CA.

The acquisition of individuals' genomes has long been the aspiration of the human genomics, and sequencing technologies promise to make this affordable within the next several years. Already, large-scale genotyping arrays are widely used in research and retail DNA tests of genetic markers have captured the public's imagination. Unfortunately, today one can do little of value with a personal genome, due to a variety of scientific, technical, legal, sociological, and ethical challenges.

We are constructing a Genome Commons Database and Genome Commons Navigator, open-source open-access research resources aiding the scientific interpretation of personal genomes. The Genome Commons Database will be an integration and augmentation of existing data sources of genetic variation and human disease, drawing from many locus-specific databases, as well as large public data sources such as dbSNP, dbGaP, OMIM, GeneTests, and PharmGKB. We are also developing a curators' toolkit to facilitate manually verified entry of data into the commons, so that individuals with expertise can readily submit data and improve data quality. The Genome Commons Navigator prioritizes and predict phenotypes of genetic variants within an individual genome, by applying knowledge from the Genome Commons Database and methods for assessment of novel variants. The Genome Commons Navigator will be an open framework that draws upon the Genome Commons database as well as an array of plug-in modules. These modules already exist as stand-alone tools, but the ability to assess and apply them has sharply limited by their inability to deploy them within a comprehensive analysis environment such as the Navigator will provide.

We plan for the Genome Commons and Navigator to be initially used in the research community to improve the methods to make new discoveries. However, with consumer and medical interest in personal genomics growing and sequencing costs plummeting, we our long term aim is to develop methods applicable to clinical practice. We will look towards the eventual prospect of the Genome Commons being deployed in within academic and commercial clinical laboratories with appropriate ethical and regulatory oversight. Ultimately, we envision the Commons as a repository of our common human inheritance would be a vast resource for research and medicine.

## 2871/F/Poster Board #803

**Genome-Wide Association Study of Fractional Excretion of Sodium (FENa).** *H.S Jin[1], K.W Hong[1], J.E Lim[1], M.J Go[2], J.Y Lee[2], S.H Lee[3], C. Shin[4], H.K Park[1], B. Oh[1].* 1) Department of Biomedical Engineering, School of Medicine, Kyung-Hee University, Seoul, Korea; 2) Center for Genome Science, National Institute of Health, Seoul, Korea; 3) Department of Internal Medicine, Kyung Hee University East-West Neo Medical Center, Seoul, Korea; 4) Division of Pulmonary and Clinical Care Medicine, Department of Internal Medicine, Korea University Ansan Hospital, Ansan, Korea.

Fractional excretion of sodium (FENa) is the percentage of sodium excreted in the urine versus the sodium reabsorbed by the kidney. Resorption of sodium constantly maintains the osmotic-pressure of plasma, which ensures the appropriate exchange of electrolytes in cells and extracellular environments. To investigate genetic factors that affect FENa, genome-wide association studies were conducted in two independent cohorts, Ansung (n=1,019), and Ansan (n=1,568) in Korea. The genotypes were determined by Korea Association Resource consortium (KARE) using Affymetrix SNP array 5.0. Linear regression analyses were conducted for FENa in each cohort with controlling covariates by age, sex, body mass index, and mean arterial pressure. Also, we performed the same analysis to the combined data set of Ansung and Ansan including cohort as an additional covariate. In this study, there was no signal reaching genome-wide significant p-value criterion ($p=5 \times 10\text{-}7$). This phenomenon may be attributed to genetic effect of FENa is low or multi-locus effects, and our samples were a small number for GWAS study. All associated SNPs showed moderated signals with (p-value < 0.01). Among the moderately associated SNPs, 17 SNPs revealed repeated association in both cohorts. These SNPs located on four known gene loci, in CSMD2 (rs1536110, $p=2.25 \times 10\text{-}5$), in CIB4 (rs935172, $p= 2.65 \times 10\text{-}5$), in XKR4 (rs9298526, $p=5.39 \times 10\text{-}5$), and in ESCO1 (rs12373434, $p=6.29 \times 10\text{-}5$), and one unknown gene in KIAA1772 (rs8086339, $p=4.25 \times 10\text{-}5$). Although, significant SNPs were not satisfied GWAS basis, this study is the first genome-wide association study of FENa. This study for the association of genetic variations with FENa in cohorts of Korean population will be worth validated in the other populations and will highlight mechanisms that influence FENa.

## 2872/F/Poster Board #804

**Determination of haplotypes and estimation of haplotype frequencies by detecting homozygotes with genome-wide SNP genotypes of 3,397 individuals from the Japanese population.** *Y. Yamaguchi-Kabata[1], A. Takahashi[1], T. Tsunoda[2], N. Hosono[3], M. Kubo[3], Y. Nakamura[4,5], N. Kamatani[1].* 1) Laboratory for Statistical Analysis, Center for Genomic Medicine, RIKEN, Tokyo, Japan; 2) Laboratory for Medical Informatics, Center for Genomic Medicine, RIKEN, Yokohama, Japan; 3) Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN, Yokohama, Japan; 4) Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; 5) Center for Genomic Medicine, RIKEN, Yokohama, Japan.

Understanding haplotype structure and haplotype frequencies is important for associating genetic polymorphisms with a given trait and for inferring genetic genealogy of alleles in a population. Haplotypes can be determined without ambiguity when an individual does not have more than one heterozygous site at the genomic region. Taking advantage of a large size of genome-wide SNP genotype data from 3,397 individuals from the Japanese population, we attempted to examine the accuracy and efficacy of the estimation of population haplotype frequencies based on the data from homozygotes for each chromosomal region. We compared the haplotype frequencies estimated by our method with the inferred haplotype frequencies estimated by two statistical methods. The comparison showed that the haplotype frequencies estimated from the proportions of homozygotes were very similar to the results obtained by the statistical methods, PHASE and SNPHAP. The haplotype frequencies were also compared to those from the 'definitive haplotypes' from 74 complete hydatidiform mole samples in D-haploDB, and the haplotype frequencies were quite similar. Determination of haplotypes from homozygotes using the genotype data from thousands of individuals appears to be useful for detecting real haplotypes that exist at relatively low frequencies. In addition, the detected haplotypes with the estimated frequencies will be a catalogue of haplotypes for the population, which is useful for study design of GWAS to search for causative variants for the trait from the most associated SNPs.

## 2873/F/Poster Board #805

**Identification of loci influencing Toll-like receptor 7-induced innate immune responses in HapMap B-lymphoblastoid cell lines.** *J.L. Strout, F. Radella, S. Stratton, M.M. Wurfel.* Division of Pulmonary and Critical Care Medicine, Harborview Medical Center, University of Washington, Seattle, WA.

**Background:** Recognition of single-stranded RNA present in viruses such as influenza and HIV is mediated by Toll-like receptors 7 and 8 (TLR7/8), and leads to activation of an innate immune response. These responses could be influenced by common genetic polymorphisms, altering susceptibility to infection. B-lymphoblastoid cell lines (B-LCL) used in the HapMap project can be stimulated to produce inflammatory cytokines via the TLR7 receptor, and therefore, are a valuable resource for the discovery of loci influencing TLR-mediated responses. **Hypothesis:** Loci affecting innate immune inflammatory responses to a TLR7 agonist can be identified using B-LCL from HapMap trios. **Methods:** We exposed Epstein-Barr Virus transformed B-LCL from 30 Caucasian (CEU), and 30 Yoruban (YRI) trios (International HapMap project populations) to CL097, an imidazoquinoline compound that is a specific agonist for TLR7. After a 24 hour incubation, we measured levels of IL6, IL10, and TNF$\alpha$ in the culture supernatants. We then used QFAM in PLINK to determine associations between genotypes present in HapMap (Release 23) and the three different TLR7-mediated inflammatory responses (TNF$\alpha$, IL6, IL10). We also evaluated the differentiation state of the B-LCLs, by measuring the expression of five cell surface markers by flow cytometry, and soluble IgG and IgM production. **Results:** We identified 29 loci in CEU and 18 loci in YRI ($p<1 \times 10\text{-}5$) that associated with TLR7-induced cytokine responses. Of these, 10 CEU, and 6 YRI loci were associated with multiple cytokine responses. While no single polymorphism was found to be significant at this level in both populations, we did identify 15 gene regions in which overlapping associations were observed. We identified only marginal (p<0.01) associations between cell surface expression of CD95 (FAS) and TLR-7-induced responses in CEU and YRI. Likewise, differentiation state, as measured by IgM production, showed only a moderate association (p<0.01) with TLR-7 induced responses, but only in CEU. **Conclusion:** HapMap B-LCLs are a valuable resource for identifying the genetic determinants of the TLR-7 mediated innate immune response.

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## 2874/F/Poster Board #806

**CFTR gene's mutational spectrum in Mexican patients diagnosed with cystic fibrosis.** *M. Chávez-Saldaña[1,2], E. Yokoyama[1], J. Lezana[3], A. Carnevale[4], M. Macias[1], R. Vigueras[1], M. López[5], L. Orozco[6].* 1) HISTOMORFOLOGIA, INSTITUTO NACIONAL DE PEDIATRIA, Mexico City, Mexico; 2) Doctorado en Ciencias Biológicas, Universidad Autónoma Metropolitana, Mexico City, Mexico; 3) Hospital Infantil de México "Federico Gómez" S.S. Mexico City, Mexico; 4) Coordinación Nacional de Medicina Genómica, ISSSTE, Mexico City, Mexico; 5) Departamento de Sistemas Biológicos. Universidad Autónoma Metropolitana, Campus Xochimilco, Mexico City, Mexico; 6) Laboratorio de Genómica de Enfermedades Multifactoriales. Instituto Nacional de Medicina Genómica, Ciudad de México, México.

Cystic fibrosis is the most common autosomal recessive disease. In Mexico, at the National Institute of Pediatrics, 20 new cases are registered annually. To date, over 1,600 mutations have been described related to the causative gene. Mutation distribution varies on the different populations world wide. In this study, we hope to establish the CFTR gene's mutational spectrum in Mexican patients diagnosed with cystic fibrosis. A total of 230 non-related patients were analyzed. The characterization of the alleles was done using a commercial kit for 36 mutations, as well as a simple chain conformational polymorphism method and DNA sequencing. With the combination of these strategies was able to characterize 77.3% of all alleles with CF and twelve different mutations were added to the 34 previously reported, making a total of 46 different mutations detected in the Mexican population. Only seven mutations showed a frequency more than 1%: DF508 (44.6%), G542X (7.4%), N1303K (2.4%), DI507 (1.52%), S549N (1.52%), R334W (1.52%), and 3849 +10 kbC -T (1.3%). The two last mutations are included in the panel of the most frequent mutations in the Mexican population, reported in this study. Is important to note that to know the ethnic and geographical distribution of CFTR mutations could improve the CF detection programs in a given population. In this way is essential to increase knowledge about of the molecular genetic and epidemiology of this disease in our country, to develop methods of diagnostic more effective and to improve the cost-benefit of studies. Support CONACYT-SALUD 2003-C01-066 and specifically CONACYT for a full scholarship number 89745.

## 2875/F/Poster Board #807

**Genomic survey of loss-of-heterozygosity status in Glioblastoma Multiforme using sequencing data.** *R. Dominguez-Vidana[1, 2], R.A. Gibbs[1], D.A. Wheeler[1], L.A. Donehower[1,3].* 1) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX; 2) Interdepartamental Program in Cell and Molecular Biology, Baylor College of Medicine, Houston, TX; 3) Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX.

Glioblastoma Multiforme (GBM) is the most common and malignant of the glial tumors. It is a mixture of poorly differentiated astrocytes located preferentially in the cerebral hemispheres. GBM primarily affects adults; and manifests mostly de novo. It is estimated that the median survival time from diagnosis is approximately 14 months; and approximately only 1 in 5,000 patients survives more than 5 years. The Cancer Genome Atlas (TCGA) pilot project reported an integrative analysis of mutations, CNVs, gene expression and DNA methylation patterns in 206 GBMs. Provided insights into the roles in the disease of *ERBB2, NF1* and *TP53*, *PIK3R1*, and revealed a link between *MGMT* promoter methylation and a hypermutator phenotype, and, most importantly, provided a network view of the altered pathways. A Johns Hopkins's University project also studied GBMs, finding recurrent mutations in *IDH1*, associated with increased survival. Both of these studies established the power of unbiased genomic analyses in the characterization of brain cancer. The purpose of this study is to identify which genes show a statistically significant loss of heterozygosity (LOH). Mutation files were compiled; and unvalidated, and silent mutations were removed from the analysis. Valid mutations were split into missense mutations, and LOH. This hits were crossed with KEGG, manually curated, and other previously delimited pathways. From these analyses several genes were identified with a recurrent LOH pattern, like: *MKI67, NRAP, C10orf54, C9orf66, PRAME, IL1RL1, ABCA13, GDF15, KIAA1632, PALM2-AKAP2, PTPRR, SERPINA6, TP53, TRPM3*. Further analyses are required to elucidate their functional roles in GBMs. This type of analyses shows that combining current pathway data with SNP data can allow identifying potential new genomic alterations that can contribute to carcinogenesis.

## 2876/F/Poster Board #808

**Genetic Control of DNA Methylation and Expression in the Human Brain.** *D.G. Hernandez[1, 2], R. Gibbs[1, 2], M. van der Brug[1, 3], B. Traynor[1], M. Nalls[1], L. Shiao-Lin[1], S. Arepalli[1], R. Zonozi[1], A. Dillman[1], J. Troncoso[4], R. Johnson[5], H. Zielke[5], L. Ferrucci[6], D. Longo[7], M. Cookson[1], A. Singleton[1].* 1) Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, MD, 20892, USA; 2) Department of Molecular Neuroscience and Reta Lila Weston Laboratories, Institute of Neurology, UCL, Queen Square House, London WC1N 3BG, United Kingdom; 3) Department of Molecular and Integrative Neurosciences, The Scripps Research Institute, 130 Scripps Way, Jupiter, FL 33458, USA; 4) Division of Neuropathology, Department of Pathology, School of Medicine, Johns Hopkins University, Baltimore, Maryland 21205, USA; 5) University of Maryland Brain Bank, University of Maryland School of Medicine, Baltimore, MD 21201, USA; 6) Clinical Research Branch, National Institute on Aging, Baltimore, MD, USA; 7) Lymphocyte Cell Biology Unit, Laboratory of Immunology, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA.

An important challenge in the post-genome era is to annotate and understand the effects of genetic variation, particularly within the context of human tissues. Fortunately, the technology is now in place to investigate epigenetic variation and gene expression from a whole-genome perspective. Comprehending how these multiple layers of control interact in a complex and heterogeneous tissue such as the brain is a challenge that can be addressed using high throughput technologies designed to capture large amounts of information for each level of variability. In this study, we investigated the effects of common genetic variability on DNA methylation, mRNA expression and microRNA expression in the frontal cortex and cerebellum regions of 400 normal human brains. We found an abundance of genetic cis regulation of mRNA expression and show novel quantitative trait loci for DNA CpG methylation. Integrating genetic, epigenetic and expression data not only allows an understanding of the normal regulation of DNA methylation and gene expression in the human brain, but acts as an essential first step toward understanding the pathobiological consequences of genetic variants linked to disease. These functional maps also offer the opportunity to form inferences regarding relationships between genes based on patterns of co-regulation and present an a more complete view of the multiple levels of gene regulation in the human brain.

## 2877/F/Poster Board #809

**Buccal Cell DNA for Population Studies: Comparative analysis with corresponding blood DNA validates the use of buccal DNA for high-throughput genotyping.** *M. Karim, S. MacLeod, C. Randolph, W. Zhao, C. Hobbs.* Dept Pediatrics, Arkansas Children's Hospital Research Institute, Little Rock, AR.

Cost-effective and non-invasive methods for DNA collection are required for high-throughput genetic analysis. Peripheral blood is an excellent source of large amounts of high quality DNA, however obtaining peripheral blood samples is a costly, invasive procedure and requires well trained, skilled professionals with proper materials. In addition, the collection of blood from newborn babies and obese individuals is often very tedious, requiring multiple needle sticks. On the other hand the buccal cell collection for DNA is easier, cheaper and usually self collected by study subjects. In order to validate the use of buccal cell-derived DNA in a high throughput genotyping platform, we used DNA from a total of 96 mothers (16 to 40 years) for whom DNA derived from both blood lymphocytes and buccal cells was available. We have evaluated and compared an alternative, less invasive protocol for isolation of DNA from buccal cells with the corresponding blood derived DNA from the same individuals and tested for the outcome of genotyping 1536 single nucleotide polymorphisms (SNPs) for each individual. Whole genome amplification (WGA) was performed on all buccal DNA samples by the use of the GenomePlex® Complete Whole Genome Amplification (WGA) Kit (Sigma, St. Louis, MO). The genotyping data showed (98.2%) concordance between genotype calls for buccal and blood extracted DNA. Our results suggested the non-invasive buccal cell collection in conjunction with WGA results in sufficient DNA for large-scale high-throughput genotyping studies with high concordance of genotype calls compared to genomic DNA derived from blood cells.

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## 2878/F/Poster Board #810

**The relation between the change of functional cardiac parameters and SNPs in GSTP1 and CBR3 after doxorubicin chemotherapy.** *M. Alikasifoglu[1], B. Volkan-Salanci[2], E. Tulumen[3], P.O. Kiratli[4], B. Oksuzoglu[5], N. Guler[6], L. Tokgozoglu[3], B. Erbas[4].* 1) Medical Genetics Dept, Hacettepe University Medical Faculty, Ankara, Turkey; 2) Institute of Childs Health, Genetics Unit, Hacettepe University Medical Faculty, Ankara, Turkey; 3) Cardiology Dept, Hacettepe University Medical Faculty, Ankara, Turkey; 4) Nuclear Medicine Dept, Hacettepe University Medical Faculty, Ankara, Turkey; 5) Internal Medicine Dept, Ankara Numune Egitim ve Arastirma Hastanesi, Ankara, Turkey; 6) Medical Oncology Dept, Bayindir Hastanesi,-Ankara, Turkey.

Background: Cardiotoxicity is an important adverse effect of anthracycline chemotherapy. Although it is known to have a dose dependent effect, studies have shown variances in individual response, due to genetic background. Glutathione S transferase P1 (GSTP1) is responsible for the detoxification of doxorubicin (Dox), and carbonyl reductase 3 (CRB3) converts Dox to doxorubicinole. Genetic variants of GSTP1 and CRB3 may be contributory to pharmacokinetic and pharmacodynamic variability of Dox. Aim of this study was to investigate the relationship between genetic polymorphisms of GSRP1 (A313G) and CBR3 (V244M) and cardiotoxic effect of Dox assessed by ECG-gated blood pool SPECT (SPECT) and echocardiography (E). Materials and methods: Sixty-eight (61 F, 7 M) patients, with normal baseline cardiac function, was included. Chemotherapy combinations contained either Dox or epirubicin as 1st line chemotherapy. Systolic and diastolic cardiac functions were evaluated before and after therapy (mean follow-up:10.4 months± 4.7) using E and SPECT. Left ventricular ejection fraction (EF), peak filling rate (PFR), peak ejection rate (PER), end systolic volume (ESV), end diastolic volume (EDV) values were calculated using SPECT data. GSTP1 and CBR3 polymorphisms were analyzed using TaqMan probes. Results: The mean received Dox dose was 511 ± 146 mg/m2 (210-1188). Fifteen patients (28 %) received adjuvant radiotherapy over the cardiac region. HER2 antagonists were given in 7 patients after chemotherapy. EF values were significantly decreased after Dox with both SPECT and E ($p<0.01$, $p= 0.043$). In 1 patient EF was below 40% after 7 months at 600 mg/m2 of Dox ESV ($p=0.028$) and diastolic parameters; (Mi) e wave deceleration time ($p<0.001$), Mi color propagation ($p=0.001$), and PFR ($p= 0.038$) deteriorated significantly after therapy. Patients who received HER2 antagonists and radiation to cardiac region, showed higher ESV % change ($p=0.015$, 0.013) compared to others. The difference between cardiac parameters and CBR3 genotypes were not significant. GSTP1 polymorphism's AA genotype revealed higher ESV % increase (9.4 ml ± 10) when compared to G allele carriers (GG and AG) (3.09 ml ± 10) after Dox treatment ($p=0.02$). Conclusion: This prospective clinical study showed a significant relationship between GSTP1 (A313G) polymorphism and ESV change after Dox treatment. GSTP1 genotypes may contribute to Dox cardiotoxicity and might be used in dose adjustment in the future.

## 2879/F/Poster Board #811

**Single Nucleotide and Structural Variant detection using paired end sequence data.** *S. Gopalakrishnan, Z.S. Qin.* Biostatistics, University of Michigan, Ann Arbor, MI.

The emergence of short read sequencing has brought us large datasets, which present us with opportunities to find rare disease predisposing variants. Sequence data includes variants that may occur only in the sequenced individual. The first step involved in analysis is identifying single nucleotide variants and separating them from sequencing errors. In our previous work, we presented methods for identifying single nucleotide variants (SNV) using single end data. Here, we propose a new method for identifying SNVs using paired end short read sequence data. Current methods use only the read pairs where both reads of the pair are mapped uniquely to the genome. In our data, about 25-30% of the read pairs are lost due to multiple mapping and/or the inability to map one read of the pair. Extending our previous work, we allow both read ends to be mapped to multiple locations, and choose the appropriate alignment of the reads based on our algorithm. We also allow orphaned reads that have only one read end mapped to the genome to contribute to the inference procedure. Our method is based on a Bayesian Gibbs sampling approach with a continuous prior based on the insert length of read pairs. We run an iterative procedure that chooses read alignments for the multiply mapped paired end and orphaned reads. We infer SNVs based on the final alignment given by the algorithm. Another consequence of using paired end data is the inbuilt information present about structural variants. In addition to SNVs, we also identify genomic locations that are candidate structural variants. We use a sliding window based likelihood ratio test based on insert length and depth of coverage to test for structural variants. Once candidate regions are found, one can carry re-sequence across the inferred boundary to verify the presence and type of structural variant. Preliminary results indicate that we find a lot more SNVs and structural variants by using our algorithm than using only the uniquely mapped paired end reads.

## 2880/F/Poster Board #812

**Follow-up with dense SNP genotyping in NTRK2 continues to show significant association to LOAD.** *R.T. Perry[1], H.W. Wiener[1], R.C.P. Go[1,2].* 1) Dept. Epidemiology, Res Asst Prof, Univ Alabama at Birmingham, Birmingham, AL; 2) Pacific Health Research Institute, Honolulu, HI.

Linkage scans of Alzheimer's Disease (AD) families have identified the 9q22 region as a candidate region. Next to the 19q13 peak where *APOE* is located, the 9q22 signal was the most suggestive from the linkage scan of the NIMH Alzheimer's Disease Genetic Initiative (ADGI) family cohort. Follow-up confirmation of this signal was demonstrated when we genotyped additional microsatellite markers, narrowing the 1 LOD region to 11.5 cm. Four candidate genes located adjacent to the proximal edge of this peak have been reported to be associated with AD from candidate gene and genome wide association studies. We reported a significant association of a three SNP haplotype (designated, SNPs 4, 5, 6) in one of these genes, *NTRK2*, in the NIMH-ADGI cohort. *NTRK2* belongs to the neurotrophic tyrosine receptor kinase (*NTRK*) family, which includes *NTRK1*, *NTRK2*, and *NTRK3*. The *NTRK* family encodes the receptors TRKA, TRKB, and TRKC, to which the neurotrophins, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) bind respectively, with high affinity. Signaling through the NTRK family of receptors appears to be compromised in AD. There are three alternatively spliced isoforms that are differentially expressed between the brain and other tissues and between the frontal cortex of AD and control brains and they involve exons located in the same area of the gene where the three SNP haplotype is located. In order to delineate this association further, we have identified and genotyped all the haplotag SNPs located in the 10 LD blocks spanning and bordering the three SNPs and additional SNPs located between the blocks (Total = 47) in LOAD families of the NIMH-ADGI cohort. Single locus results were suggestive of association around SNP 4; therefore, we performed haplotype analyses from adjacent SNPs to increase heterozygosity and genetic information. Under a dominant model, we found significant association for two SNP haplotypes adjacent to SNP 4 and upstream of SNP 5 (pvalue 0.0006, 0.0005, respectively). Similar results for SNPs in these two areas were obtained for three SNP haplotypes. We are now in the process of confirming these associations by genotyping the most significant SNPs in a separate LOAD family cohort of 899 samples in 201 families from the National Cell Repository for Alzheimer's Disease (NCRAD). Results will be presented at the meeting.

## 2881/F/Poster Board #813

**Ascertainment biases in SNP chips affect measures of population divergence.** *A. Albrechtsen[1], F. Nielsen[2], R. Nielsen[3].* 1) Dept biostatistics, Copenhagen Univ, Copenhagen, Denmark; 2) Department of Clinical Biochemistry, Rigshopitalet, Copenhagen,Denmark; 3) Department of Integrative biology and Statistics, University of California Berkeley, USA.

Chip based high throughput genotyping has facilitated genome-wide studies of genetic diversity. Many studies have utilized these large data sets to make inferences about the demographic history of human populations, using measures of genetic differentiation such as FST or principal component analyses. However, the SNP chip data suffer from ascertainment biases caused by the SNP discovery process in which a small number of individuals from selected populations are used as discovery panels. In this study, we investigate the effect of the ascertainment bias on inferences regarding genetic differentiation among populations in one of the common genome-wide genotyping platforms. We generate SNP genotyping data for individuals, that previously have been subject to partial genome-wide Sanger sequencing and compare inferences based on genotyping data to inferences based on direct sequencing. In addition we also analyze publicly available genome-wide data. We demonstrate that the ascertainment biases will distort measures of human diversity and possibly change conclusions draw from these measures in some times unexpected ways. We also show that details of the genotyping calling algorithms can have a surprisingly large effect on population genetic inferences. We present a correction of the spectrum for the widely used Affymetrix exttrademark SNP chips but also show that such corrections are difficult to generalize among studies.

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## 2882/F/Poster Board #814

**Population Genomics of Human Gene Expression using Next-Generation RNA Sequencing.** *S.B. Montgomery[1,2], C. Beazley[1], C. Ingle[1], J. Nisbett[1], R. Lach[1], J. O'Brien[1], S. Searle[1], S. White[1], E.T. Dermitzakis[1,2].* 1) Population & Comparative Gen, Wellcome Trust Sanger Inst, Cambridge, United Kingdom; 2) Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland.

Understanding the genetic basis of gene expression variability is a fundamental component in building our understanding of the etiology of complex traits. We have previously investigated the proximate causes of expression variability in natural populations genotyped as part of the HapMap project (Stranger et al. Science 2007 and Nat Genet. 2007). Recent advances in next generation RNA sequencing (RNA-seq) have enabled increased resolution of expression variability and transcriptional complexity providing access to features such as alternative splicing, isoform quantitation and allele-specific expression. We have investigated population differences in gene expression in lymphoblastoid cells from 60 HapMap CEU individuals of European descent, also sequenced in the 1000 genomes project, using 37-bp paired-end RNA-seq. Using a single lane of an illumina sequencing reaction, we have obtained 10 million reads per individual which cover ~ll,000 known genes and ~95,500 known exons. We detect high correlation of read counts across individuals and have investigated technical sources of discordance. We have investigated the potential of imputation of read counts between well-quantified exons (>50 reads) and have seen intermediate correlation (SRC: 0.4-0.5). We have detected expression quantitative trait loci (eQTLs) using the HapMap3 genotypes and compared them to those obtained from the same individuals in an array-based study. We find a significant enrichment of sharing in association between the two but have also identified specific differences which are likely due to higher resolution of the RNAseq data. RNA-seq eQTLs are distributed around the transcription start site as observed in array data but we have also seen a substantial enrichment within the gene and outside. Known splice variants also seem to have a large impact on levels of expression from different exons supporting the idea that the resolution of the RNAseq data allows for the identification of the effect of splicing variants. Finally, we will discuss our observations of de novo gene structure and allele-specific expression and eQTLs discovered by integrating the 1000 genomes low coverage genotype data.

## 2883/F/Poster Board #815

**High-throughput Polymorphism Detection and Genotyping Using Next Generation Sequencing Technology.** *Z. Deng[1,2], M. Yeager[1,2], L. Amundadottir[2], L. Qi[1,2], J. Boland[1,2], C. Matthews[1,2], A. Hutchinson[1,2], S.J. Chanock[2].* 1) Core Genotyping Facility, Advanced Technology Program, SAIC-Frederick Inc., NCI-Frederick, Frederick, MD; 2) Division of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, MD.

Targeted re-sequencing of genomic regions associated with human diseases is required to follow-up signals conclusively identified by genome-wide association studies. Sequence-based variation discovery makes it possible to produce a comprehensive list of genetic polymorphisms and subsequent functional studies can be preformed to identify potential causal variants from the regions of interest. Recently, massively parallel sequencing technologies have become the preferred choice for targeted re-sequencing. However, the high-throughput nature of the next generation sequencing has posed new challenges to downstream data analysis. We have developed a high-throughput computational pipeline to process next generation sequence data for single nucleotide polymorphisms (SNP) and insertion/deletions (INDEL) discovery and genotyping. Briefly, sequence reads that passed QC were aligned to the target genomic region by MOSAIK software. The alignment parameters were optimized based on the repetitive nature of the target region. The resulting assembly was analyzed in a column-by-column approach and potential polymorphic sites and most likely genotypes were called based on a set of heuristic rules. Next, the completion rates were determined and loci with low completion rates were removed from further analysis. Lastly, manual inspections aided by the NextGENe software and CONSED were performed to QA the results and resolve ambiguous cases. To validate this computational pipeline, we have analyzed sequence data from three targeted re-sequencing projects covering a total of 290 kb genomic regions. Genotype calls on more than 2,500 polymorphic sites were made with high accuracy (>98% for SNP). Comparisons with dbSNP indicated that about half of the variants are novel. Minor Allele Frequencies distribution analysis and Hardy-Weinberg fitness analysis showed no major deviations for most polymorphic sites. To further validate the genotype results, concordance checks were performed on 41 HapMap samples included in the sequencing projects. The results showed near perfect concordant rates (>98%), indicating high accuracy of our approach. In summary, we have developed and validated a high-performance computational pipeline and demonstrated its utility in targeted re-sequencing projects. Funded by NCI Contract No. HHSN261200800001E.

## 2884/F/Poster Board #816

**Rapid Detection of Ashkenazi Jewish Carrier Screening Using the AutoGenomics INFINITI BioFilmChip Microarray.** *M. Procter[1], L.Z. Chou[1], A. Marchese[3], S. Moledina[3], R. Mao[1, 2].* 1) Res & Development, ARUP Lab, Salt Lake City, UT; 2) Department of Pathology, University of Utah, Salt Lake City, UT; 3) AutoGenomics, Carlsbad, CA.

Background: Jewish persons of eastern European descent, referred to as Ashkenazi Jews, are at higher carrier risk of certain genetic diseases than the general population. The American College of Obstetricians and Gynecologists (ACOG) recommends genetic testing for prospective parents of Ashkenazi Jewish descent for mutations associated with the following conditions: Tay-Sachs, Canavan, Familial Dysautonomia, and Cystic Fibrosis. Furthermore ACOG recommends that testing be offered to these same individuals for mutations associated with 5 additional diseases. These diseases are all autosomal recessive, 20-100 times more frequent in the Ashkenazi Jewish population, and associated with debilitating or life-threatening conditions. In a collaboration between ARUP and AutoGenomics, a BioFilmChip Microarray was developed to screen 30 mutations and 1 polymorphism in all these diseases except Cystic Fibrosis, which is usually a stand-alone test. We evaluated the feasibility and utility of using this assay in our molecular genetics laboratory. Methods: The polyester film-based chip consists of 240 spots contained replicates of the 30 loci. These mutations are detected using PCR followed by hybridization to a chip. Samples were hybridized, incubated and detection took place in an automated Infiniti instrument manufactured by AutoGenomics. To determine accuracy of this assay, DNA from 35 samples with various mutations for Ashkenazi Jewish diseases detected by this panel was used. We used DNA from Coriell Cell Repositories and known-positive and known-negative samples from ARUP's clinical genetics laboratory. Results and conclusion: One-hundred percent concordance was observed in the samples tested, except for one sample with 3 no-call results. Our study showed this test to be, reliable and robust in the detection of a large number of mutations associated with Ashkenazi Jewish diseases. This BioFilmChip Microarray assay can simultaneously detect many mutations in a single reaction while the automated 24-well format allows high sample throughput for a large population screening. Further testing for reproducibility and limit of detection is in progress.

## 2885/F/Poster Board #817

**Genetic Associations with Lactase Dehydrogenase, Anion Gap, and Hepatitis in the Third National Health And Nutrition Examination Survey (NHANES III).** *M.J. Rieder[1], K. Brown-Gentry[2], J. Canter[2], D.C. Crawford[2].* 1) Dept Genome Sciences, University of Washington, Seattle, WA; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Serum lactate dehydrogenase (LDH) is a heritable (35-60%) intermediate phenotype that is associated with trauma mortality and hemolysis. LDH catalyzes the interconversion of pyruvate and lactate, and is a multimeric protein encoded by *LDHA*, *LDHB* and *LDHC*. Pyruvate production is controlled by the pyruvate dehyrodrogenase complex, encoded by *PPM2C*. One measure of anion imbalance and LDH levels is calculated anion gap ($[Na^+]+[K^+]$)-($[Cl^-]+[HCO3^-]$). We hypothesized that genetic variation in the enzymes for LDH production influences serum levels, calculated anion gap (AG), or tissue injury (e.g hepatitis infection). We selected and genotyped 13 *LDHA/B/C* and *PPM2C* tagSNPs in 7157 participants from the Third National Health and Nutrition Examination Survey (NHANES III). NHANES III is a diverse, population-based sample (2631 non-Hispanic **W**hites; 2108 non-Hispanic **B**lacks; and 2073 **M**exican-**A**mericans) linked to health and lifestyle variables, including serum LDH, cation/anion values, hepatitis. Assuming an additive genetic model, we performed linear regressions adjusted for BMI, sex, and age and stratified by race/ethnicity. For serum LDH, we had four significant associations: *PPM2C* rs911 ($\beta$=3.21; p=0.028) in **B** and *PPM2C* rs911 ($\beta$=3.61; p=0.012), *LDHC* rs2721126 ($\beta$=4.60; p= 0.020), and *LDHA* rs11601413 ($\beta$=-5.39; p=0.005) in **MA**. For calculated AG, the two significant associations were *PPM2C* rs4735258 ($\beta$=0.23; p= 0.036) and *LDHC* rs10832934 ($\beta$=-0.84; p=0.009) in **B**. Tissue injury due to acute hepatitis was quantitated from the presence/absence of hepatitis A, B, and C antibodies (Hep A/B/C), and tested using logistic regression using the same covariate adjustments. Consistent with the calculated AG results, *PPM2C* rs4735258 (OR= 1.18; 95% CI: 1.01-1.4; p = 0.038) and *LDHC* rs10832934 (OR=0.58; 95% CI: 0.36-0.93; p = 0.024) were associated with HepA in **B** (n=772 cases and 937 controls). In **MA** (n=1424 cases and 324 controls), *LHDA* rs2896526 (OR=0.77; 95% CI=0.60-0.98; p = 0.035) was associated with HepA. Furthermore, *LDHA* rs2896526 was significantly associated with HepB (OR=1.34; 95% CI: 1.1-1.8; p=0.021) in **B** (n=242 cases and 1467 controls), and *LDHA* rs2403279 was significantly associated with HepC (OR=0.61; 95% CI: 0.37-0.99; p=0.05) in **W** (n=42 cases and 2357 controls). These results suggest that genetic variation associated with serum LDH, calculated AG, and presence of hepatitis exposure may provide information on pathways and diseases associated with cell turnover.

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## 2886/F/Poster Board #818

**Genome wide association study on leptin levels in the KORA study.** *H. Grallert[1], C. Gieger[1], C. Herder[2], B. Thorand[1], C. Meisinger[1], H.E. Wichmann[1,3], W. Rathmann[5], W. Koenig[4], T. Illig[1,3].* 1) Instiute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Bavaria, Germany; 2) Institute for Clinical Diabetes Research, German Diabetes Centre, Leibniz Centre at Heinrich Heine University Düsseldorf, Düsseldorf, Germany; 3) Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-Universität, Munich, Germany; 4) Department Internal Medicine II-Cardiology, University of Ulm, Medical Center, Ulm, Germany; 5) Institute of Biometrics and Epidemiology, German Diabetes Centre, Leibniz Centre at Heinrich Heine University Düsseldorf, Düsseldorf, Germany.

Leptin inhibits hungry feeling and has a major role in regulation of metabolism. Leptin levels increase with adiposity degree. In the KORA study we investigated the association between leptin levels and genetic variation in a genome wide approach. We used a sample of 1644 participants of the follow up of KORA survey 3 (F3) and a second sample of 1814 participants of KORA F4 for our analysis. Genotyping was done with the Affymetrix 500K Arrayset in KORA F3 and with the Affy 6.0 Chip in KORA F4. For better comparison of the different genotyping methods genotype data were imputed. In both surveys none of the SNPs reached genome wide significance level. However, we found 10 SNPs that were associated with leptin levels with p-values lower than 0.0009 in both surveys. These SNPs were located in three regions on chromosome 4 and 5. Two SNPs on chromosome 5 are located near genes coding for proteins that might interact with leptin (SNP1: OR=1.12 p=4.7x10-4 (F3), OR=1.11 p=6.8 x10-4 (F4)); SNP2: OR= 1.11 p=4.2x10-4 (F3), OR=1.14 p=8.3x10-5 (F4)). One SNP on chromosome 4 is located in proximity to a gene of interest (SNP3: OR=1.12 p=4.0 x10-4 (F3), OR=1.11 p=6.7 x10-4 (F4)). The other seven SNPs are located in a region on chromosome 5 far away from known genes. Thus, speculations on these SNPs are impossible up to now. In conclusion our results give evidence for two loci that are potentially associated with leptin levels and thus might be involved in leptin regulation and energy metabolism. However, the detailed mechanisms have to be elucidated in further studies.

## 2887/F/Poster Board #819

**Genome-wide Screening for Novel Alu Retrotransposons.** *D. Hedges, S. Zuchner, N. Rocco.* Miami Institute for Human Genomics, Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami, Miami, FL 33136.

Transposable elements (TEs) make up a significant fraction of primate genomes. While most of this genomic content is comprised of older elements that have lost their capacity to generate new copies, multiple lineages of TEs continue to actively propagate in extant primates, including humans. These elements generate de novo insertion events which can result in pathogenic alleles. TE activity has now been implicated in over 20 genetic disorders. The discovery of disruptive TE insertions within genes may assist in elucidating the genetic architecture of complex disorders. However, there currently exists no feasible method for high throughput screening of populations of mammalian-sized genomes for novel TE insertions. Here we describe a method for the discovery of all novel Alu insertions within a given human genome. Our approach, which combines targeted enrichment with Next-generation sequencing, will make it feasible to screen significant numbers of individuals for insertion events. We are currently validating the method by examining the transmission of detected Alu insertions within a pedigree structure.

## 2888/F/Poster Board #820

**A High Resolution Association Mapping Panel for the Dissection of Complex Traits in Mice.** *E. Eskin[3,4], B.J. Bennett[1], C.R. Farber[8], L. Orozco[1], H.M. Kang[5], A. Ghazalpour[1], T. Kirchgessner[6], E.P. Gargalovic[6], L.W. Castellani[1], E. Kostem[4], N. Furlotte[4], T.A. Drake[7], A.J. Lusis[1,2,3].* 1) Department of Cardiology, David Geffen School of Department of Cardiology, David Geffen School of Medicine at UCLA, University of California, Los Angeles CA; 2) Department of Microbiology, Immunology and Molecular Genetics, UCLA; 3) Department of Human Genetics, David Geffen School of Medicine at UCLA, University of California, Los Angeles CA; 4) Department of Computer Science, UCLA, University of California, Los Angeles; 5) Computer Science and Engineering, University of California, San Diego; 6) Bristol Myers Squibb; 7) Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA,University of California, Los Angeles CA; 8) Department of Medicine, Department of Biochemistry and Molecular Genetics and Center for Public Health Genomics, University of Virginia, Charlottesville, VA.

Linkage analysis for the identification of genes contributing to complex traits in mice has been only modestly successful due to low mapping resolution. Association analysis in mice has the potential of much better resolution but is confounded by population structure and inadequate power to map traits that explain less then 10% of the variance, typical of mouse quantitative trait loci (QTL). We report a novel strategy for association mapping that combined classic inbred strains for mapping resolution and recombinant inbred strains for mapping power. Using a mixed model algorithm to correct for population structure, we validate the approach by mapping about 3000 cis- expression SNPs with a resolution 1 to 2 orders of magnitude narrower than QTL analysis. We report the fine mapping for metabolic traits such as plasma lipids. This resource, termed the Hybrid Mouse Diversity Panel (HMDP), has sufficient power to map traits that contribute to 10% of the overall variance. Importantly, the resolution of the panel can approach up to an order of magnitude better than that achievable using linkage analysis of complex traits. In addition, each strain is renewable and, therefore, diverse molecular and phenotypic data can be collected ad infinitum. In addition to greatly increased ability to narrowly map and identify genes for complex traits, this panel should be useful for the analysis of gene-by-environment interactions where multiple individuals of the same genotype need to be studied. Moreover, the fact that the data involving clinical traits, expression traits, proteomic traits, and metabolic traits are cumulative makes this resource ideal for systems biology.

## 2889/F/Poster Board #821

**Therapeutic drug discovery in dystrophin deficient zebrafish.** *G. Kawahara[1], M. Alexander[1], J. Guyon[2], L. Kunkel[1, 3].* 1) Children's Hospital Boston, Boston, MA; 2) Alaska Fisheries Science Center, Auke Bay Laboratories, Juneau, AK; 3) Howard Hughes Medical Institute, Boston, MA.

Zebrafish animal models of human diseases are powerful tools for screening a large number of chemical compounds for therapeutic efficiency. The zebrafish *sapje* mutant has been shown to carry a nonsense mutation in exon 4 of the zebrafish dystrophin gene and *sapje like* ($sap^{c/100}$) is a second allele of dystrophin deficiency caused by exon 62 skipping. Both alleles are excellent models of human Duchenne Muscular Dystrophy (DMD) and each shows disturbed muscle structure and a severe reduction of birefringence (the ability to refract polarized light) at 3-7 days post fertilization (dpf). The birefringence is a result of dorsal skeletal muscle deterioration and weakness that ultimately results in lethality of the most *sapje* mutants at 10 dpf. To identify potential therapeutic chemicals for treating DMD, we undertook chemical screening of a small, commercial molecular library using the *sapje* fish. Embryos from mating heterozygous *sapje* fish were cultured in normal fish water containing pools of the chemical library and were incubated from 1 dpf to 4 dpf. To examine the effects of chemicals for the recovery of the muscle phenotypes, birefringence assays were examined at 4 dpf. Fish treated with some pooled chemicals in the chemical library that were genotypically confirmed as *sapje* mutants showed no defects in birefringence compared to the control, suggesting that we may have successfully identified compounds that are effective for the recovery of phenotypes caused by dystrophin gene mutation. We have identified several pools of compounds that are capable of extending the lifespan of the embryos of *sapje* fish and reduce muscle weakness. Currently the individual components of the pools are being tested and also screened against the second allele of dystrophin deficient zebrafish. Those influencing both alleles will be tested for affect on their function in myoblast fusion in primary myoblast cultures isolated from wild type and *sapje* fish. The results of this screen may lead to new candidate molecules and pathways towards better therapeutic strategies for DMD.

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## 2890/F/Poster Board #822

**High-density, genome-wide profiling of over 600k single nucleotide polymorphisms (SNPs) and over 900k Copy Number Variations (CNVs) using the Affymetrix Mouse Diversity Genotyping Array.** *A.J. Williams[1], H. Yang[2], F. Pardo-Manuel de Villena[3], G.A. Churchill[2], T. Webster[1], A. Pirani[1].* 1) Informatics, Affymetrix, Inc., Santa Clara, CA; 2) The Jackson Laboratory, Bar Harbor, ME; 3) The University of North Carolina at Chapel Hill, Chapel Hill, NC.

A new Mouse genotyping array has been developed, the first tool that enables researchers to study the complexity of the mouse genome and strain diversity in terms of both SNPs and copy number variation. Previously mouse researchers had a relatively limited ability to conduct whole-genome association studies and find markers for disease. This comprehensive array will be an effective tool for scientists using the mouse as a model for human disease and as a tool for researchers looking to better understand the mouse genome.

We describe the analysis methods developed for the array, using a Gaussian mixture model named BRLMM-P to perform genotype calling. Analysis has been tuned to meet the particular needs of mouse genetic data resulting in very high call rates and accuracy. SNP-specific models have been created by analyzing a large set of training data to optimize clustering for each SNP individually. The training data consists of classic Laboratory strains as well as their crosses and crosses between them and other strains. In an assessment of a prototype form of the system, the average sample concordance is 99.65% with an average sample call rate of 98.9%. For inbred strains, the average sample heterozygous rate (which is equivalent to an error rate) is 0.34%.

Applications for the array include but are not limited to mapping the phylogenetic origin for each segment of the genome, identifying structural variation in laboratory mice, observing genetic variation among substrains and sister strains, identifying de novo mutations and studying gene conversion.

## 2891/F/Poster Board #823

**Use of Zebrafish (Danio rerio) for identification of human genes related to aging.** *J.M. Keller, J. Escara-Wilke, E.T. Keller.* Urology & Pathology, Univ Michigan, Ann Arbor, MI.

**Background:** Zebrafish (Danio rerio) are an outstanding animal model to explore vertebrate development and genetics. Systematic phylogenetic analysis has defined mammalian orthologs of mapped zebrafish genes and comparison of map positions in zebrafish and mammals identified significant conservation of synteny. Zebrafish have been primarily utilized to explore development and are underexploited for evaluation of pathophysiology of age-related diseases. We believe that evaluation of mature zebrafish will provide novel information that can link genes to a variety of disease processes in the aged-human. However, there is an extensive gap in knowledge regarding age-specific morbidity and population lifespan data of zebrafish. Accordingly, the goal of our project was to characterize zebrafish demographics and age-related disease so that genetic screens can be applied to these phenotypes to identify human gene candidates for age-related diseases.

**Methods:** We established a colony of 3500 zebrafish to evaluate the population aging and age-related diseases. These zebrafish were divided into low and high density populations which consisted of 10 and 20 fish/tank, respectively. Zebrafish were observed daily for signs of disease or death.

**Results:** Currently, the colony is on year 6, with less than 1% of the population remaining for each density group. The median lifespans are approximately 35 and 28 months of age for the low and high density groups, respectively. We found several age-related diseases in large proportions of the population. Specifically, male zebrafish developed seminoma with similar histological attributes as human seminoma. This started to occur at approximately 25 months of age. Other tumors were observed including intestinal carcinomas and spindle cell sarcomas, but at much lower frequency. Another finding in a large proportion of the age-population was the occurrence of kyphosis and osteoporosis at fish greater than 36 months of age.

**Conclusion:** These data set the groundwork for age-related diseases of zebrafish and provide phenotypes that can be targeted for genetic screening to identify zebrafish genes that regulate age-related diseases. These genes can than lead to identification of candidate human genes that impact aging and age-related diseases.

## 2892/F/Poster Board #824

**Resequencing of the whole candidate region for 16q22-linked spinocerebellar ataxia in Japanese individuals using next-generation sequencing.** *T. Kaname[1,2,4], M. Tsukahara[3,4], K. Yanagi[1,4], K. Fujimori[4,5], I. Kikuzato[4,6], M. Teruya[4,7], Y. Imada[3,4], M. Nezuo[3,4], S. Yano[3,4], Y. Sato[4,6], Y. Miwa[3,4], T. Hirano[4,5], R. Hirano[8], H. Takashima[8], K. Yoshiura[2,9], N. Niikawa[2,10], K. Naritomi[1,2].* 1) Dept Med Gen, Univ Ryukyus, Nishihara, Japan; 2) SORST, JST, Tokyo, Japan; 3) Tropical Technology Center Ltd., Okinawa, Japan; 4) Okinawa Cutting-Edge Genome Project; 5) Advanced Industrial Science and Technology, Tsukuba, Japan; 6) OSTC, Okinawa, Japan; 7) Okinawa Industrial Technology Center, Okinawa, Japan; 8) Dept Neurology and Geriatrics, Kagoshima Univ Sch Med, Kagoshima, Japan; 9) Dept Hum Genet, Nagasaki Univ Grad Sch Biomed Sci, Nagasaki, Japan; 10) RIPHS, Health Sci Univ Hokkaido, Hokkaido, Japan.

Next Generation Sequencing technologies have brought new approaches of genetic analyses for personal genome or human diseases. The technologies can accurate giga-base order of sequence within a single run, allowing for complete resequencing of whole candidate region of a genetic disease in patients, whose responsible gene is not identified. We picked one candidate region of a neurologenetic disorder, the autosomal dominant spinocerebellar ataxia linked to chromosome 16q22 (16q ADCA), as an example of the resequencing approach. The candidate region of 16q ADCA in Japanese individuals was extracted and amplified by long-range PCR or other techniques spanning the whole region. Then, a fragment library or a mate-paired library from the region was constructed and sequenced to a depth >100-fold using the SOLiD 3 system. Comparison of each sequences and the genome database allowed us to find novel variations in Japanese individuals in addition to known SNPs or variations. We compared the results of fragment library and mate-paired library as well. The approach of 'Resequencing of whole candidate region for a genetic disease' might be a powerful tool for searching mutations in patients to identify the responsible gene.

## 2893/F/Poster Board #825

**Deep clinical resequencing to detect simple and complex mutations using Next Generation Sequencing.** *E.L.H. Chin, C. Alexander, L. Bean, B. Coffee, M. Hegde.* Department of Human Genetics, Emory University, Atlanta, GA.

Full gene sequence analysis to detect mutations in disease genes is common place in clinical diagnostic laboratories. Current technology, Sanger dideoxy termination sequencing, allows rapid development and implementation of sequencing assays in the clinical laboratory, but has limited throughput and mainly because of cost constraints only allows analysis of one or a few genes in a patient. Since mutation identification is of paramount importance for diagnosis confirmation, genetic counseling, risk assessment and carrier screening, efforts must be made to perform accurate and sensitive mutation analysis. Next generation sequencers have evolved rapidly, but have mainly been used for large scale genome sequencing projects and are just beginning to be used in clinical diagnostic testing. One advantage of next generation sequencers is that many genes in a patient can easily be analyzed at the same time, allowing for mutation detection when there are many possible candidate genes for a specific phenotype. In addition, regions of a gene typically not tested for mutations, deep intronic and promoter mutations can now be analyzed routinely. In parallel with the advances in sequencing technology, bioinformatic tools have been developed to assemble sequences from short reads. The technology is now sufficiently mature to begin implementation in a clinical laboratory setting where highly accurate data is required. We describe here the application of next generation sequencing technology in clinical diagnostic testing at Emory Genetics Laboratory (EGL). EGL specializes in testing for rare disorders, sequencing over 150 genes individually or in panels using the Sanger dideoxy termination methodology. EGL recently acquired a SoLiD platform (ABI) and is currently validating twenty previously characterized positive controls in disease causing genes where nucleotide changes were detected by Sanger sequencing. The positive controls chosen for validation range from simple substitution mutations to complex deletion and insertion mutations occurring in autosomal dominant and recessive disorders. These validation studies will include sequence quality, depth, accuracy, and cost analysis. This data will serve as a model for implementation for next generation sequencing in clinical laboratories by defining the strengths and weaknesses of the methodology.

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## 2894/F/Poster Board #826

**Informatic Challenges for Human Whole-Genome Next-Generation Resequencing.** *N. Homer, B. Merriman, SF. Nelson.* Dept Human Gen, Univ California, Los Angeles, Los Angeles, CA.

Next-generation sequence technology has allowed for the cost-effective resequencing of human genomes. Nevertheless, the size and complexity of data can be overwhelming when performing informatics analysis. Many recent alignment and variant calling algorithms have been developed to help analyze next-generation sequencing data. These algorithms and tools can be used together to create a complete analysis workflow that enables accurate and efficient variant calling that identifies protein coding change mutations, structural variants, and other potential differences. We present our experiences pertaining to human glioblastoma whole genome resequencing. In particular, we detail informatics challenges and their solutions to whole genome sequencing using the Applied Biosystems SOLiD sequencer. We also describe the application of these tools to the resequencing of paired tumor/normal cancer samples. The informatics challenge begins with retrieving and archiving any raw data produced by the sequencer. Next, we align the data to the human reference genome, tolerating errors as well as variants such as single base changes, insertions, and deletions. Finally, we apply various variant-calling algorithms to identify potential coding change mutations, as well as cataloguing intra and inter chromosomal translocations. Utilizing such a workflow, we are able to help reveal the mutational spectrum of human glioblastoma multiforme.

## 2895/F/Poster Board #827

**Interleukin 7 alpha receptor mutation in an infant with Severe Combined Immunodeficiency of South American decent.** *A. Shanmugham, H. SHUHAIBER HANS H., E. GUZMAN, K. ANYANE-YEBOA.* PEDIATRICS, COLUMBIA UNIVERSITY, NEW YORK, NY.

Severe Combined (SCID) is a fatal disorder if untreated, characterized by severe lymphopenia and lack of adaptive immunity. SCID occurs with an estimated incidence of 1 in 75,000 births and is considered a pediatric emergency of potentially lethal outcome due to recurrent and persistent infections. Early diagnosis is critical, since Hematopoietic stem-cell transplantation ( HSCT) results in survival rates above 90% when performed serious infections develops. A major X-linked locus and sever autosomal recessive loci have been identified for SCID. A 5 month old girl with no significant past medical history was admitted for and loose stools and weight loss of 700 grams in 1 month. She was the full term product of an uncomplicated pregnancy. She had experienced upper respiratory infections. Laboratory workup revealed a total T-cell count of 3 (normal 2500-5600), CD4: 1 (1800-4000), CD8: 2 (590-3000). B-cells were 1596 (590-3000), Natural Killer 242 (of 17-830). Her plain chest x-ray revealed prominent interstitial markings consistent with Pneumocystis jerovicii. A primary immunodeficiency was considered. The coding sequence of Interlukin 7 receptor was amplified from genomic DNA and sequenced in 9 segments. She was found to have a homozygous splice site nucleotide change, c.83(-2a>t). Subsequently, both parents were confirmed to be heterozygous for the same mutation. This mutation, similar to one previously reported by Giliani et al (2005), disrupts the required “at” splice acceptor site at the 5’ end of exon 2 of the IL7R gene,encoding the IL-7Rα receptor (also known as CD127). Several nucleotide polymorphisms have been reported in the IL7RA gene, some of which result in amino acid changes . The IL-7Rα cDNA is composed of 1379 nucleotides and encodes for an 80-kDa type I membrane glycoprotein of 440 amino acids. Following binding of IL-7 to IL-7R, one of the key events is the activation of Jak1 (that is coupled to IL-7Rα) and Jak3 (associated with γc), which can phosphorylate the IL-7Rα, thus allowing recruitment of STAT5 and PI3K. Gilani et al 2005 reported 16 novel cases of SCID due to IL7RA gene defects. Our case further identifies new splice defect mutation (splice mutations contribute 24% to total IL-7RA mutations) in an Argentinian patient with a typical presentation. Relatively few IL7R mutations have been published, and the true incidence is unknown. Many US states are considering including SCID in their newborn screening programs.

## 2896/F/Poster Board #828

**Identification of novel sequence variants in *FMR1* in developmentally delayed males.** *S.C. Collins, D.J. Cutler, J. Visootsak, M.P. Adam, B. Coffee, M.E. Zwick, S.T. Warren.* Department of Human Genetics, Emory University School of Medicine, Atlanta, GA.

Fragile X syndrome (FXS) is a common form of mental retardation resulting from the functional loss of the *FMR1* gene product. While this is most often due to transcriptional silencing triggered by trinucleotide repeat expansion, there is one report of FXS caused by a missense mutation, I304N (De Boulle et al. 1993). To determine if other sequence variants in *FMR1* associate with the more general diagnosis of developmental delay, we used the Illumina GAII to resequence pools of LPCR products covering the promoter, exons, and a portion of the introns of *FMR1* in 963 developmentally delayed males referred to our clinical laboratory for fragile X testing. The probability of the minor allele frequencies in each pool exceeding the base error rate was calculated using a binomial distribution, and the threshold for variation detection was empirically set at $p < 1x10^{-4}$. The resulting positive predictive value, as determined by Sanger sequencing confirmation, was 78.2%, with a false negative rate of 23.5%. Within the tested clinical population, we identified and verified a novel missense change in *FMR1*, R138Q, which alters a conserved residue within the nuclear localization signal of FMRP. Clinical characterization of the proband reveals severe MR, seizure disorder, and recurrent otitis media, but no striking FXS-like physical features. Family studies have been inconclusive, but do not rule out the segregation of the R138Q allele with the phenotype. In addition to the R138Q missense change, we have also identified novel synonymous changes in codons 125 and 217, a novel 5’UTR variant downstream of the CGG repeat tract, three novel variants in defined promoter elements, and hundreds of novel variants in the introns and 3’UTR of *FMR1*. Although functional characterization and control genotyping of these novel variants will be necessary to determine association with developmental delay, this study more than doubles the number of known sequence variants in *FMR1*. Furthermore, this study is the first to conclusively show that missense, nonsense, and promoter mutations in *FMR1* are rare in patients who are currently tested for FXS by repeat tract analysis, suggesting that there is limited clinical utility for *FMR1* resequencing in these patients.

## 2897/F/Poster Board #829

**Exome sequencing of patients with Kabuki syndrome.** *C. Gilissen, A. Hoischen, N. Wieskamp, P. Arts, W.A. van der Vliet, M. Buckley, B.B.A. van Bon, B. de Vries, H. Scheffer, H.G. Brunner, J.A. Veltman.* Radboud University Nijmegen Medical Centre, Nijmegen, Gelderland, Netherlands.

Up to half of all rare genetic diseases remain unexplained at the molecular level. Identification of the causative genes in these disorders has been difficult because of their low frequency (not allowing association studies), de novo occurrence (not allowing linkage analysis), and lack of frequent occurring structural genomic variants (not allowing microarray-based CNV approaches). The recent development of massive parallel sequencing technology, combined with whole exome enrichment, now for the first time allows the unbiased analysis of all coding sequences in the human genome. We apply this strategy by capturing and sequencing the exome of three patients with Kabuki syndrome. For each patient we capture 34 Mb of genomic sequence using the Roche NimbleGen Exome capture array representing ~180,000 human exons and 550 miRNAs. The captured fragments are processed by the Roche 454 FLX Titanium, resulting in up to 1Gb uniquely map-able data per patient. More than 85% of the captured sequence can be mapped near or on the target sequence, indicating efficient enrichment. We find up to 7100 exonic variations per patient compared to the reference genome including 0.2% nonsense, 34% missense mutations and 13.9% small insertions and deletions. These numbers are consistent with those reported on the sequencing of other individual genomes [1]. Array based genotyping confirms on average 98% of the SNP variations, indicating the genotyping accuracy of this novel approach. After exclusion of all known SNPs, variations are classified based upon information from splice sites, amino acid changes, evolutionary conservation, protein truncating potential, and protein structure predictions. Currently we are in the process of validating the high ranking mutations by Sanger sequencing, testing for de novo occurrence, and testing disease candidate genes in a cohort of more than 50 Kabuki patients.

[1] Ng PC, Levy S, Huang J, Stockwell TB, Walenz BP, et al. (2008) Genetic Variation in an Individual Human Exome. PLoS Genet 4(8):e2000260. doi:10.1371/journal.pgen.1000160.

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## 2898/F/Poster Board #830

**A highly annotated whole genome sequence of a Korean Individual.**
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Sequencing technologies have much progressed in recent years, and initiated an era of personal genome sequences. To date, human genome sequences have been reported for individuals with ancestry in three distinct geographical regions: a Yoruba African, two individuals of Northwest European origin, and a person from China. Here, we provide a highly annotated, whole genome sequence for a Korean individual (AK1). The genome of AK1 was determined by a combined approach that included whole genome shotgun sequencing (27.8x coverage), targeted bacterial artificial chromosome (BAC) sequencing, and high-resolution comparative genomic hybridization (CGH) using custom microarrays featuring over 24 million probes to provide the most detailed analysis of an individual human genome to date. Alignment to the NCBI reference, a composite of multiple ethnic clades, disclosed nearly 3.45 million single nucleotide polymorphisms (SNPs) including 10,162 non-synonymous (ns) SNPs, and 170,202 deletion or insertion polymorphisms (indels). SNP and indel densities were strongly correlated genome-wide. Applying very conservative criteria yielded highly reliable copy number variants (CNVs) for clinical considerations. Potential medical phenotypes were annotated for nsSNPs, coding domain indels, and structural variants. Integration of human whole genome sequences, particularly from groups of individuals with minimal ethnic admixture, will assist in understanding genetic ancestry, migration patterns, and population bottlenecks.

## 2899/F/Poster Board #831

**Solution-Based Enrichment of Genomic Loci for Massively Parallel Sequencing on the SOLiD System.**
*K. Li[1], D. Roberts[2], T. Sokolsky[3], A. Antipova[3], B. Novak[2], C. Pabon[2], L. Degoricija[1], M. Visitacion[2], C. Lee[3], C. Clouser[3].* 1) Life Technologies, 850 Lincoln Centre Dr., Foster City, CA, USA; 2) Agilent Technologies, 5301 Stevens Creek Blvd., Santa Clara, CA, USA; 3) Life Technologies, 500 Cummings Center, Beverly, MA, USA.

The identification of genetic variants and mutations associated with human disease requires the development of a robust and cost-effective approach for systematic resequencing of candidate regions in the human genome. The SOLiD System acquires tens of gigabases of mappable sequence within a single run, allowing for accurate resequencing of large genomes. When combined with the Agilent SureSelect Target Enrichment System, the ultra-high throughput of the SOLiD System facilitates deep sequencing of target genomic regions of interest. The SureSelect method extracts target regions from genomic DNA by hybridization to in-solution biotinylated cRNA probes, or "baits." The target enrichment workflow is streamlined to incorporate this enrichment step after libraries are constructed. Post-enrichment material is amplified and used directly for downstream steps including emulsion PCR and sequencing on the SOLiD System. Sequence capture of a 2.7 Mb region from Yoruba DNA resulted in 700-fold enrichment with 65% of reads mapping uniquely to the target region, as compared to 0.1% for an un-enriched control. Due to the inherent scalability and potential for automation of SureSelect in-solution enrichment, the coupling of this method with the SOLiD System platform provides a useful solution for targeted resequencing applications.

## 2900/F/Poster Board #832

**Exon capture approaches for gene discovery in callosal agenesis.**
*J. Li[1], M. LEE[1], G. Da Gente[1], R. Yeh[2], E. Sherr[1].* 1) Dept Neurology, UCSF, San Francisco, CA; 2) Dept Biostatistics, UCSF, San Francisco, CA.

Resequencing of high-value genomic regions promises to accelerate the identification of disease-associated mutations. Here we present an approach that combines customized, high-density Agilent microarrays and Illumina 1G massively parallel sequencing to selectively sequence 3.5Mb of exons identified by homozygosity mapping in patients with autosomal recessive callosal agenesis. We selected exons from genomic fragments identified through homozygosity mapping. These fragments and flanking regions were analyzed using the program OligoWiz2.0 to design probes for our custom array that cover these candidate genomic regions and the unique sequences are identified by the NCBI Browser with repeats masked by Window Masker program. The full set (244K) of probe sequences was exported to Agilent's eArray platform to produce the custom array. Genomic DNA from patients was prepared according to the Illumina/Solexa sample preparation protocol. Briefly 5 ug of recently purified high quality genomic DNA suspended in TE was noninvasively sonicated (Misonix sonicator 3000) in eppendorf tubes. After fragmentation, DNA samples were ligated with 1G sequencing adaptors and the products size purified (100-400 bp) and quantified. The fragments were PCR amplified to yield 5ug of DNA using adaptor-based primers followed by hybridization to the Agilent custom arrays. Prior to hybridization to the array, to assess the genomic representation of the purified DNA, fragments were cloned into blunt end vectors before hybridization and a sample of bacterial clones selected, purified and sequenced. Hybridization to the custom designed Agilent chip, was conducted according to the Agilent CGH protocol (version 2.0) and enriched DNA was eluted from the chips and reamplified with 10-12 PCR cycles before Illumina sequencing. For data analysis, we use MAQ to align the sequence reads with the reference. Among the 14818199 reads generated, 46.09% reads mapped to the target sequences and 85.6% of the target sequences were covered with average depth of 91.58. We identified 375 candidate SNPs of which 26 are non-synonymous changes. Overall, the method we present allows a flexible, cost effective approach to disease-oriented gene discovery and resequencing projects.

## 2901/F/Poster Board #833

**High-throughput, High-accuracy Targeted Multiplex Amplification for Medical Resequencing Using Spacer Multiplex Amplification ReacTion (SMART).**
*P. Shen, S. Krishnakumar, W. Wang, C. Scharfe, M. Mindrinos, R. Davis.* Genome Technology Ctr, Stanford Univ, Palo Alto, CA.

Efficient and cost-effective targeted resequencing studies are highly dependant on the reduction in human genome complexity that can be achieved by targeted multiplex amplification. We have developed a novel, robust, and reproducible methodology to amplify human sequences in parallel for use in downstream multiplexed sequence analyses. We call the methodology SMART (Spacer Multiplex Amplification ReacTion); it is based, in part, on padlock probe technology. A significant feature of the SMART assay is the use of Long Padlock Probes (LPPs that are greater than 300 bases in length for the assay). The LPPs have sequences that hybridize to the sequences flanking a target sequence, and capture the target by extension of the probe, followed by ligation to form a circular molecule. The target sequences are then amplified using primer sequences in the probe that are common to all the circular molecules. We applied the SMART amplification to 5376 exons up to 600 bp in length in genes involved in mitochondrial function and associated clinical disease phenotypes. The multiplex PCR products were hybridized to a custom-designed Affymetrix MRS array containing the coding sequences (>0.8Mb) of 524 mitochondrial genes. Using a maximum-intensity base caller of >90% as the cutoff for the success of target amplification, we were able to sequence 93% of the exons by the MRS arrays. The failed exons mostly have higher GC content. For the 3312 exons with GC content less than 50%, the successful rate of our multiplex PCR is close to 99%. Currently we are using NGS (next-generation sequencing) platforms to test amplicon amount distribution across all exons. In comparison with other target enrichment systems such as Roche's Sequence Capture arrays and Agilent's SureSelect, we believe that our SMART technology reaches equivalent multiplex fold at higher specificity, requires less DNA, and is more flexible to run and cost-effective.

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## 2902/F/Poster Board #834

**Robustness in the Face of Complexity, Single Molecule Real Time DNA Sequencing.** *S. Turner.* Pacific Biosciences, Menlo Park, CA.

New DNA sequencing technologies are needed in order to fulfill the goals of projects like the 1000 genome project and better understand human disease. We present such a technology. Single molecule real time (SMRT) DNA sequencing is a novel high-throughput method for sequencing DNA. It harnesses the intrinsic power of DNA polymerase enzymes as sequencing engines by eavesdropping on template directed synthesis in real time. Using two technological innovations, phospholink nucleotides and ZMW confinement, this method enables fast, single molecule DNA sequencing at very long readlengths. Through the combination of these innovations, our technology allows the speed, processivity, efficiency and fidelity of the enzyme to be exploited. The accuracy in SMRT sequencing is equally good throughout a read because after each base incorporation, the labeled portion of the phospholink nucleotide is cleaved away and the system reverts completely back to its initial condition. We show application of this technology to shotgun sequencing of human and bacterial DNA resulting in high consensus accuracy and unprecedented readlength. We will present a novel sample prep concept based on DNA hairpin ligation to double-stranded DNA that facilitates whole genome shotgun sequencing directly from genomic DNA with near-Poisson limited coverage uniformity and practically no GC bias. This sample prep will be demonstrated to enable consensus sequencing based on just one DNA molecule, allowing high accuracy sequencing at the molecular limit and without amplification. This capability is applied to detect SNPs in mixed samples with fractions as low as 1%. We will present an alternative to paired-end reads called strobe-sequencing in which insert sizes are dynamically adjustable at run-time and a variable number of linked reads inside one molecule are possible. This will allow elucidation with sub-one-fold coverage of structural variations including complex tangles that with paired ends require multiple libraries with different insert sizes and high fold coverage.

## 2903/F/Poster Board #835

**Functional Significance of Novel Genetic Variants of PON1 in a Mexican-American Population.** *K. Huen, K. Harley, S. Rose, A. Bradman, B. Eskenazi, N. Holland.* Center for Children's Environmental Health, School of Public Health, University of California, Berkeley, CA, USA.

Paraoxonase 1 (PON1) is a high density lipoprotein (HDL) - associated enzyme, which can inactivate the toxic oxon derivative of organophosphate (OP) pesticides. Additionally, it can prevent oxidation of LDL. PON1 genotype frequencies vary widely between ethnic populations. Over 250 SNPs have been previously identified in the PON1 gene (mostly in Caucasians), yet studies of PON1 genetic variation and effects on PON1 phenotypes focused primarily on promoter SNPs (-108,-909 and -162) and coding SNPs at positions 192 and 55. Previously, we sequenced the PON1 gene in Mexican-American subjects (60 chromosomes) and identified over 99 genetic polymorphisms with MAF > 5%, including several novel variants (7 SNPs, 3 insertions, and 8 deletions). We genotyped the newly identified polymorphisms in addition to 37 SNPs across the PON1 gene (chosen by Tagger) and measured PON1 phenotype using three substrate-specific assays (arylesterase, chlorpyrifos-oxonase, and paraoxonase) in 339 children and 361 mothers from the Mexican-American birth cohort CHAMACOS. Single marker association tests adjusting for multiple testing (FDR) were performed in PLINK to determine the functional significance of the PON1 variants. We also tested for haplotype-based associations employing a sliding window approach. Twelve polymorphisms were significantly associated with arylesterase activity in both mothers and seven-year old children (FDR adjusted p-value <0.05). Of those, two were newly identified deletions (located in the five prime UTR region and intron 7) that were not in strong LD with the well known promoter SNP at position -108. Over 30 polymorphisms were significantly associated with POase activity, however only 10 were in weak LD with both PON1-108 and PON1192. We identified several novel PON1 genetic variants that affect enzyme activity and thus may impact its ability to protect against OP pesticides and oxidative stress. This is particularly important for minority populations with high pesticide exposures.

## 2904/F/Poster Board #836

**Optimal Algorithm For Haplotype Assembly From Whole-Genome Sequence Data.** *D. He, E. Eskin.* Univ. of California, Los Angeles, Los Angeles, CA.

Haplotypes (the sequence of alleles present on a single chromosome) in human populations contain the complete information for DNA variation in an individual. Many studies has shown that haplotypes are very useful for whole-genome association studies and for inferring human evolutionary history. In this study, we examine how haplotypic information can be explained using DNA sequence fragments. Current sequencing technology is cost-effective enough to generate large number of DNA reads. The problem of "haplotype assembly" is a challenging problem of assembling the two haplotypes for a chromosome given the collection of such fragments, or reads. Errors in reads significantly increase the difficulty of the problem and it's been shown that the problem is NP-hard even for reads of size 2. Existing greedy and stochastic algorithms are not guaranteed to find the optimal solutions for the haplotype assembly problem. In this paper, we proposed a dynamic programming algorithm, which is able to assemble the haplotypes optimally with time complexity O(m*2^k*n), where m is the number of reads, k is the length of the longest read and n is the total number of SNPs in the haplotypes. Our experiments show that the optimal solution makes on average a 1.1% improvement over the current best-known solution.

## 2905/F/Poster Board #837

**Using next generation sequencing technologies to identify variants in the neuroligin pathway associated with autism spectrum disorder.** *K.M. Steinberg[1, 2], M. Zwick[1].* 1) Dept Human Gen, Emory Univ, Atlanta, GA; 2) Graduate Program in Population Biology, Ecology and Evolution, Emory Univ. Atlanta, GA.

Neuroligins are post-synaptic cell adhesion molecules that bind to neurexins in the pre-synaptic density. Recent studies demonstrate that mutations in the X linked genes neuroligin 3 (NLGN3) and 4X (NLGN4X) and the autosomal gene neurexin 1beta (NRXN1β) contribute to autism spectrum disorder (ASD). However other studies failed to find these associations in individuals with ASD. To comprehensively survey the common and rare variation in these regions, we are resequencing NLGN3, NLGN4X and NRXN1β in individuals from families with two or more affected male sibpairs from the Autism Genetic Resource Exchange (AGRE) using two different next generation sequencing platforms. Affected male sibpairs were chosen based upon sharing identical markers near NLGN4X (DXS9895 and 9902) with each other as well as with their mother. One male from each sibpair was selected for resequencing; data from these affected males will be presented. In the first part of our study we examined 21 affected males and 20 controls (the affected males' fathers). Target DNA was isolated using Microarray-based Genomic Selection and then hybridized to custom resequencing arrays containing all unique coding and non-coding DNA from the three genes. SNPs identified in our study are partitioned into functional classes (UTR, silent, replacement, intron, intergenic) and compared within and between these classes. We identified and validated 3 exonic variants: 2 synonymous and 1 non-synonymous; however, the non-synonymous variant was identified in a control. Validation of variants in other functional classes is ongoing. In the second part of the study we are examining 152 affected males. Target DNA was isolated using long range PCR and then sequenced using the Illumina Genome Analyzer. All variants will be examined for evolutionary conservation using the phastCon program, severity of functional mutations using the Panther program and presence or absence from dbSNP. We are currently in the process of validating variants as well as identifying the presence and/or absence of these mutations in control males.

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## 2906/F/Poster Board #838

**Discovery and analysis of short sequence variants from population resequencing of two genes in the human genome.** *V. Bansal[1], O. Harismendy[1], S.S. Murray[1], N.J. Schork[1], J. Deleuze[2], E. Topol[1], K.A. Frazer[1].* 1) Scripps Genomic Medicine - Scripps Translational Science Institute - The Scripps Research Institute, 3344 N. Torrey Pines Court, La Jolla, CA 92037; 2) Sanofi Aventis, France.

Resequencing of candidate genomic loci in large populations represents a promising approach for the discovery of low frequency DNA variants that are unlikely to be captured in genome-wide association studies. Using the Illumina GA sequencing platform, we have resequenced 200 kilobases from two genes in a sample of 289 individuals of European Ancestry (146 individuals with Body Mass Index (BMI) below 30 and 143 individuals with BMI above 40). These two genes, Fatty acid amide hydrolase (FAAH) and monoglyceride lipase (MGLL) are important genes in the endocannabinoid pathway and a polymorphism in the FAAH gene has previously been associated with drug abuse in a population of European ancestry. Using the short read analysis package MAQ, we identified 1451 single nucleotide variants in our population. We leveraged the sequence data across all individuals to filter out false variants, resulting in ~ 1350 SNVs. These include more than 800 SNVs that have a minor allele frequency below 1 percent. We evaluated the accuracy of sequencing based genotype calls by sequencing individual samples multiple times and estimate an error rate of ~ 7% for genotype calling. Comparison of sequencing based genotype calls to genotype calls using the Sequenom platform showed that the sequencing derived genotype calls are robust to the presence of neighboring variants. We also developed a pipeline to detect short insertions/deletions using paired-end mapping and de novo sequence assembly of the short reads. Using this strategy, we identified a set of 143 reliable indel variants in our population. Finally, case control association analysis identified a set of rare single nucleotide variants with moderate association with BMI (p-value < 0.01). Accordingly, population sequencing appears to be useful for identifying rare sequence variants associated with morbid obesity.

## 2907/F/Poster Board #839

**Targeted Enrichment Of Genomic Loci Using Custom Microarrays For Massively Parallel Sequencing.** *J.J. Corneveaux[1,2], H.J Friedman[1,2], A.N. Allen[1,2], S. Szelinger[1], D.W. Craig[1,2], J.V. Pearson[1,2], A. Caruso[3], A. Keller[4], A. Borries[4], J.T. Leonard[3], M.J. Huentelman[1,2].* 1) The Translational Genomics Research Institute (TGen), Neurogenomics Division, 445 N. 5th Street, Phoenix, Arizona 85004, USA; 2) Arizona Alzheimer's Consortium, 901 E. Willetta Street, Phoenix AZ 85006; 3) febit inc., 99 Hayden Avenue, Lexington MA, 02421 USA; 4) febit biomed GmbH, Im Neuenheimer Feld 519, D-69120 Heidelberg, Germany.

Massively parallel sequencing technologies have ushered in a new era of genomic research including whole genome and linkage/association peak resequencing. When coupled with "DNA bar-coding" strategies, this technology can sequence tens to hundreds of individuals across several hundred kilobases in a single sequencing run. A key milestone in achieving these multiplexed assays is the selective enrichment of the regions of interest from the genome. Long range PCR has shown to be an effective means of achieving enrichment, however this approach is often challenging to optimize and, depending on the number of individuals to be sequenced and the number of regions investigated, is not always time or cost effective. In this light, we have collaborated with febit (Heidelberg, Germany) to generate custom DNA microarrays to capture regions of interest from the genome for downstream sequencing. Using the febit Geniom One instrument to create custom capture biochips, we can synthesize microarrays with >120,000 probes overnight and selective hybridization of genomic DNA can be achieved in as little as 24 hours. The sequencing library is prepared using standard Illumina protocols prior to hybridization-capture; therefore, we can elute from the biochip, perform standard linear PCR and load directly onto the Genome Analyzer instrument for sequencing.

Using a single febit biochip and a single Illumina Genome Analyzer II flow cell, we performed a pilot study to capture genomic DNA from 7 HapMap individuals across two contiguous genetic loci on different chromosomes; one 210Kb in size (*WWC1*) and one 20Kb in size (*TP53*). An average of 8.8 million ±3.7 million paired-end reads were generated per flow cell lane following quality control for a total of 72 bases sequenced per cluster. We show across our target regions an average of 327X ±139X coverage per individual with 95.1% ±8.3% of target bases with 1X or greater coverage. 46.5% ±6.2% of total quality filtered reads align to the target loci. We further show that the majority of off-target reads map to genomic repeat regions. Our results demonstrate the utility of such an approach for target loci enrichment and we show the theoretical potential to multiplex 10 or more samples per lane on a flow cell while still achieving 20X or greater coverage per sample across a target of 230Kb. Using these data as a benchmark, we provide some estimates for calculating depth of coverage for a variety of target sizes.

## 2908/F/Poster Board #840

**Molecular barcoding schema for medical and metagenomic SOLiD sequencing applications.** *P. Gilles[1], J. Kilzer[1], J. Bodeau[2], H. Breu[2], T. Sokolsky[3], J. Johnson[1], A. Harris[1], R. Bennett[1].* 1) Genetic Systems, Life Technologies, Carlsbad, CA; 2) Genetic Systems, Life Technologies, Foster City, CA; 3) Genetic Systems, Life Technologies, Beverly, MA.

As the SOLiD sequencing platform rapidly increases its throughput to the 250GB/run range - it is essential to efficiently utilize this expanding capacity for targeted sequencing of decomplexed human samples and sequencing of a multitude of species and strains of microorganisms. To this end, a specialized set of 10bp - 96 color-space-balanced barcodes have been developed that enable clear discrimination between samples for maximum sequencing accuracy and are designed for use as a "nested" set that allows 20-plex sequencing with 5 base barcode reads and 96-plex runs with 10 base sequence reads. Importantly, validation runs have demonstrated color balance by Satay plots of sequenced DNA fragment libraries and multiple sequence tests are experimentally designed for sensitive detection of complete sequence coverage and barcode sequence accuracy. Furthermore pilot early access developments have been tailored for combined and routine use of decomplexing systems such as the Agilent SureSelect target enrichment system on the SOLiD sequencing platform. The 96-plex barcoding system for the generation of DNA fragment libraries described herein provides validated color-balanced barcodes and primers which enable obtaining optimal quality, efficiency and cost effectiveness for enhanced depth and breadth of medical resequencing and metagenomic applications.

## 2909/F/Poster Board #841

**Massive parallel sequencing of ataxia genes after array-based enrichment.** *A. Hoischen[1], C. Gilissen[1], W. van der Vliet[1], P. Arts[1], N. Wieskamp[1], S. Vermeer[1], R. Meijer[1], M. Buckley[1], B. Kremer[2], N. van Slobbe-Knoers[1], J. Veltman[1], H. Scheffer[1].* 1) Department of Human Genetics, UMCN St. Radboud, Nijmegen, Netherlands; 2) Department of Neurology, UMCN St. Radboud, Nijmegen, Netherlands.

Targeting multiple disease genes by massively parallel sequencing has tremendous diagnostic potential but requires new 'front-end' methods to enrich templates to be sequenced. Here we validated the array-based sequence capture method for medical sequencing approaches in heterogeneous genetic disorders. As a model disease we chose autosomal recessive ataxia and selected 7 patients with known mutations in ataxia genes. Genomic sequences of all known disease genes, including all intronic sequences and 5kb up- and downstream of each gene, as well as a novel ataxia candidate locus were represented on a single oligonucleotide array, comprising 2.5 Mb of genomic sequence in total. After enrichment each of the patients DNA was analyzed by one quarter of a Roche GS FLX Titanium sequencing run, resulting in up to 100 Mb sequence data per patient. This was sufficient to reach an average per base coverage of 25-fold in all targeted regions. Enrichment showed high specificity, as on average 85% of all reads mapped uniquely to the targeted regions. Very few single reads mapped to non-targeted regions. Importantly, this approach enabled an unbiased detection of known mutations in all 7 patients, deletions and point mutations, as well as known SNP variants (hetero- and homozygous). These results show that massive parallel sequencing of enriched samples enables tailor-made genetic diagnosis of heterogeneous disorders.

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## 2910/F/Poster Board #842

**Comprehensive identification of SNPs, indels and structural variants by sequence capture and deep re-sequencing of large genomic regions in pooled DNA samples.** *K.A Hunt[1], L. Franke[1,2], G.A.R. Heap[1], J.H.M. Yang[3], N. Bockett[1], V. Mistry[1], C.A. Mein[4], R.J. Dobson[4], Z. Albertyn[5, 6], C. Chelala[7], C. Hercus[6], D.A. Van Heel[1].* 1) Gastroenterology, Inst Cell/ Molec Sci, Barts and the London, London, United Kingdom; 2) Department of Genetics, University Medical Centre Groningen, 9700 RB Groningen, the Netherlands; 3) Diabetes and Inflammation Laboratory, Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, Addenbrooke's Hospital, Cambridge, United Kingdom; 4) The Genome Centre, Barts and The London School of Medicine and Dentistry, Charterhouse Square, London, United Kingdom; 5) Centre for Comparative Genomics, Murdoch University, Perth, Australia; 6) Novocraft Technologies Sdn Bhd, Kuala Lumpur, Malaysia; 7) Institute of Cancer & CR-UK Clinical Centre, Barts and The London School of Medicine and Dentistry, Charterhouse Square, London, United Kingdom.

Comprehensive definition of regional genetic variation in a large sample collection is essential for pharmacogenetics; deep exon resequencing for coding mutations; and fine mapping after genome wide association studies (GWAS). We assessed three genomic regions (total 1.26Mb, selected to include RGS1, IL12A, IL2, IL21 from a recent GWAS), using 10 pools of 8 human DNA samples, enriched each pool using Nimblegen sequence capture microarrays, and high-throughput Illumina sequenced DNA fragments. We developed gapped alignment (Novoalign) and variant calling algorithms, to identify: 3457 SNPs (45.8% previously known); 472 small (≤7bp) insertion-deletion variants (6.6% known); and 10 larger structural variants including a common 980bp deletion between IL2 and IL21. Excellent correlation between known genotypes in each sample and sequence called genotypes in each pool was observed. A 5.0% per-SNP false negative rate was determined using 121 genotyped HapMap SNPs. A 6.5% per-SNP false positive rate was determined using dideoxy capillary resequencing of 46 randomly selected SNPs. A subset of insertion-deletion and structural variants was validated using a second method. DNA pooling is cost-effective for both sequence capture and sequencing stages, without compromise in accuracy. We show common and rare variants of multiple types (not just SNPs) can be defined in large sample numbers from targeted genomic regions.

## 2911/F/Poster Board #843

**Multiple sample pooling and next-generation sequencing to determine *RET* sequence variation within a population.** *R.L. Margraf[1], J. Durtschi[1], S. Dames[1], D.C. Pattison[1], J. Stephens[1], R. Mao[1,2], K. Voelkerding[1,2].* 1) ARUP Institute for Clinical & Experimental Pathology®, Salt Lake City, UT; 2) Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT.

Background: The determination of sequence variation within a genetic diagnostic locus is critical for molecular assay design and clinical test interpretation. Next generation sequencing (NGS) offers a new approach for characterizing sequence variation within a population by cost-effectively pooling multiple samples before re-sequencing. Using the *RET* proto-oncogene as a model; we examined multi-sample pooling coupled with NGS to determine gene sequence variation within a population. Methods: We used 10 de-identified human DNA samples with known sequence variations determined previously by Sanger sequencing *RET* exons 10, 11, and 13-16, since sequence variation within these exons can be causative of Multiple Endocrine Neoplasia type 2. Long range PCR was used to amplify the 8.8 kb fragment spanning intron 9 to intron 16. Amplicons were equimolar pooled, fragmented by sonication, and sequenced on a single flow cell lane of the Illumina Genome Analyzer to generate 36 base length reads. Alignment and data analysis employed DNAStar's SeqMan and NGen programs. Results: Although the pooled data set averaged 18,000 read coverage, there were four areas of lower coverage (<6000); two areas were immediately 36 base pairs from amplicon ends and the two internal areas of low coverage had high GC content (yet averaged approximately 250 reads per allele). Each of the 10 samples in the pool had at least one unique single nucleotide variation, each with a singleton allele frequency of 5% (1/20 alleles). Using ≥20 for the phred-like quality score criterion, all 12 unique *RET* variants in the pool were detected with an average read frequency of 4.8%. Two samples each had two unique variants; one sample had two variants immediately adjacent on the same allele (GC>TG) with frequencies of 4.4% and 4.3%, while the other sample had variants in exon 13 and exon 14 with frequencies of 4.3 and 4.6%, respectively. These read frequencies per singleton alleles were well above the average random nucleotide error of 0.09 +/- 0.14%. Conclusions: Known sequence variations in the *RET* proto-oncogene were accurately detected near expected allele frequencies of 5% in a pool of 10 samples. Based on allele frequencies, random error, and the areas of low read coverage; we predict 20 to 30 samples could be pooled to reliably detect singleton alleles by NGS. We will continue to investigate improvements in sample pooling technique, Illumina library preparation and sequencing, and data analysis.

## 2912/F/Poster Board #844

**Genome-wide survey of common variants in pre-microRNA regions in humans.** *L.M. Reynolds[1], S.H. Wu[3], M.T. McManus[2], W.C. Hsueh[3].* 1) Dept of Biopharmaceutical Sciences, UCSF, San Francisco, CA; 2) Dept of Microbiology and Immunology, UCSF, San Francisco, CA; 3) Depts of Medicine and Epidemiology & Biostatistics, UCSF, San Francisco, CA.

MicroRNAs (miRNAs) are a novel class of genes that regulate gene expression by base pairing with target mRNAs and affecting translation. Since 30% or more of human genes might be regulated by miRNAs, their involvement in human diseases could be quite common. Therefore, we set out to identify common variants (MAF ≥ 5%) in all human pre-miRNA genes, in order to evaluate their association with complex traits in humans.

We identified 461 validated and 747 predicted pre-miRNAs (candidates) from the UCSC Genome Browser (as of June 2006). Primers could be designed for 1,071 regions, and 893 were successfully sequenced using the Sanger method, in 24 CEU samples used by the HapMap project. Of these 893 sequenced regions, 342 are validated pre-miRNAs (50% reported in miRBase 11, 2009). The variant discovery results are summarized in the table below. In conclusion, we identified many novel common variants in pre-miRNA regions to be included in future miRNA association studies. Furthermore, as our sequencing study was carried out using HapMap CEU samples, our findings can be readily used by all other scientists. *P<0.001 compared to (Pre-miRNA - miRNA)

| Region | n | Mean size (range) | # snp (% novel) | snp freq/kb | # Indel (%novel) | Indel freq/kb |
|---|---|---|---|---|---|---|
| Nonpre-miRNA | 876 | 458 (411-511) | 497 (24%) | 1.26 | 32 (72%) | 0.08 |
| Pre-miRNA - miRNA | 342 | 67 (39-114) | 19 (26%) | 0.86 | 1 (100%) | 0.04 |
| miRNA | 392 | 22 (18-27) | 5 (40%) | 0.58* | 0 | 0 |
| Candi-date | 551 | 88 (58-137) | 36 (22%) | 0.74 | 5 (80%) | 0.10 |

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## 2913/F/Poster Board #845

**Reliable recovery of mutations and CNVs (incl. all known) using array-based sequence capture of 112 MR-genes.** *R. Almomani[1], J. van der Heijden[1], Y. Ariyurek[2], J. den Dunnen[1], M.H. Breuning[1].* 1) Center for Human and Clinical Genetics, Dept. of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands; 2) Leiden Genome Technology Center LUMC, The Netherlands.

Although sequencing of a human genome gradually becomes an option, cost remains a prohibitive factor and zooming in on the region of interest is attractive. We tested array-based sequence capture prior to massive parallel sequencing to select genes potentially involved in mental retardation (MR) and congenital malformation. Fragmented, patient-specific tagged sequence-ready DNA was hybridized to custom 385K long oligonucleotide NimbleGen arrays to enrich the protein-coding and immediate intron flanking sequences of 112 genes. After hybridization, washing, elution and PCR-enrichment, captured DNA was sequenced using Illumina's technology. Arrays were successfully re-used and hybridized with pooled samples. For data analysis we have built a pipeline automatically reporting sequence variants, checking their presence in known databases (e.g. dbSNP) and predicting the potential consequences at the level of RNA splicing and protein translation. All known variants were reliably detected, incl. the pathogenic variants from control cases and SNPs derived from array experiments. Of the new variants, 24 exonic and 93 intronic, nearly all were confirmed using Sanger sequencing; analysis of the potential pathogenic consequences is ongoing. Although overall coverage was highly variable, it was reproducible per region and facilitating the detection of large deletions and duplications (CNVs), incl. a partial deletion in the B3GALTL gene from a control sample. To improve results further, and to get a more even coverage over the targeted region, future arrays should contain probes from both DNA strands and fewer probes from densely and more probes from sparsely covered regions.

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## 2914/F/Poster Board #846

**Population-Based Detection of Rare, Surfactant Associated Gene Variants Using Pooled Sample Next Generation Sequencing.** *H. Heins[1], D. Wegner[1], S. Robison[1], T. Druley[1,2], R. Mitra[2], F. Vallania[2], A. Hamvas[1], F.S. Cole[1]*. 1) Pediatrics, Washington Univ, St. Louis, MO; 2) Genetics, Washington Univ, St. Louis, MO.

**Background:** Rare mutations in the genes encoding surfactant protein C (*SFTPC*) and ATP-binding cassette family submember A3 (*ABCA3*) cause respiratory disease in infants and children. Previous technologies have prevented cost-effective estimation of the population attributable fraction of childhood respiratory disease associated with these mutations. **Objective:** To use pooled sample next generation sequencing to determine the population-based frequency of genetic variants in *SFTPC* and *ABCA3*. **Methods:** Equal amounts of DNA extracted from 1110 Guthrie cards from the Missouri newborn screening program were combined into four pools: one African American (AA) pool (n=197) and three European American (EA) pools (n=304, n=304, n=305). PCR products from each pool spanning the entire *SFTPC* (6.2 kb) and 32 exons (13.8 kb) of *ABCA3* were normalized, pooled, and sequenced using an Illumina Genome Analyzer. SNP detection was performed using an in-house software program (Splinter). Functional analyses of non-synonymous SNPs were performed with SIFT and PolyPhen. **Results:** In *SFTPC*, we found 24 novel SNPs out of a total of 67 (44 promoter, 2 splice site, 2 non-synonymous, 1 synonymous, 18 intronic). Both non-synonymous SNPs were common and predicted to be nonfunctional. In *ABCA3*, we found 69 novel SNPs out of a total of 106 in the AA cohort (6 splice site, 11 synonymous, 14 non-synonymous, and 75 intronic). Of the non-synonymous SNPs, 4 were previously found in infants with surfactant deficiency or predicted to be functionally disruptive by both programs. In the EA cohort, we found 33 novel *ABCA3* variants out of a total of 66 variants (9 splice site, 6 synonymous, 15 non-synonymous, and 36 intronic). Of the non-synonymous SNPs, 7 were previously found in infants with surfactant deficiency or were predicted to be functionally disruptive by both programs. Four of these functionally disruptive SNPs were identified in more than one individual, and four were novel. The population-based frequencies of functionally disruptive mutations were 2.5% (5/197 in AA) and 1.9% (17/913 in EA). **Conclusions:** Functionally disruptive mutations in *SFTPC* are very rare, but the overall frequency of disruptive mutations in *ABCA3* (2%) suggests that the prevalence of respiratory disease attributable to these mutations is significant. This technology will allow the identification of the population-based frequency of genetic variants in other surfactant-related genes.

## 2915/F/Poster Board #847

**WHOLE EXOME RESEQUENCING IN A MULTIGENERATIONAL PEDIGREE WITH HEREDITARY SPASTIC PARAPLEGIA.** *S. Züchner[1], J. Huang[1], C. Almonte[1], E. Powell[1], S. Young[2], D. Burges[3], M. Schmidt[1], B. Boese[3], E. Szekeres[3], X. Zhang[3], M. Pericak-Vance[1], E. Rampersaud[1], E. Martin[1], T. Harkin[3], D. Hedges[1]*. 1) Miami Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) Center for Computational Sciences, University of Miami Miller School of Medicine, Miami, FL; 3) Roche Inc.

Classic approaches to gene mapping in Mendelian disease require the availability of large informative pedigrees. We are studying a relatively small autosomal-dominant family with five affecteds suffering from Hereditary Spastic Paraplegia with the aim to identify the causative gene using microarray-based whole exome capture followed by next-generation sequencing. In eight individuals we were able to cover up to 98% of the 180,000 targets at a read depth of >= 3, 86% at a read depth of >=10, and over than 50% of all targets with read depth of >=20. In average we identified 8500 variants per individual, with approximately 450 of these being novel. To further reduce the number of potentially significant novel variants, we applied a novel genomic convergence strategy using linkage exclusion mapping. Currently, we are confirming the <50 remaining novel variants in unrelated cases and controls to ultimately identify the underlying genetic cause. By comparing the variant calls derived from sequencing with 1M Illumina genotyping data we estimated a false negative error rate of <4%. The three-generational pedigree structure will allow us to further explore false-positive error rates and de-novo changes per meiosis. The results enabled us to develop realistic strategies for the efficient application of whole exome capture-arrays. This is of particular interest for genetic studies of diseases with high phenotypic penetrance but also rare variant studies of complex diseases.

## 2916/F/Poster Board #848

**Approaches for targeted sequencing of genes associated with healthy aging.** *E.F. Kirkness[1], T. McIntosh[1], S. Scherbakova[1], K. Beeson[1], P.C. Ng[1], R.L. Strausberg[1], S.S. Murray[2], N.J. Schork[2], E.J. Topol[2], S. Levy[1]*. 1) The J. Craig Venter Institute, Rockville, MD; 2) Scripps Genomic Medicine, The Scripps Research Institute, La Jolla, CA.

We have initiated a project to sequence human cohorts, targeting 81 genes potentially associated with healthy aging. Two approaches have been assessed for optimal detection of sequence variants in coding exons. The first employs RainDance technology to assemble a 1,526-plex PCR reaction in microdroplets. After a single PCR reaction, the 1,526 amplicons (mean length 370 bp) were sequenced via the Roche-454 platform. The amplicon pools from different individuals were barcoded to enable sequencing of multiple samples in a single sequencing run. In order to test the completeness and accuracy of the methodologies, we initially targeted a DNA sample that had been sequenced previously (HuRef). The analysis of HuRef DNA revealed that 93% of the targeted bases (exons and flanking intronic sequence totaling 563 kb) were covered to a depth of more than 10x after sequencing of the pooled products to an average depth of 148x. Two distinct approaches identified 90% of the 211 SNPs originally identified in the targeted regions of the 81 genes via Sanger sequencing. The remaining loci were either located in regions of high GC content, and had insufficient sequence coverage for calling SNPs, or were located in homopolymeric stretches that were incorrectly aligned, or had no alternate allele. Notably, the amplicon sequence data revealed an additional 23 SNPs that had not been detected by previous analysis of the HuRef genome assembly. A majority of these were confirmed as SNPs in dbSNP. In order to assess the utility of WGA-amplified DNA, we performed RainDance amplification and Roche-454 sequencing on two WGA-amplified case samples. After sequencing to an average depth of 68x and 25x, only 5.6% and 6.5% of the target bases remained unsequenced, and at least 80% of the target bases were covered by 30% of the average coverage. Sequence analysis revealed 192 and 183 SNPs respectively. The second capture approach employed solution hybridization of probe baits (Agilent SureSelect). Sample DNA was sheared and adapters for downstream sequencing were ligated before hybridization. The captured DNA was then sequenced using the AB SOLiD platform. Targeted DNA was sequenced to an average mapped depth of 200x, with 80% of the target region covered at 20% of the normalized coverage. Finally, we have compared the ability to detect variants via targeted capture versus whole genome sequencing (Sanger and SOLiD), and compare the biases inherent in each approach.

## 2917/F/Poster Board #849

**Whole-Genome SNP Detection and Haplotype Phasing with Mate Pairs via Ligation-Based Dibase Sequencing.** *S. McLaughlin[1], H. Peckham[1], S. Ranade[2], C. Lee[1], C. Clouser[1], J. Manning[1], C. Hendrickson[1], L. Zhang[1], E. Dimalanta[1], T. Sokolsky[1], J. Ichikawa[1], J. Warner[1], M. Laptewicz[1], B. Coleman[1], F. Hyland[2], F. De La Vega[2], A. Blanchard[1], J. Malek[3], G. Costa[1], K. McKernan[1]*. 1) Applied Biosystems, Beverly, MA; 2) Applied Biosystems, Foster City CA; 3) Weill Cornell Medical College in Qatar, Doha, Qatar.

The HapMap project along with next-generation sequencing technologies provides unprecedented opportunities to fully characterize whole-genome polymorphism events comprising many individuals across multiple populations. Genetic variants such as single-nucleotide-polymorphisms (SNPs), small indels, large-scale indel events on the order of several kibobases, genomic rearrangements such as inversions and translocations, and even full-scale de novo sequencing can be characterized rapidly and at per-base cost orders of magnitude less than the original Human Genome Project. We sequenced a Yoruba HapMap sample, NA18507, using mate-pair libraries (14.9x) with various insert sizes (600bp-3.5kb) as well as several fragment libraries (3x). SNP detection was performed via a heuristic approach which considers the number of reads per allele as well as a weighted score for each base call. We found 2.33M heterozygous SNPs and 1.53M homozygous SNPs with an overall dbSNP concordance of 81% (dbSNP v129). We evaluated mate pairs which cover 6.18M distinct potential genotypes (our whole genome SNP calls and HapMap genotypes which include reference-allele homozygous loci) and we observed 4.03M potential genotypes covered by 6.77M pairs. Nearly 2/3 of the genotypes are covered by at least 1 mate pair read that is in phase with another genotyped location and 43% that we detect as heterozygous are in phase with another that we also detect as heterozygous. We compared annotated heterozygous HapMap phases to these data and found they are in 98.95% agreement. We also attempted to resolve phases for our 634,568 novel heterozygous SNP calls by interrogating mate pairs pairing to both a novel heterozygous and a HapMap phased heterozygous and found that 76,300 (8.32%) of the novel heterozygous SNPs paired to a HapMap-phased heterozygote. For the 15,946 of these SNPs that have both alleles paired to a HapMap phased heterozygous, the two alleles are in opposite phase as expected 99.52% of the time. The average size of these haplotype blocks is 1.6Kb with blocks extending to as long as 215Kb using multiple mate pairs which overlap with multiple heterozygous loci. We have demonstrated that SOLiD mate-pair data is highly amenable to resolving haplotype phases at single-base pair resolution.

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### 2918/F/Poster Board #850

**Assembly of *E. coli* using Single Molecule Sequencing™ Paired Reads.** *J. Reifenberger, C. Hart, D. Lipson, K. Steinmann, T. Raz, K. Kerouac, J. Kepler, J. Lessard, P. Wellman, S. Letovsky, J. Thompson, P. Milos.* Helicos Biosci Corp, Cambridge, MA.

Single molecule DNA sequencing provides a novel method for interrogating DNA molecules with minimal manipulation of the sample. However the short reads, typically 25 - 50 bases in length, are difficult to fully assemble into a genome. Paired reads can provide additional linkage information to aid in de novo assembly and we have developed a paired reads strategy using the Helicos Single Molecule Sequencing (tSMS)™ platform. Sample preparation for a paired reads experiment is just as it is for single pass sequencing experiments and includes only shearing and terminal tailing of template DNA molecules. To create paired reads, DNA strands are attached to a surface and sequenced-by-synthesis for a known number of cycles. A spacer is then added to the DNA strands in a controlled manner by alternating between the addition of a trio of natural dCTP, dTTP, dATP (i.e. non-fluorescing nucleotides) and dTTP, dATP, dGTP to the individual strands on the surface. The length of the spacer is sequence dependent and aids in the eventual assembly of the reads. Once the spacer has been added, sequencing by synthesis continues for as many cycles as desired. The simple sample preparation, which does not require ligation of primers or any PCR amplification, results in the pairs being free of the artifacts and biases that complicated sample preparations can introduce. In this work, we demonstrate the viability of the technique and our ability to tightly control the length of the spacer between the two reads on an individual DNA molecule. Using our paired read strategy we have produced data that has allowed us to assemble *E. coli* using single molecule paired read data. We present assembly results using third party tools, such as Velvet, and results rendered using a novel de Bruijn graph assembly strategy. We focus on extending de Bruijn assembly methods that can utilize two unique features of the Helicos paired read strategy including both variable read lengths and the additional sequence information that is contained within our spacer.

### 2919/F/Poster Board #851

**Analysis of human Dorsal Root Ganglion neurons.** *I. Belfer, K. Motoyama, M. Poddar, C. Lee, M. Gold.* Dept Anesthesiology, Univ Pittsburgh, Pittsburgh, PA.

Study: Evidence on molecular biological, biochemical and physiological properties of dorsal root ganglia (DRG) neurons, the neurons responsible for somatosensation, has been obtained through the study of lower species. Genetic studies of human pain require data on human DRG neurons properties. The purpose of the study was to determine the extent to which human tissue obtained from two sources within the University of Pittsburgh Medical Center could be used for genetic, biochemical and functional analysis. Methods: Tissue samples: Human DRG (L4-L5 bilaterally) were taken from regular autopsies and organ donors according to criteria provided by the Committee for Oversight of Research Involving the Dead (CORID). Of the four ganglia collected from each donor, one was fixed in paraformaldehyde for subsequently histological analysis, two were snap-frozen on dry ice and used for subsequent analysis of DNA, RNA and protein, and one was placed in ice-cold culture media and processed for subsequent functional analysis. This last procedure was only attempted with tissue obtained from organ donors. Results: Tissue was obtained from 10 organ donors and 35 autopsies. The maximum delay between cross clamp and the collection of donor tissue was 3 hrs with an average of 2hrs. Autopsy tissue was obtained on average 18-hour post-mortem (28 hour maximum and 5 hour minimum). As anticipated, based on the stability of DNA, the integrity of DNA was excellent in all samples. RNA was largely degraded in tissue obtained via autopsy with a post-mortem delay of 15 hour or more. RNA integrity was excellent in the organ donor samples. Western blot analysis indicated that even relatively low abundance, high molecular weight proteins such as NaV1.7 and NaV1.8 were detectable in tissue obtained via organ donation and autopsy. Finally, functional analysis of isolated DRG neurons obtained from organ donors was possible, with neurons surviving in culture for at least a week. Conclusion: 1) Quantitative analysis of mRNA expression levels is possible with tissue obtained from both autopsies and organ donors; 2) Preliminary analysis indicates that it will be possible to perform quantitative between subjects analyses of tissue obtained via organ donation and autopsy. Histological analysis will be particularly important for these experiments to confirm the protein of interest is in DRG neurons; 3) It is possible to obtain live DRG neurons from organ donors.

### 2920/F/Poster Board #852

**Linear whole genome amplification method produces templates suitable for quantitative diagnostic analyses.** *A. Brooks[1], M. Stapleton[2], I. Aranovich[1], P. Van Hummelen[1], S. Wang[2], N. Kurn[2].* 1) Rutgers Cell & DNA Repository, Rutgers Univ, Piscataway, NJ; 2) NuGEN Inc., San Carlos, CA.

DNA based diagnostics from limiting and poor quality samples continue to be a challenge for a variety of qualitative and quantitative tests. Traditional whole genome amplification (WGA) approaches have been demonstrated for a variety of DNA based applications; however, none of the current approaches amplify the DNA template in a sufficiently linear fashion to permit utilization of WGA products for clinical applications. This study describes the development and application of a linear whole genome amplification approach, whole genome-SPIA, for limiting amounts (<10ng) of genomic DNA to be used for both quantitative real time PCR (QPCR) and microarray based technologies as well as its potential use in next generation sequence analyses. We demonstrate the utility of this approach using a trisomy 21 model which is indicative of the sensitivity and linearity needed for subtle quantitative analysis. Whole-genome single primer isothermal amplification (whole-genome-SPIA) technology was used to generate linearly amplified DNA templates from 6 trisomy 21 and 6 normal fetal gDNA samples. Each amplification uses 10ng of gDNA template and yields an average of 10ug of amplified product with no positional or deletional bias. This amplification technology employs chimeric DNA-RNA primers for the random generation of substrates which are linearly amplified by the single primer isothermal amplification (SPIA). To test the reproducibility, linearity and fidelity of the amplified product both QPCR and QFPCR were performed for multiple chromosome 21 targets on all amplified samples as well as gDNA amplified with commercially available amplicon-based and multiple strand displacement WGA products. The results demonstrate that whole-genome-SPIA amplified products from all cases result in products suitable for quantitative analysis. QPCR analysis of all regions explored has demonstrated statistical segregation for all trisomy 21cases from controls in a ratio similar to unamplified templates. Additionally, QFPCR analysis has demonstrated that in all cases there is a statistical difference in peak height for all chromosome 21 markers and not for markers interrogating chromosomes 18 and 13. This data demonstrates the potential clinical utility of linear whole genome-SPIA amplification approach and the value of limiting DNA samples for genetic analyses where conservation of genomic loci (or genomic copy number) is essential.

### 2921/F/Poster Board #853

**Novel method to produce partial trisomy mice using a human artificial chromosome (HAC): an application to Down syndrome.** *J. Kudoh[1,2,3], K. Miyamoto[2], N. Suzuki[4], K. Sakai[2], S. Asakawa[2,5], T. Okazaki[4], N. Shimizu[3], M. Ikeno[2,4].* 1) Center for Bioinformatics, Keio University School of Medicine, Tokyo, Japan; 2) Department of Molecular Biology, Keio University School of Medicine, Tokyo, Japan; 3) Advanced Research Center for Genome Super Power, Keio University, Tsukuba, Japan; 4) Chromo Research Inc., Nagoya, Japan; 5) Laboratory of Aquatic Molecular Biology and Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan.

Down syndrome (DS), or trisomy 21, is caused by the inheritance of three instead of two copies of human chromosome 21 (HC21). Individuals with DS show characteristic faces, mental retardation, congenital heart defect, increasing risk of leukemia in postnatal periods and early onset type of Alzheimer disease in adulthood. It is difficult to identify critical gene(s) for each phenotype because the number of partial trisomy 21 is limited and the phenotype is highly variable even with full trisomy 21. Therefore mouse models have been used to study phenotype-genotype correlations in DS. Here we report the development of novel method to produce mice harboring an extra copy of gene(s) on a human artificial chromosome (HAC). HAC is a recently developed cloning vector for mammalian cells and animals. As HACs are maintained extrachromosomally, the genes carried on HACs are expected to express under the authentic regulation without insertional mutagenesis of cellular chromosomes. We developed a HAC vector to insert any target genes into the fixed site of a HAC by Cre/lox recombination. Bacterial artificial chromosome (BAC) clones containing the entire target gene(s) were selected and inserted into HAC vector maintained in mouse embryonic stem (ES) cells. From ES cells containing HAC, we produced mice harboring HAC that carries human chromosome 21 gene(s). Subsequent backcrossing with C57BL/6 showed that HAC is stably transmitted to progeny mice. These novel mouse model will be useful to identify a gene(s) responsible for a phenotype(s) of DS and analyze gene-dosage effects at the levels of transcriptome, proteome and metabolome.

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## 2922/F/Poster Board #854

**High-throughput DNA quality control: Allelic discrimination panel for determining sample contamination, gender and ethnicity in a biorepository setting.** *J. Tischfield[1], M. DiCola[2], Q. Wang[2], P. Van Hummelen[2], A. Brooks[1,2].* 1) Human Gen Inst, Life Sci Bldg, Rutgers Univ, Piscataway, NJ; 2) Environmental and Occupational Health Science Institute, Bionomics Research and Technology Center, RWJMS-UMDNJ, Piscataway, NJ.

The quality of genomic DNA (gDNA) is a core issue for biorepositories. Many different approaches have been used for both the quantitative and qualitative appraisal of gDNA but there is no standardization for the functional assessment of DNA quality in terms of sample identity and utility for present and future downstream applications. Most gDNA quality measurement assays provide no specific identifiers of samples. Rather, they assess the global quality of nucleic acids while yielding no information on subject gender and probable ethnicity or potential contamination with other samples, all of which are important metrics for any lab managing large numbers of samples in a repository setting. At present, most laboratories use gDNA for discovery applications that involve analysis via SNP genotyping or NextGen sequencing technologies, both of which have a PCR component in their workflow. We have implemented a rapid and cost effective means for assessing the quality of each gDNA in terms of its suitability for PCR, while capturing critical information concerning subject identity. We developed and validated a panel of 96 SNPs using the Fluidigm 96.96 dynamic array to determine gDNA subject gender and putative ethnicity as well as contamination during processing. The flexibility of the Fluidigm dynamic array allows for rapid quality control testing while retaining the relatively easy option to employ study specific supplementary panels that can be used to generate more in depth information on ethnicity (126 SNPs) or parentage (136 SNPs), as needed. There is complete correlation between the Fluidigm BioMark system and our standard QPCR validation. The correlation to SNP data from DNA microarray and capillary electrophoresis analysis is 100%. This quality control approach allows the Rutgers University Cell and DNA Repository to easily provide data for over 10,000 gDNA samples per month while reducing operating costs and generating essential information that may be used for sample utility assessment and subsequent study management. The 96 SNP database can also be used to validate GWAS data in the event of a discrepancy given that >90% of SNPs in this panel are represented on a variety of commercial array platforms. We describe the selection of SNPs for the quality control panel, standard operating procedures for sample processing and a quality assessment schema for the rapid processing and analysis of genotype data.

## 2923/F/Poster Board #855

**Diagnostic validation of an integrated DNA extraction platform for large volumes of blood.** *T. Janssens[1,2], I. Salden[1], E. Dequeker[1,2], G. Matthijs[1,2].* 1) Center for Human Genetics, University Hospital Leuven, Leuven, Belgium; 2) EuroGentest, Leuven, Belgium.

Reliable and fast DNA extraction is a prerequisite in molecular genetic testing. Automation of this process has some important benefits, including increased throughput, more consistent and reproducible processing and improved sample tracking. Several manufacturers have produced a plethora of platforms, with different extraction chemistries. However, the extraction of large blood volumes (>1 ml) is still a challenge, for which only a few systems are available. This EuroGentest project involved the validation of the Chemagic Magnetic Separation Module I extraction robot (Chemagen), integrated with a Perkin Elmer liquid handler robot. The extraction technology is based on the use of paramagnetic beads with a DNA binding coating. Using magnetic rods, these beads can be transferred from one DNA washing buffer to another when applying an electro-magnetic field. The aim of this validation was to thoroughly validate the system, through verifying the physico-chemical qualities of the extracted DNA. In addition, we wanted to compile instructions for the in-house validation, in order to avoid duplication of effort in other laboratories while warranting a uniform level of validation. Over 180 whole blood samples on EDTA (not older than two weeks) were extracted under different conditions. The DNA concentration was measured and the yield was determined. The 260/280nm ratio was calculated as a parameter for the quality of the DNA. All DNA samples were checked in a multiplex drop-out PCR. Furthermore, samples were run on agarose to detect degradation of the DNA. Stability of the DNA was evaluated by repeating the multiplex PCR and the degradation test on randomly selected samples, stored at different conditions, during several months. Finally, the performance of the DNA for downstream diagnostic tests was assessed. Our study has shown that the integrated Chemagen-Perkin Elmer DNA extraction platform is an easy-to-use, flexible system resulting in good quality DNA, high yield and satisfying performance for diagnostic purposes. A few minor adaptations had to be made to the protocol(s) to warrant optimal performance. We suggest that some of the most critical parameters are locally verified when a diagnostic laboratory implements the system under accreditation.

## 2924/F/Poster Board #856

**Association of Biomolecular Resource Facilities (ABRF): Advancing Human Genetics through Research, Communication, and Education.** *T. Hunter[1], M. Detweiler[2], G. Grills[3], K. Sol-Church[4].* 1) University of Vermont, Burlington, VT; 2) Roswell Park Cancer Institute, Buffalo, NY; 3) Cornell University, Ithaca, NY; 4) Center for Pediatric Research, NCC-Delaware.

Improvements in cutting edge and emerging technologies, such as the introduction of next generation sequencing to biomedical research, have dramatically changed the landscape of genetics and the way genetic research is performed. Today, genetic research is often a collaborative effort that is performed in association with shared resource biotechnology (core) laboratories. These cores represent centers of technological excellence that bridge discovery and translational research by integrating state-of-the-art molecular genetics and proteomics technologies into current research programs. The Association of Biomolecular Resource Facilities (ABRF) is an international society dedicated to advancing biotechnology laboratories through research, communication, and education. ABRF Research Groups conduct multi-institutional studies that focus on comparison and optimization of the state-of-the-arts biotechnologies and methodologies available to biomedical researchers and geneticists. An overview of the aims and results of ABRF Research Group (RG) studies will be presented to illustrate the challenges and opportunities of genetic technologies. For example, the results of an RG gene expression study, based on reverse transcription quantitative real time PCR, underscores the importance of sample quality, optimal priming strategies, qPCR assay location and data troubleshooting. An RG study on microRNA expression highlights the strengths and weaknesses of some of the most popular array platforms and compares data from conventional microarray platforms with Taqman Low Density Arrays (TLDA) and Illumina GAII next generation sequencing. An RG study on SNP discovery and validation provides performance comparison of two major next generation sequencing platforms (Illumina GAII and ABI SOLiD) and highlights the strengths and weaknesses of these platforms in dealing with allelic discrimination in a diploid organism. This presentation will provide a forum for discussion of cutting edge methodologies and approaches in molecular genetics and will illustrate how they can be used to facilitate human genetics research.

## 2925/F/Poster Board #857

**Single Tube Amplification of Large Sets of Genes.** *O. Ericsson[1, 2], M. Isaksson[1], H. Johansson[1], J. Stenberg[1], M. Nilsson[1].* 1) Molecular Diagnostics, Genetics and Pathology, Uppsala, Sweden; 2) Olink Genomics, Dag Hammarskölds väg 54A, Uppsala Sweden.

PCR has dominated the sample preparation for Sanger sequencing due to the advantages of 1) high specificity 2) excellent limit of detection and 3) a very simplistic reaction format. These traits have enabled analysis of highly homologous regions in the genome and sequencing of DNA from very scarce sources such as biobanks and ancient biological remnants. However, because the reaction is limited to single- or low-plex amplification, PCR is not suited for targeted re-sequencing using the new sequencing instruments. Instead, several new technologies have been developed in order to provide multiplex sample preparation of larger interesting genomic regions for the new sequencing instruments. It has proven difficult to solve the bottleneck of PCR without compromising one or several of the original advantages. The currently available solutions either lack the specificity and detection limit provided by enzymatic amplification or depend on expensive and complicated instrumentation and in some instances involve both. We present the Selector Technology, a solution phase protocol for amplification of thousands of target fragments using pools of oligonucleotides that in combination with enzymes enable amplification of target regions. Following restriction enzyme digestion of the target DNA and specific circularization of target fragments, directed by the probe library, circularized targets are amplified specifically. The protocol can be scaled up using a standard 96 or 384 well reaction format and is compatible with the Illuminja, SOLiD and 454 sequencing instruments. Each oligonucleotide can capture up to kbp regions, allowing analysis of redundant genomic elements, and repeats. The protocol enables amplification of as little as 100 ng starting material, which together with scalable single tube amplification provides a protocol suitable for parallel analysis scarce biobank samples. Sequencing data from presented probe designs, targeting exons using renewable probe pools, capturing 3000 and 1500 fragments respectively, show that in average 87% of amplification products align to amplified regions and 95% of regions are recovered. In average, first-pass designs returned targeted sequencing coverage for analysis for 84% of amplified regions of interest with a ten fold sequencing bias. The reproducibility of sequencing read distribution in targeted regions is very good ($R2 = 0.92$) providing reproducible coverage and target regions are enriched over 10.000 fold.

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## 2926/F/Poster Board #858

**Optimization of library preparation methods for next-generation sequencing.** *A.F. Scott, D.W. Mohr.* Genetic Resources Core Facility, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, MD.

Next-generation sequencing has great potential for medical resequencing and as a follow-up to examine candidate genes in regions identified by GWAS. Most next-gen DNA sequencing involves the creation of libraries of molecules with appropriate adapters that are captured, amplified in situ and used to create templates for sequencing. Several alternative protocols for library creation have been published or posted online. We have attempted to evaluate which procedures are key to successful library preparation for the Illumina Genome Analyzer so that the process can be routinely applied to large numbers of samples. Library preparation involves several steps which begin with DNA fragmentation, creating blunt ends, adding an overhanging 3' A, ligation of appropriate adapters, size selection and purification of ligated molecules, PCR amplification and removal of unincorporated primer. Major impediments to success include the inability to produce molecules of a defined size range, loss of DNA during the multiple purification and handling steps, and quantitation and validation of the libraries prior to sequencing. Modifications that we have evaluated include fragmentation using a Covaris AFA sonicator, alternate sources for enzymes and other reagents, a substitute agarose gel buffer and use of paramagnetic beads for PCR primer removal. We are also evaluating the Illumina multiplexing primers that allow library indexing as well as extended paired-end sequencing to maximize data generated per run. Lastly, we are examining the use of long-range PCR products for targeting genomic regions, esp. those not easily selected with solution or array-based capture technologies.

## 2927/F/Poster Board #859

**Evaluation of performance of gDNA from saliva collected with Oragene®DNA for the purpose of SNP and CNV analysis on the Affymetrix Genome-Wide Human SNP Array 6.0.** *R.M. Iwasiow[1], C. James[1], P. Hu[2], R.F. Wintle[2], S.W. Scherer[2].* 1) DNA Genotek Inc, Ottawa, Canada; 2) The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Canada.

Genome-wide association studies allow for rapid scanning of markers across entire genomes. The Affymetrix Genome-Wide Human SNP Array 6.0 includes more than 906,000 single nucleotide polymorphisms (SNPs) and more than 946,000 probes for detection of copy number variation (CNV). Such studies deliver most value when samples originate from a well defined population (i.e. Age group, Disease state, Socio-economic status). Often individuals of such groups are dispersed in the general population making sample collection challenging and expensive. OrageneDNA facilitates such collection by providing an alternative to blood collection, eliminating phlebotomy cost and complexity. OrageneDNA is a non-invasive, self-collection device intended for collection of large quantities of high-molecular weight genomic DNA (gDNA) from saliva. DNA is stabilized at room temperature for extended periods enabling sample collection and transport via regular mail and flexibility to process in batches. The current study evaluates the use of gDNA from saliva on the Affymetrix 6.0 Genechip. Saliva from the same four donors was collected using the OrageneDNA kit on three separate days and the gDNA extracted from each of these collections was analyzed. Performance of the gDNA from saliva was assessed by evaluating the SNP and CNV calls from each sample and the reproducibility of the data was determined by comparing the results from three separate collections from the same donor. We report QC call rates > 98% and concordance between samples from the same donor >99.8%. We also used the median of the absolute values of all pairwise difference (MAPD) to evaluate whether the chip/array produce data that is useful for copy number analysis and report MAPD <0.26. Integrated CNV results based on two algorithms (Birdseed, and Partek HMM) identified < 30 CNV per genome. The results indicate that genomic DNA isolated from saliva collected with OrageneDNA can be used as a source in the Affymetrix Genome-Wide Human SNP Array 6.0.

## 2928/F/Poster Board #860

**Successful Transformation of Cryopreserved B Cells by Epstein Barr Virus Ensures Efficient Development of a Renewable Resource for Genotype/Phenotype Studies.** *A. Ansbach[1], K. Reeves[1], K. Gwinn[2], J. Andrews[1], L. Gutmann[3], L. Mamounas[3], R. Zhang[3], C. Beiswanger[1], M. Keller[1], R. Corriveau[1].* 1) NINDS Repository, Coriell Institute for Medical Research, Camden, NJ 08103; 2) Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030; 3) NINDS/NIH, 6001 Executive Boulevard, Bethesda, MD 20892.

Biorepositories that establish lymphoblastoid cell lines (LCL) from whole blood usually do so by infecting freshly prepared mononuclear cells (MNC) with Epstein Barr virus (EBV) to obtain a transformed B cell line. The processing paradigm established in 2002 for the NINDS Repository, which supports discovery of genetic risk factors for complex neurological diseases, was for submitters to send two 8.5 ml ACD tubes of blood per unique individual. Both tubes were used to obtain MNC via density gradient-separation of peripheral blood. Fresh MNC from one tube were immediately transformed with Epstein Barr virus (EBV) to establish an LCL; MNC from the other tube were cryopreserved as a back-up sample. We previously reported that, on average, for samples collected in 8.5 ml ACD tubes with ≥ 4 mls blood volume and in transit ≤ 6 days since venupuncture, the 1st attempt success rate using fresh MNC is 94% (n=8,879). Here we report two new results. First, if the 1st attempt at transformation starting from freshly prepared MNC fails, the success rate for a second attempt using cryopreserved MNC is 75% (n=332). Second, when there is no attempt using fresh MNC, rather, the first attempt to establish an LCL uses cryopreserved MNC, the success rate is 92% (n=1,959), a result that is virtually indistinguishable from the success rate using fresh MNC. This finding has major significance for LCL-based repository science. Establishment of LCLs represent the best available practice for obtaining a renewable resource of biospecimens from a specific individual, yet establishing LCLs from fresh MNC is labor-intensive and time-inflexible. Our results demonstrate that it is not necessary to immediately establish an LCL upon receipt of the peripheral blood sample. Rather, this part of the process can be deferred until after phenotypic and preliminary laboratory data are complete and reviewed for quality control. The NINDS Repository now uses this cost-efficient demand-driven paradigm for sample processing. Two 8.5 ml ACD tubes of blood are received from an individual; one is used for extraction of DNA from blood for distribution via the public web-catalog (http://ccr.coriell.org/NINDS), and the other is used to prepare cryopreserved MNC for demand-driven LCL production.

## 2929/F/Poster Board #861

**Next generation genetic tests: from artisan genetic testing to uniform, streamlined, fully quality-assured and automatic processing of genetic tests using next generation sequencing.** *H. Cuppens[1], T. De Staercke[1], S. Chen[1], L. Vliegen[1], Y. Moreau[2].* 1) Center for Human Genetics, KULeuven, Leuven, Belgium; 2) ESAT, KULeuven, Leuven, Belgium.

Implementation of next generation sequencing in routine genetic testing for monogenetic diseases can be only achieved if robust multiplex amplifications are set up; if samples can be pooled in a cost-effective way; if quality-assurance is guaranteed; and if data can be processed automatically. With respect to pooling, in the established 1-step PCR protocol, primers carry an amplicon specific target region, a tag, and sequencing adapters. For sequencing N amplicons in x individuals, 2N*x primers are needed. We developed a 2-step PCR protocol, the first primers carry amplicon specific sequences and an intermediate adapter which is common in all primers, the second PCR only needs one pair of primers per sample that carry this intermediate adaptor sequence, a sample tag, and the sequencing adaptor. This 2-step protocol only needs 2N+2x primers. Moreover, the second pair of primers are not test-specific and can be used for any gene. For sequencing of a gene of 30 amplicons in 100 individuals, only 260 primers are needed in our 2-step PCR protocol compared to 6000 primers in the 1-step PCR protocol. A quality-assured system and automatic processing was realized by spiking samples with DNA molecules. A DNA library was generated using a 25 nucleotide long random tag, yielding $4^{25}$ different tag molecules. These molecular bar codes can be processed simultaneously with the DNA fragments under investigation. The test will be quality-assured from the moment that the sample is spiked. Spiking is currently performed in the blood sample when it arrives in the lab. The goal will be to collect a blood sample in a tube that already contains the molecular bar code. The only potential error that then still can be made is the moment at which the blood sample is taken. The test will be only valid when the correct sample tag is associated with the molecular bar code at the end of process. Not only can sequencing reveal a mutation, it will also identify the sample carrying the mutation. This allows automatic generation of a report, thereby excluding potential errors compared to reports generated by human intervention when test result and patient information are brought together in a genetic report. In this format, the only difference between different genetic tests will be only the tubes containing the gene-specific amplification primers. For the first time, different genetic tests can now be streamlined in a high-throughput, quality-assured and automated format.

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## 2930/F/Poster Board #862

**Genetic Analysis of Single Cells.** *J.-B. Fan[1], C. April[1], H.-I. Chiang[2], H.L. Fung[2], J. Chen[1], B. Klotzle[1], K. Zhang[2].* 1) Department of Applied Genomics, Illumina, Inc, San Diego, CA; 2) Department of Bioengineering, University of California San Diego, La Jolla, CA.

We are developing highly sensitive and quantitative genomic technologies for genetic and expression analysis of single cells or trace amount of DNA/RNA materials, using microarray and next-generation sequencing platforms. We have tested various Multiple Displacement Amplification (MDA)-based protocols under a variety of reaction conditions. With our current protocol and a 300K-SNP chip readout, we were able to obtain 97.2% call rate and 99.98% call accuracy when 750 pg of human genomic DNA was used; we obtained 77% call rate and 97.7% call accuracy with direct cell lysis from 5 cells. To further improve the call rate (for genotyping) and genome coverage (for sequencing) in the single cell amplifications, we added a post-MDA normalization step to our protocol. We are also developing RNA amplification methods for expression profiling of single cells. With our current protocol, we were able to generate reproducible expression profiles ($R^2$ = ~0.85) with 25 cells (250 pg total RNA). In addition, the profiles correlated well with those obtained with standard 100 ng total RNA input ($R^2$ = ~0.78). We are currently using these technologies to study medical specimens such as circulating tumor cells and cancer stem cells. We will report the comparison results of our methods with other commercial kits.

## 2931/F/Poster Board #863

**A novel microfluidic Assay for denaturating high-resolution DNA electrophoresis of MLPA and STR-Typing samples.** *M. Gassmann[1], R. Salowsky[1], J. Coffa[2], K. De Groot[2], M. Aboud[3], B. McCord[3].* 1) Agilent Technologies, Waldbronn, Germany; 2) MRC-Holland, Amsterdam, Netherlands; 3) Florida International University, Department of Chemistry, Miami.

The Agilent 2100 Bioanalyzer instrument platform is designed for performing fast electrophoretic analysis of DNA, RNA, and proteins based on microfluidic chip technology. It is widely used for the analysis of PCR products and restriction digests. However, the resolution of the current DNA assays is not sufficient to separate DNA fragments generated in applications like e.g. short tandem repeat (STR) DNA-typing (microsatellite analysis) and MLPA (Multiplex Ligation-dependent Probe Amplification). As these methods require a very high resolving power in the electrophoresis involving denaturing conditions, usually capillary-based DNA sequencers are used to separate the DNA fragments. However, due to a growing number of new applications, there is a strong demand for an alternative separation platform that is fast, flexible and easy to use. Over the last decade, MLPA and short tandem repeat (STR) DNA-Typing have been established as standard methods in the analysis of genomic DNA. While MLPA is mainly used in the detection of copy number variations causing genetic hereditary diseases, STR-typing is applied in DNA forensics, kinship and population studies. Here we describe a novel assay that is able to perform fast, sensitive, high resolution separation of Cy5-labeled DNA fragments on the Agilent 2100 Bioanalyzer. A new denaturating gel matrix has been developed that enables the separation of single-stranded DNA fragments between 10 and 500 bp with a resolution approaching 2 basepairs. The significantly increased resolution of the new assay permits the Bioanalyzer to successfully separate and analyze MLPA and STR-typing samples.

## 2932/F/Poster Board #864

**Whole genome and transcriptome amplification in large biobanks.** *N. Klopp[1], T. Illig[2], C. Korfharge[3], HE. Wichmann[4].* 1) Klopp N, Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany; 2) Illig T, Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany; 3) Korfharge C, QIAGEN GmbH, Hilden, Germany; 4) Wichmann HE, Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany.

Biobanks are a key resource in unravelling the molecular basis of diseases, identification of new targets for therapy and improvement of attribution in drug discovery and development. The scientific trend in biobanking shows the need for stable techniques for amplification of biomaterials, which can be used for samples stored under very different conditions. The focus of the project is the standardisation and validation of the innovative techniques of whole genome amplification (WGA) and whole transcriptome amplification (WTA) in the context of biobanks. A general standardized protocol for WGA and WTA procedures that use Phi29-DNA-polymerase in biobanking will be developed.The major aims of our project are: 1. To establish standardized WGA protocols for large biobanks 2. To develop standardized WGA tools to recover genomic DNA, which is in plasma or serum samples and from FFPE- tissue or blood spots 3. To optimize the WGA procedure by extensive quality control measures of WGA products 4. To develop and establish WTA of large biobank samples 5. To optimize WTA procedures by extensive quality control of WTA products Furthermore, the concept of the project is to transfer the results of WGA and WTA solution to national and international organisations in the field of biobanking. The development of the proposed, innovative and specialized tools and customized solutions will help to expand and secure biobanks.

## 2933/F/Poster Board #865

**High-density mapping of susceptibility genes to multifactorial diseases using DigiTag2 assay.** *N. Nishida[1], M. Sageshima[1], A. Suyama[2], K. Tokunaga[1].* 1) Dept Human Genet, Univ Tokyo, Tokyo, Japan; 2) Dept Life Sciences, Univ Tokyo, Tokyo, Japan.

DigiTag2 assay performs multiplex SNP typing by encoding all of the SNP genotypes to the well-designed oligonucleotides, named DNA coded numbers (DCNs). The DCNs are assigned to the target SNPs in an unconstrained manner, therefore, the DNA chips prepared to read out the types of DCNs are universally available for any types of SNPs. Moreover, the primers and probes for DigiTag2 assay are designed using fixed designing parameters to carry out SNP typing under the same experimental conditions without any optimization.

DigiTag2 assay is a suitable multiplex SNP typing platform to genotype with a number of samples (adequate genotyping throughput: 192 samples per day) in a 96-plex or a 32-plex form. DigiTag2 assay has other advantages against the conventional platforms in the running cost (for oligonucleotides, reagents, DNA microarrays, etc.), which is estimated to be less than $0.06/genotype, and in the conversion rate (defined by the proportion of successfully genotyped SNPs in the number of SNPs examined), which reaches to 90.72% (929/1,024 SNPs). Moreover, we made a SNP typing using DigiTag2 assay for over 1,000 SNPs with over 26,000 samples in total and revealed that the average call rate are 99.53% in both of 32-plex and 96-plex forms.

The genotyping platform with high conversion rate plays an important role for the replication studies to identify the disease associated genes from candidate loci found in the GWAS (genome-wide association study) stage. The DigiTag2 assay will facilitate a high-density mapping of susceptibility genes to multifactorial diseases in the replication studies.

## 2934/F/Poster Board #866

**A new approach to identify sequence-specific protein-DNA interactions using surface arrays and mass spectrometry.** *M. Olivier, Y. Zhang, S.P. Mirza.* Biotechnology and Bioengineering Center, Med Col Wisconsin, Milwaukee, WI.

A method that can efficiently identify DNA-bound proteins is an indispensable tool for the study of mechanisms involving DNA-protein interactions, such as transcriptional regulation which significantly impacts cellular function. Traditional methods such as electrophoretic mobility shift assay (EMSA) that are usually implemented to test the binding of proteins to short DNA fragments will not help determine the identity of the DNA-bound protein(s). The identity of individual DNA-binding proteins may be confirmed by super shift assays if the protein is known and a quality antibody is available. However, for the vast majority of DNA-bound protein(s), these assays fail to uncover their IDs. Here, we report a novel approach to identify DNA -bound proteins, combining an oligonucleotide surface array and mass spectrometry (MS) for protein identification. We have applied our approach to identify the protein(s) differentially bound to DNA sequences containing single nucleotide polymorphism (SNP) that have been shown to be associated with plasma TG levels in humans. We covalently linked a 5'-amine- modified double-stranded 41-mer oligonucleotide to an aldehyde-terminated self-assembled monolayer on a gold surface. To identify DNA-bound proteins from nuclear extracts, peptides were trypsin digested and analyzed on an ion trap mass spectrometer. A full range MS spectrum was acquired followed by MS/MS of the six most abundant ions from the full MS scan, followed by database searching using the SEQUEST algorithm. The resulting identified proteins were compared between the two allelic forms of the oligonucleotide, and proteins bound only to one oligonucleotide were identified. For SNP rs3734967 of a brain-expressed serontonin receptor, HTR5A, our approach reproducibly (n=3) detected a nuclear protein, Poly(rC)-binding protein 1, bound to the G allele that also demonstrated a differential binding of proteins using EMSA. For SNP rs2721 of a feedback regulator gene for cholesterol synthesis, INSIG1, we identified three major liver nuclear proteins that are differentially bound only to the T allele. Whether or not the SNPs truly modulate the binding to these identified protein candidates awaits further investigation. Nonetheless, our novel surface array-MS approach has been able to identify proteins captured selectively by oligonucleotides in a sequence-specific manner, and could facilitate the high-throughput characterization of protein-DNA interactions.

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## 2935/F/Poster Board #867

**Quality Control of Affymetrix Genome Wide Human SNP 6.0 Processing at a High-Throughput Facility.** *J. Romm, B. Marosy, K. Hetrick, B. Craig, M. Zilka, C. Ongaco, S. Pottinger, J. Gearhart, J. Goldstein, D. Leary, M. Barnhart, L. Watkins, E. Pugh, K. Doheny.* Center for Inherited Disease Research, Johns Hopkins University, Baltimore, MD.

The Center for Inherited Disease Research (CIDR), established at Johns Hopkins University in 1996, provides high quality genotyping services and statistical genetics consultation to investigators working to discover genes that contribute to common disease. CIDR has recently begun offering an Affymetrix GenomeWide Human SNP 6.0 genotyping service. We have developed quality control measures and defined workflow to obtain sample QC metrics quickly. We have strict sample acceptance criteria which includes blood or cell line genomic DNA, assaying samples for concentration (>50ng/uL by OD) and for lack of degradation (high molecular weight band >4kb on a 2% agarose gel). CIDR developed a Laboratory Information Management system (LIMs) for tracking workflow and reagent creation and use. There are QC steps in the LIMs that require verification to proceed, and the option to exclude a sample from hybridization to an array. LIMs provides a real-time informatic log of sample ID/array barcode association during manual injection of samples. Before an entire 96 well plate is hybridized, we perform 4 'Test-Hybs' and review gender, barcode and HapMap concordance, Contrast QC (CQC), CIDR QC birdseed call rate and DM QC call rate. In order to quickly generate these statistics, CIDR has developed an AffyCall pipeline which automatically parses and archives output files and updates QC statistics for each sample. To obtain the CIDR QC birdseed call rate, each sample is individually called with a group of 95 standard samples in order to mimic calling data by plate. This allows assessment of each sample independent of the overall batch performance and reduces variables involved in troubleshooting chemistry or sample quality issues. We evaluate the correlation of the STY CQC/NSP CQC at a plate level to confirm that there was no mispooling of a PCR plate before purification. CIDR has defined more stringent internal QC for data that is released to a PI and to public databases than currently recommended by Affymetrix. A releasable sample is one having a DM QC call rate >86%, a CIDR QC birdseed call rate of >98% and a CQC value of >1.0. If a sample does not perform well the first time through the assay, it is repeated once. Our first Affymetrix 6.0 project, a case-control study of T2DM, resulted in a 99.72% blind duplicate reproducibility rate, HapMap concordance of 99.76%, and a total release of 97% of experimental samples. The mean call rate for released samples was 99.6%.

## 2936/F/Poster Board #868

**Maternal Cell Contamination (MCC) Testing on the new 3500 Capillary Electrophoresis System using STR/Microsatellite Markers.** *S.C. Hung[1], C.J. Davidson[1], L. Pique[2], E.S. Nordman[1], B.F. Johnson[1], R.A. Padilla[1], R.N. Fish[1], L.K. Joe[1], S.R. Berosik[1], A. Chhibber[1], J. Lee[1], S.R. Santhanam[1], A.C. Felton[1], A.A. Pradhan[1], I. Schrijver[2].* 1) Life Technologies Corporation, Foster City, CA; 2) Molecular Pathology Laboratory, Stanford University Medical Center, Stanford, CA.

Fetal gDNA samples, isolated from amniotic fluid (AF) or chorionic villus samples (CVS), are commonly used for prenatal genetic testing. AF and CVS samples are extremely valuable samples that are collected via invasive surgical procedures, and as a result could be subject to maternal cell contamination (MCC). Due to the sensitivity of PCR, MCC can potentially contribute a significant source of error in prenatal testing with misinterpretation of a prenatal test thought to be possible at levels of MCC as low as 1-2%. Commercially available forensic human identification kits that employ fluorescent multiplex PCR amplification of polymorphic microsatellite markers are suitable for the MCC detection. To interrogate several informative markers when analyzing samples for MCC, we utilized the AMPFLSTR® Minifiler PCR Amplification Kit, which examines 8 microsatellite loci across four distinct fluorescent dyes. In order to evaluate the new 3500 capillary electrophoresis (CE) instrument, synthetic MCC sensitivity controls (10%, 5%, 2% and 1%) as well AF samples were analyzed. Semi-quantitative calculations of the level of MCC contamination were performed by comparing the peak area of the informative maternal allele to the unique fetal allele; the new CE instrument provided adequate sensitivity for detecting a synthetic MCC control at a level of 2% or greater. Further, we discuss common pitfalls to the analysis of MCC results such as broad peak errors, allelic drop out (ADO), preferential allelic amplification, and the influence of stutter peaks.

## 2937/F/Poster Board #869

**Co-amplification at Lower Denaturation temperature-PCR (COLD-PCR) can be used to increase mutations detection sensitivity of genetic testing.** *A. Milano, A. Goodeve, A. Dalton.* Sheffield Diagnostic Genetic Services (SDGS), Sheffield Children's NHS Foundation Trust, Sheffield, United Kingdom.

Background: One of the main limitations of the most common PCR based techniques used in genetic diagnosis (e.g. Sanger sequencing, Pyrosequencing technology, TaqMan) is the sensitivity of detection for low levels of somatic mutations. However, the identification of these low levels alterations can be critical in several areas of medicine including oncology, virology, microbiology and prenatal diagnosis. Recently, Wang et al (Nature Medicine 2008; 14:579-84) have reported that a major improvement in detection sensitivity can be achieved by the use of "COLD-PCR". This modified PCR is based on the principle of using a sequence specific lower denaturation temperature "Tc" at which only heteroduplexes (of mutant plus wild type alleles) are selectively denatured and amplified.

Methods: We compared our conventional PCR methods with COLD-PCR to determine any improvements in detection sensitivity of the JAK2 p.V617F mutation by TaqMan and KRAS p.G12V mutation by Pyrosequencing analysis. Sensitivity was assessed using serial dilutions of DNA from cell lines homozygous for the above mutations.

Results: COLD-PCR successfully detected mutations in all samples that were positive by conventional PCR, and enhanced the mutant-to-wild-type ratio by an average of 5-fold. This resulted in an increase in sensitivity from 0.5% to 0.1% for TaqMan based analysis of the JAK2 p.V617F and from 5% to 1% for Pyrosequencing analysis of the KRAS p.G12V mutation. All samples were genotyped correctly and no mutations were detected in any of the wild type control samples.

Conclusions: The implementation of COLD-PCR required only minimal optimisation comprising determination of the Tc denaturation temperature; no changes in reagents or instruments were necessary. We can confirm that COLD-PCR is specific and reproducible and that its use results into a significant increase in assay sensitivity, which will have an important impact on clinical decision making.

## 2938/F/Poster Board #870

**High Throughput Targeted Resequencing of Human Genomic Disease Loci using Solution Sequence Capture.** *R. Selzer[1], M. Rodesch[1], H. Halvensleben[1], J. Wendt[1], B. Thomson[1], L. Freeberg[1], G. Weissenberger[2], A. Sabo[2], D. Muzny[2], M. Bainbridge[2], M. D'Ascenzo[1], X. Zhang[1], T. Richmond[1], L. Nazareth[2], D. Burgess[1], T. Albert[1], R. Gibbs[2].* 1) Roche NimbleGen, Inc., Madison, WI; 2) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX.

Oligonucleotide microarrays have been successfully coupled with next generation sequencing platforms for the efficient targeted resequencing of disease candidate loci from ~100Kb up to the entire human consensus coding exome of ~35Mb. These solid phase approaches permit functional genomic elements to be sequenced to high depth, and enable identification of rare variation in human inherited disease, and in cancer. Although the microarray-based methods exhibit superior reproducibility and sequence coverage uniformity compared to solution-based hybridization methods, their utility for studies of very large sample numbers has so far been limited by the lack of automated, high-throughput, processing routines. This has been alleviated to some extent by sample pooling strategies, including the use of sequence tags (MIDs), but there is still a need to be able to process samples more rapidly. We have devised a highly scalable, novel sequence capture method that combines the advantages of solution-based hybridization approaches with the desired functional attributes of microarray-based techniques. Application of this method to analyze a 400Kb contiguous target region on human chr15 resulted in greater than 70% of the subsequent ~600,000 454 GS FLX Titanium sequence reads mapping to the capture target. More than 95% of the target bases were covered, with average and median coverage each greater than 300X, sufficient for the automated calling of rare sequence variants with high confidence. Targets larger than 5Mb have also been successfully captured and targets of 30Mb are theoretically possible. This rapid, scalable and cost-effective method provides an efficient means of characterizing chromosomal regions identified through genome-wide association studies of many individuals, and will facilitate the simultaneous analysis of common and rare sequence variation in inherited diseases.

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## 2939/F/Poster Board #871

**Miniaturizing RNAi Assays: Acoustic Droplet Ejection (ADE) Enables Efficient and Reproducible siRNA Library Screens at Low Volumes.** *C. Jarman[1], V. Bruzzel[2], A. Davies[2], S. Pickett[1].* 1) Labcyte Inc., Sunnyvale, CA, USA; 2) Institute of Molecular Medicine, Trinity College, Dublin, Ireland.

The use of RNA interference (RNAi) for loss of function studies has revolutionized our ability to characterize gene function or to identify potential therapeutic targets for drug discovery. To facilitate large-scale, high-throughput functional genomics studies using RNAi, we have investigated the use of acoustic droplet ejection (ADE) technology for siRNA library based screening. As the number of siRNA molecules increase per target, cost is quickly becoming the prohibitive factor in conducting a successful genome-wide screen. Previously we have shown the use of ADE technology (unique tipless and touchless liquid transfer technology) in delivering sub-microliter volumes of siRNA molecules, transfection reagent and various cells to conduct library-based screens in standard 96-, 384- and 1536-well plate formats. Recently we have been investigating the use of ADE (Echo(R) liquid handler, Labcyte Inc.) for reverse transfecting cells on microscope slides in volumes as low as 25 nanoliters. Here, we used ADE to deposit siRNAs in a transfection matrix onto glass slides in an array, overlaid this with a monolayer of adherent cells and incubated the slides to allow reverse transfection. We then assessed for the effects of gene silencing by digital image analysis at a single cell level. Results suggest that ADE technology meets the current demands of siRNA library-based assays to enable high-throughput siRNA screening. In addition, by using ADE in an arraying capacity we have significantly reduced the amount of siRNA needed for each assay. This microarray approach further increases the data acquisition speed whilst reducing the reagent and consumables costs of each experiment.

## 2940/F/Poster Board #872

**Testing SNP Markers for Human Identification Application.** *J. Chen, D. Pinnamaneni, C. Wright.* Dept Biol, Claflin Univ, Orangeburg, SC.29115.

Developing a detection system for degraded DNA samples presents a big challenge for the field of DNA-based human identification. Currently commercial human DNA identification methods are based on the use of short tandem repeat (STR) DNA markers. Applied Biosystems' AmpF/STR Identifier and Promega's PowerPlex 16 systems for human identification generate STR PCR fragments up to 370 base pairs and 470 base pairs respectively. When DNA samples are degraded, however, neither kit is able to generate the PCR products required for the identification. We have investigated the use of single nucleotide polymorphism (SNP) markers for human identification. In the SNP approach much shorter DNA fragment are required, this will allow the system to work on degraded DNA samples. We select SNP markers with allele frequencies ranging from 0.35 to 0.5 among the Asian, African and Caucasian populations and test these markers on DNA samples from these populations. We calculated that fifty SNP markers can provide discrimination power about one in 1000 trillion, so the odds that two individuals will have the same 50 SNP DNA profiles are about one in one thousand trillion (except identical twins). The discrimination power from fifty SNP is much higher than the current commercial products. Another advantage of SNP technology is that the SNP probes can be put on DNA chip or array platform. We have tested ten SNP markers on three DNA panels from Asian, Africa America, and Caucasian. The information will be used for SNP Human Identification technology development.

## 2941/F/Poster Board #873

**Next generation SNP genotyping using the Affymetrix platform.** *M. Shapero, R. Mei, J. Zheng, M. Purdy, L. Wang, D. Giberson, M. Borodkin, J. Burrill, M. Yamamoto, S. Cawley, A. Williams, K. Jones.* Genotyping Research, Affymetrix, Santa Clara, CA.

**Purpose**: DNA microarray technology continues to play an important role in identifying the genetic basis of common and complex human diseases through genome wide association studies (GWAS). As large scale sequencing efforts across multiple populations continue to add to the characterization of both common and rare genetic variation in the form of single nucleotide polymorphisms (SNP) , microarrays are well poised to capitalize on this new information content. To this end, Affymetrix has continued to develop new technological approaches to facilitate large-scale GWAS. Here an overview of our next generation genotyping platform is presented: an integrated, high throughput system that includes a highly automated workflow, a new enzymatic-based assay, and novel SNP content that was derived from a large diversity screen.

**Methods**: DNA target preparation, DNA amplification, and enzymatic fragmentation of post-amplification products are all automated on the Beckman ArrayPlex platform. Hybridization of DNA target occurs on peg arrays contained in either 24-well or 96-well plate configurations, with subsequent interrogation of the polymorphic nucleotide via a ligation reaction that uses two differentially labeled solution probes. Following a stringent wash, array staining occurs in concert with a multi-color, CCD-camera based detection scheme. All steps from array plate hybridization to array plate scanning are fully automated using the Affymetrix GeneTitan System. Lastly, all results are displayed via a comprehensive user-friendly software suite.

**Results**: The current SNP content for this platform is derived from a screen of dbSNP Build 128 across a diverse set of 1300 samples. This ligation-based SNP genotyping platform has been tested using several different kinds of input DNA, ranging from cell-line derived DNA to DNA extracted from human blood and saliva. Assay performance was evaluated using overall call rate, trio concordance, and concordance to independent DNA genotype information (HapMap).

**Conclusion**: The next generation platform for GWAS provides comprehensive and accurate genotyping of hundreds of thousands of SNPs in a single assay and offers a sample throughput coupled with minimal manual intervention consistent with the needs of large scale studies being conducted as part of the search for the underlying genetic basis of complex human disease.

## 2942/F/Poster Board #874

**Specificity of TaqMan® Drug Metabolism Genotyping Assays using DNA sequencing by capillary electrophoresis.** *P. Tsang, B. Ching, J. Au-Young, T. Hartshorne, T. Ceccardi.* Applied Biosystems, Foster City, CA.

Genetic polymorphisms in the cytochrome P450 genes are of pharmacogenetics importance due to the significant roles the P450 enzymes have in drug metabolism. Genotyping using TaqMan Drug Metabolism (DME) Genotyping Assays and DNA sequencing are two common methods used for detecting single nucleotide polymorphisms (SNPs), insertions and deletions (indels), and multi-nucleotide polymorphisms (MNPs) in P450 genes for pharmacogenetics applications. Bioinformatics methods were used to design assays that specifically target the polymorphism of interest. The TaqMan DME assays were tested on a total of 180 Coriell samples from four different populations and the frequency of the alleles and genotypes were compared to reported results in public databases and the literature, when available. However, the specificity of TaqMan DME genotyping assays were not further investigated by other concordant laboratory methods. Of particular interest are the DME genes showing significant homology to other loci in the genome, such as pseudogenes or related gene family members. Our objective was to demonstrate the specificity of a subset of TaqMan DME genotyping assays for polymorphisms in CYP2D6, CYP2C9 and CYP2C19 genes by showing concordance between genotyping results obtained with the TaqMan DME assays and DNA sequencing. PCR primers with M13 tails were designed for 39 selected alleles and were used to amplify genomic DNA samples from Caucasian and African American Coriell cell lines. The predicted size and specificity of each amplicon was corroborated by gel electrophoresis. Amplicons of the predicted sizes were sequenced by capillary electrophoresis and their genotypes were determined by aligning the TaqMan DME assay target sequences with the amplicon sequences. By showing concordance between the genotypes obtained from sequencing and TaqMan DME assays, we were able to confirm that correct genotypes were obtained using TaqMan DME genotyping assays. Therefore, TaqMan DME genotyping assays provide a reliable and fast genotyping method for identifying many P450 genetic variants for pharmacogenetics applications.

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## 2943/F/Poster Board #875

**Y-SNPs analysis in Japanese male (Tokushima area).** *Y. Yoshida[1,2], S. Kubo[3], Y. Nakahori[2].* 1) Tokushima Police Headquaters, Tokushima, Japan; 2) Department of Human Genetics, Institute of Healthbiosciences, The Tokushima University Graduate School, Tokushima, Japan; 3) Department of Forensic Medicine, Faculty of Medicine, Fukuoka University, Fukuoka, Japan.

In recent years, many researches to discriminate Japanese people are investigated on the basis of the racial classification using SNP. Similarly, it is effective to detect Y-SNP in order to discriminate a Japanese male. In the Y-SNP classification, it is reported that ration of O, D, and C type occupy most in the Japanese male, and N and P system are minority. In general D type peculiar to Japanese is called “Jomon”, and most famous O type in Japan is called “Yayoi”. Also in our previous study, same three types s observed were high frequency, such as O type: 48.8%, D type: 27.3%, C type: 12.4% in the male of Tokushima prefecture. In our comparative study of 4 areas (Gunma, Fukui, Tokushima, Kagoshima), we experienced that a frequency difference was in these three types on Japan . And we could find the characteristics of each type, especially the C type was guessed moving linearly in the inside of Japan from the Y-STRs data of 9loci. Then, in this research we detected not only the 4 loci of P31 (O2), M122 (O3), M216 (C) and M174 (D) which have been used until previous study, but also the loci of M8 (C1), M217 (C3), P186 (O), M231 (N), and P230 (P). The detection method was improved so that regional difference could be examined in detail. We analyze quickly and simply by the Cycleave PCR method (Takara) using real-time PCR equipment. Since this method using a chimera probe has high sensitivity, prepared primer and the probe so that only mutant type could be detected, and detected 2 loci by PCR at once . The result was divided into each type of O, D, C, and N by 1st PCR, and further, the type of C and O were able to be separated into C1 / C3 and O2 / O3 by 2nd PCR. Moreover, the sample which has not been detected to the 1st PCR detected P type by P230. We introduce our examined method and its data about the male of Tokushima area in Japan. Reference: Yoshida Y. et al. Leg Med, 10 243-252 (2008).

## 2944/F/Poster Board #876

**Evolution, expansion and genomic impact of the human retrotransposon SVA.** *D.C. Hancks, A.D. Ewing, J.E. Chen, H.H. Kazazian, Jr.* Genetics, University of Pennsylvania, 515 Clinical Research Building, 415 Curie Blvd, Philadelphia, PA. 19104.

Although most human retrotransposons are inactive; both inactive and active retrotransposons drive genome evolution and may influence transcription through various mechanisms. In humans, three retrotransposon families are still active, but one of these, SVA, remains mysterious. Here we report the identification of a new subfamily of SVA, which apparently formed after an alternative splicing event, where the first exon of the Mast2 gene spliced into an intronic SVA and subsequently retrotransposed. After molecular and computational experiments, we found a number of functional splice acceptor sites within many different transcribed SVAs across the human and chimpanzee genome along with additional examples of splicing followed by retrotransposition in other primates. Likewise, using a mini-gene splicing construct containing an SVA in cell culture, we are able to observe splicing, along with exonization events that shift the reading frame introducing premature termination codons (PTC). Moreover, these results imply that an SVA residing within an intron in the same orientation as the gene may alter normal gene transcription either by gene-trapping or by introducing PTCs through exonization, thereby affecting genome evolution.

## 2945/F/Poster Board #877

**Rapid scale up of a whole genome genotyping platform while implementing a modified management strategy.** *A. Crenshaw, M. Parkin, W. Brodeur, T. Minnetyan, J. Carey, G. Grant, D. Gage, W. Winckler, S. Gabriel.* Gen Analysis Platform, Broad Inst/MIT & Harvard, Cambridge, MA.

The Broad Institute Genetic Analysis Platform was tasked with a rapid scale up of one of its whole genome platforms, which included QC and QA steps taken to ensure quality throughout the process. This was done in real time to accommodate a ~48,000 sample project as well as other projects being planned. This was made possible with the addition of minimal capital equipment and informatics support. In analyzing the process we were able to identify pinch points in the process so that we could increase efficiency and quality without too much upfront costs. We will discuss methods used to ensure high data quality by requiring implementing stringent QA and QC procedures to monitor sample quality, sample identity, and processing controls. We will also discuss the informatics support that is wrapped around this process to enable monitoring of a high throughput environment in easily digestible formats to identify and resolve any issues that might arise in a timely manner. These processes and monitoring have allowed us to produce data in a robust and scalable fashion in a short period of time.

## 2946/F/Poster Board #878

**Non ionizing radiation may induce genomic imbalances in cultured human tissues after being submitted to a cell-phone radiation environment above international exposure limits.** *J.K. Heinrich[1], C.L Campanhol[1], R.M. Rodrigues-Peres[1], W.R. Heinrich[2], A.M. Almeida[2].* 1) CAISM - Women’s Hospital - State University of Campinas - UNICAMP , Campinas, SP, Campinas, Brazil; 2) CPqD Foundation , Campinas, SP, Brazil.

Radiation may cause several cell injuries and genetic alterations such as chromosomal rearrangements, aneuploidy, micronuclei frequency increase, microsatellite instability, mutations, gene amplification, gene transcription changes which culminates in cell transformation and death in the first and following generations of cells after the first insult. The objectives of this study were to access genotoxic and clastogenic effects of non-ionizing radiation, the same kind of radiation employed in cell-phone devices. Conventional karyotyping, micronuclei frequency and gene copy number status of the genes TP53, ERBB-2 and C-MYC through FISH analysis and genome-wide studies through mCGH were applied to 24 blood samples and skin tissue from healthy donors with normal genomic profiles. Cells were submitted to a non-ionizing radiation experimental source during different periods of time. The radiation system was specially designed for the experiment with strict conditions, controls and calibration. The power values were calculated to allow SAR values (specific absorption rates) of 0,8 W/kg, 2W/kg (international limit for public exposure), 5W/kg and 10W/kg with AMPS, CDMA and GSM modulation. Control samples and sham-exposed controls were also analyzed. Chromosomal abnormalities and micronuclei frequency showed to be dose-dependant with higher frequencies for the highest SAR levels after 5W/kg. We failed to observe imbalances for the TP53 gene although a slight number of aneuploid cells were found. Hybridization controls indicated that those copy changes were specific related to radiation. Interclass coefficients and correlation analysis were perfomed. As a correlation between the exposure of non-ionizing radiation and genomic imbalances was observed above international exposure limits, our findings should help for the future proposals of new public policies regarding exposure limits and international guidelines as the number of cell phone users has duplicated in the last years. SUPPORT: FUNTTEL/Brazil.

## 2947/F/Poster Board #879

**Genomic technologies in the analysis of historical relics: identification of remains of Nicholas II Royal family and their servants.** *E.I. Rogaev[1,2,3], A.P. Grigorenko[1,2,3], Y.K. Moliaka[2,3], G. Faskhutdinova[2], E.L.W. Kittler[4], I.Y. Morozova[1,2].* 1) Department of Genomics and Laboratory of Evolutionary Genomics, Vavilov Institute of General Genetics, Russian Academy of Science, Gubkina Street, 3, Moscow, 119991, Russian Federation; 2) Brudnick Neuropsychiatric Research Institute, University of Massachusetts Medical School, 303 Belmont Street, Worcester, MA 01604; 3) Research Center of Mental Health, Russian Academy of Medical Science, Zagorodnoe Shosse 2/2, Moscow, 113152, Russia; 4) University of Massachusetts Medical School, CFAR, Worcester, MA, USA.

We have performed the genomic analysis of historical specimens from two graves found in 1993 and 2007 presumably belonging to Nicholas II royal family members and their attendants who were murdered during the Russian Civil War in 1918. We successfully reconstructed the sequences from complete mitochondrial (mt) genome and determined Y-chromosome and autosomal chromosome profiles for the old skeleton specimens. We extended our analysis of the Royal family to testing of skeleton remains presumably belonging to their three servants and a court physician. As the reference samples the specimens from the distant relatives of the servants were tested along with the living descendants of the European Royal families. In addition, ~117 year old blood traces of Nicholas II from museum historic relic were examined. We developed multiplex PCR assays and high-throughput sequencing technologies for analysis of highly degraded nuclear and mitochondrial DNA from badly damaged old biological specimens. To predict the historical and population origin of the remains the SNPs haplotypes were determined for the Y-chromosome and mt genomes. In addition to individual STR and SNP profiles the genotype-phenotype correlations (hair and eye color) were made by direct re-sequencing of ultra-short amplicons generated for some nuclear genes. The results of our study demonstrated that comprehensive genomic analysis can provide a very accurate and efficient individual and kinship identification for very old historical relics and severely damaged forensic specimens. Our data provide evidence without reasonable doubt that the remains of Royal Family attendants and all members of Romanov family (father, mother and five children), including Anastasia and Alexei, have been identified.

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## 2948/F/Poster Board #880

**The detailed genomic and epigenetic characterization of human 15q11-13 in an autism endophenotype family.** *S.G. Gregory[1], A.J. Towers[1], L.L. Sullivan[2], H.A. Cope[1], M.L. Cuccaro[3], M.A. Pericak-Vance[3], B.A. Sullivan[2], G.R. DeLong[4].* 1) Dept Med, Duke Ctr Human Gen, Durham, NC; 2) Department of Molecular Genetics and the Institute for Genome Sciences & Policy, Durham, NC; 3) Miami Institute for Human Genomics, University of Miami, Miami, FL; 4) Department of Medicine, DUMC, Durham, NC.

Classic autism (AutD) comprises a spectrum of behavioral and cognitive disturbances of childhood development. The core AutD phenotype includes deficits in social interaction, impaired language development and patterns of repetitive behaviors and/or restricted interests. Estimates of population prevalence range between 1/300 to 1/150, with a male to female ratio of 4:1. The disorder has been shown to be highly heritable with the relative risk for siblings being 50-100 times that of the general population. Despite the considerable efforts of previous genetic studies only a small percentage of AutD cases (<10%) have been ascribed to single gene disorders. Although this likely reflects the underlying genetic heterogeneity of the disorder, investigators are rethinking the model of inheritance associated with autism to include epigenetic mechanisms, including genomic imprinting and epimutations. We have adopted a highly innovative, multi-faceted approach to profile the genomic and epigenetic signatures of a multi-generational, co-morbid family that has a clinical history of autism, albinism, splinter skills and bipolar disorder. A number of previous genetic studies have linked 15q11-13 with autism however, additional studies have singularly established a link between genes within 15q11-13 to bipolar disorder, via the GABA(A) receptor cluster, oculocutaneous albinism, via OCA2, and special memory abilities or splinter skills to the region. We have used state of the art high-resolution Nimblegen oligo tilepath microarrays to carry out copy number, ChIP-chip and DNA methylation analysis of DNA from peripheral blood from our co-morbid autism family. Additionally, we have correlated epigenetic profiles with gene expression using Illumina's genome-wide expression arrays. Here we report these analyses and the DNA methylation and histone modification (H3K4me2/H3K4me3/H3K9me2/H3K9me3) profiles within proximal 15q of 12 members from the co-morbid autism family.

## 2949/F/Poster Board #881

**Global analysis of chromatin marks in human pancreatic islets provides insights to type 2 diabetes susceptibility loci.** *D.S. Pearson[1], M.L. Stitzel[1], P. Chines[1], M.R. Erdos[1], L. Song[2], T. Furey[2], G.E. Crawford[2], F.S. Collins[1].* 1) Genome Technology Branch, NHGRI, Bethesda, MD; 2) Institute for Genome Sciences & Policy, Duke University, Durham, NC.

Genome-wide association studies have so far identified 18 variants associated with type 2 diabetes (T2D). Two thirds of these variants are associated with impaired insulin secretion or processing, and the vast majority reside in non-coding portions of the genome. These data suggest that altered regulatory function in the pancreatic islet may play an important role in T2D pathophysiology, but the regulatory landscape of the human islet epigenome is largely unknown. Recent chromatin studies in cell lines and other tissues suggest that regulatory elements are found in open chromatin regions and are enriched for certain histone H3 modifications. To characterize the islet epigenome and identify important regulatory elements that may be disrupted in T2D, we characterized the genome-wide distribution of a host of histone H3 lysine methylation marks (monomethylation of lysine 4, dimethylation of lysines 9 and 79, and trimethylation of lysines 4, 27, and 36) and regions bound by p300, CHD7, and CTCF using chromatin immunoprecipitation coupled with next generation sequencing (ChIP-Seq). Regions of open chromatin were defined using genome-wide DNase hypersensitivity mapping. These approaches were highly successful in identifying known promoters and enhancer elements in human islets. Extending these analyses to 18 regions of T2D association, our data suggest the existence of approximately 80 putative regulatory elements. For several of these, a SNP associated with increased T2D risk is located within or near the newly identified regulatory element. Currently, we are evaluating the function of wildtype and variant versions of a subset of these elements using dual-luciferase assays that assess enhancer, enhancer blocker, and silencer activity. Characterizing the entire repertoire of human islet regulatory elements will enhance our understanding of gene regulation in the human islet and should provide critical insight into the molecular mechanisms involved in diabetes susceptibility.

## 2950/F/Poster Board #882

**Feasibility for Detecting Epigenetic Abnormalities in Autism Brain.** *R. Person, S. Kim, X. Zhang, Y.H. Jiang, R. Chen, Y. Li, W. Li, A. Beaudet.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

There is increasing evidence that de novo and recent mutations at any one of many heterogeneous loci cause a substantial fraction of autism. These findings are entirely consistent with the high heritability of autism, as evidenced by the high concordance between monozygotic (MZ) twins. Yet, the etiology of autism remains unknown for a large fraction of individuals, particularly for nondysmorphic, higher functioning males. Epigenetics refers to regulation of gene expression without change in the underlying DNA sequence. Important, analyzable components of epigenetic expression include expression of mRNA and miRNA, DNA methylation, and histone modifications. Epigenetics plays at least some role in the etiology of autism as evidenced by the fact that usually maternal but not paternal interstitial duplications of chromosome 15q11-q13 cause autism. Epigenetic abnormalities could be more widely important in the etiology of autism, and could contribute to the heritability seen in MZ twins if epimutations arose prior to MZ twinning. As a proof of principle, we wished to demonstrate the feasibility of using existing methods to detect the known epigenetic aberrations within 15q11-q13, responsible for Prader-Willi syndrome (PWS) and Angelman syndrome (AS) syndromes. To investigate DNA methylation, MBD2 and MBD1 proteins were used to enrich methylated and unmethylated CpG rich DNA respectively. Histone modifications were examined using native chromatin immunoprecipitation (N-ChIP), followed by analysis either using Agilent microarrays or massively parallel Solexa DNA sequencing. Finally, the expression of 723 human and 76 human viral miRNAs were examined using the Agilent human miRNA (V2) microarray system. Using MBDs to examine the status of DNA methylation, we confirmed the ability to detect the known abnormalities in PWS and AS brain. Using NChIP to detect histone H3 lysine 4 trimethylation, a marker of active transcription, we confirmed its presence exclusively on the paternal SNRPN allele - present in AS but not PWS individuals. Finally, we found a significant upregulation of three miRNAs in 6 of 20 autism and in 0 of 20 control cerebellum samples. Thus, we have demonstrated the ability to use these methodologies to detect known epigenetic abnormalities in PWS and AS brain and the feasibility of detecting changes in autism brain if they occur. Additionally, we've shown a significant elevation of three miRNAs in a subset of autism cerebellum samples.

## 2951/F/Poster Board #883

**Genome-wide comparison of high resolution maps of nucleosome organization from purified human cells uncovers substantial complexity in the chromatin landscape.** *A. Valouev[1], S. Johnson[3], C. Smith[2], S. Boyd[2], A. Fire[1], A. Sidow[1].* 1) Department of Pathology, Stanford University, Stanford, CA; 2) Department of Genetics, Stanford University, Stanford, CA; 3) Department of Microbiology & Molecular Biology, Brigham Young University.

Chromatin organization of a cell is defined by complex interactions of nucleosomes packaging the DNA, transcription factors binding to their response elements and chromatin remodeling complexes changing the chromatin landscape according to the needs of the cell. What forces affect positioning of nucleosomes and to which degree this positioning is reproducible across different tissues is currently an actively debated topic in the chromatin field. While significant positioning and phasing of nucleosomes has been observed in synchronized yeast cells (Lee et al 2007, Segal et al 2006), seemingly very little positioning is present in samples from multi-stage whole worm pellets (Valouev et al 2008) suggesting that although positioned nucleosomes can be ubiquitous, they are also extremely cell-type specific ruling out the possibility of a universal DNA code dictating genome-wide organization of nucleosomes. To further investigate the extent of this phenomenon and precisely identify forces involved in chromatin organization, we have generated almost 2 billion SOLiD sequencing reads to produce high-resolution maps of nucleosome positioning within three human cell types purified from the blood of a single individual (granulocytes, CD4 and CD8 T-lymphocytes). Additionally, to specifically address the DNA sequence contribution to the positioning of nucleosomes, we also generated a high-resolution positioning map for the nucleosomes reconstituted in-vitro on human DNA. For dissecting functional effects on nucleosome positioning, we have generated ChIP-Seq data to precisely map behavior of several DNA binding proteins within these blood cells. Our data reveals a complex organization of chromatin and confirms that positioned nucleosomes are common within tissues, but their positions may differ significantly across different tissue types. Our in vitro reconstitution data suggests that although many nucleosomes can be positioned by the sequence, stereotypic positioning of many nucleosomes in vivo is not encoded by the sequence, but can be explained by other forces affecting chromatin in vivo, such as binding of transcription factors.

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## 2952/F/Poster Board #884

**Investigating the Pathogenesis and Therapy of Friedreich Ataxia Using Mouse Model Cell Lines.** *C. Sandi, R. Mouro Pinto, V. Ezzatizadeh, S. Al-Mahdawi, M. A Pook.* CCCB/BICGP, Division of Biosciences, School of Health Sciences and Social Care, Brunel University, Uxbridge, Middlesex, UB8 3PH United Kingdom.

Friedreich ataxia (FRDA, OMIM 229300) is an autosomal recessive trinucleotide repeat disease caused by expanded GAA repeats in the first intron of the FXN gene. Normal individuals have 5 to 30 GAA repeat sequences, whereas affected individuals have 70 to 1600 repeats. The effect of the GAA expansion mutation is to reduce the expression of frataxin, a mitochondrial protein. The exact mechanism by which the GAA repeat expansion leads to decreased frataxin expression is unknown and also the function of frataxin is still partly controversial but it is believed to be involved in iron-sulphur cluster biosynthesis (ISCs). Frataxin insufficiency leads to oxidative stress, mitochondrial dysfunction and reduced activities of several enzymes involved in iron-sulfur cluster synthesis. Bisulfite sequence analysis of the FXN flanking GAA regions reveals a shift in the FRDA DNA methylation profile, with upstream CpG sites becoming consistently hypermethylated and downstream CpG sites becoming consistently hypomethylated. It has also been suggested that GAA repeats may produce a heterochromatin-mediated gene silencing effect. There is no effective therapy for FRDA. In order to gain further understanding of FRDA pathogenesis, the physiological function of frataxin and to develop an effective system for testing potential therapies, mouse models of FRDA are considered essential. We have recently generated a GAA repeat expansion mutation-based FRDA mouse model that exhibits GAA repeat instability, epigenetic changes and progressive mild pathology representative of FRDA. We now describe the establishment of neural stem cells (neurospheres) and fibroblasts from this FRDA mouse model. The neurospheres show reduced levels of FXN expression and the fibroblasts show significantly more sensitivity to hydrogen peroxide-induced oxidative stress than control cells. We further show how these cultured cells are now being used to investigate epigenetic-based drug therapies for FRDA. In particular, we demonstrate increased FXN expression by the use of DNA hypo/demethylating agents and histone deacetylase inhibitors (HDACi) for the development of novel therapies for FRDA.

## 2953/F/Poster Board #885

**Epigenetic Profiling in healthy Aging and Exceptional Longevity.** *G. Atzmon[1,2], A. Bergman[3], T. Budagov[1], R. Thompson[1], N. Barzilai[1,2], J.M. Greally[1,2].* 1) Dept Med, AECOM, Bronx, NY; 2) Dept Genetic, AECOM, Bronx, NY; 3) Dept Systems & Computational Biology, AECOM, Bronx, NY.

Epigenetic alterations may play a role in extension of lifespan and incidence of age-related degenerative diseases. We systematically assess the contribution of epigenetic changes, and in particular DNA methylation status to the aging process and phenotype. We have piloted for the first time Epigenome Wide Association Study (EGWAS) in 15 Ashkenazi subjects (8 centenarians, and 7 young unrelated controls), analyzing more than 1.3 million features across the human genome utilizing the genome-wide cytosine methylation assay-HELP and Nimblegen array. In the young unrelated controls 79% of loci fail to cut by the methylation-sensitive restriction enzyme HpaII (indicating hypermethylation) vs. 87% in centenarian. These initial results support the hypothesis that subjects with exceptional longevity may exhibit different levels of DNA methylation than controls that change globally with age. Three percent of the total epigenetic loci exhibit two (or greater) fold changes between the groups, of which 5% of loci showed a complete transition (from hyper to hypo-methylation or vice versa). The majority of loci with two fold or greater changes in methylation status, showed increasing methylation with age. These preliminary results indicate for the first time that although most of the methylation loci may not change significantly, some changes can be seen with respect to age. The role of epigenetics in human aging and life span has not yet been elucidated. Here we bring together a novel technology and a unique genetically homogenous population (Ashkenazim) between ages 60-110 years. We are therefore ideally poised to investigate the role of epigenetic changes in life span. Our exiting preliminary results demonstrate the power and advantage of utilizing whole genome epigenetic association study in aging and longevity.

## 2954/F/Poster Board #886

**Comprehensive DNA methylation analysis in neuronal and non-neuronal cells.** *K. Iwamoto[1], M. Bundo[1], J. Ueda[1], Y. Nakano[1], M. Oldham[2], W. Ukai[3], E. Hashimoto[3], T. Saito[3], D. Geschwind[2], T. Kato[1].* 1) RIKEN Brain Science Institute, Wako, Japan; 2) University of California Los Angeles, CA, USA; 3) Sapporo Medical University, Sapporo, Japan.

DNA methylation in the brain cells likely reflects their developmental history, neuronal activity, and environmental exposures, and is associated with behavior. Despite the previous efforts of DNA methylation profiling in the human brain, little is known about epigenetic differences between neurons and non-neurons. However, such knowledge is critical to a more complete understanding of the role of the genome in brain functions. We performed comprehensive DNA methylation analysis in neuronal and non-neuronal nuclei obtained from the human prefrontal cortex by cell sorter based method. The analytical methods we used included global methylation assay by pyrosequencing reaction, site-specific methylation quantification using Illumina beads technology, and Affymetrix promoter tiling array analysis. We found that while non-neuronal nuclei showed similar DNA methylation pattern to bulk cortex, neuronal nuclei manifested qualitatively and quantitatively distinctive DNA methylation patterns. Through the tiling array data analysis, we also found that genes related to neuronal activity were methylated in non-neuronal nuclei, while genes expressed in astrocytes were methylated in neuronal nuclei. Such DNA methylation differences may contribute to the phenotypic differences between neurons and non-neurons.

## 2955/F/Poster Board #887

**Design Principles for Methylation Sensitive High Resolution Melt Assay and Successful Direct Sequencing.** *A. Tobler, M. O'Donoghue, N. Koch.* Applied Biosystems - part of Life Technologies, Foster City, CA.

DNA methylation plays a critical role in the regulation of gene expression in development, differentiation, and disease. While methylation of promoters correlates with suppression of gene expression, global hypomethylation of genomic DNA has been observed in tumor cells. Most of the techniques available to study DNA methylation do not offer enough sensitivity to confidently detect methylated DNA levels less than 10%. Methylation Sensitive High Resolution Melting (MS-HRM) is an effective technique to screen samples for estimated DNA methylation levels. MS-HRM amplicons can further be sequenced to determine the exact methylation pattern. Awareness of some basic factors that influence detection sensitivity will improve the chance of a successful MS-HRM assay. In this report, MS-HRM assay design principles are discussed and the methylation of the MTA1 promoter is examined. Sensitivity to detect samples with as low as 0.1% methylation is demonstrated with primers containing methylation sites. We further present sequencing data of DAPK1 amplicons that were directly sequenced after MS-HRM analysis. These results demonstrate the sensitivity and suitability of the Applied Biosystems MS-HRM Workflow to screen samples for differences in methylation levels, and the option to directly sequence MS-HRM amplicons.

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## 2956/F/Poster Board #888

**Genomic Methylation Variation Correlated with Metabolic Syndrome-Related Phenotypes.** *M.A. Carless, T.D. Dyer, D.L. Rainwater, A.G. Comuzzie, M.C. Mahaney, J. Blangero.* Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX.

Metabolic syndrome is a complex disorder that is reaching pandemic proportions worldwide and is a strong predictor of diabetes and cardiovascular disease. Few studies have assessed involvement of genomic methylation in metabolic syndrome and very little is currently known about the importance of epigenetic variation in disease risk. In this study, we quantified relative methylation in 190 individuals of Mexican American descent, within the San Antonio Family Heart Study, at 1,505 gene-centric CpG sites using an Illumina GoldenGate assay to identify epigenetic correlates with metabolic syndrome-related phenotypes. Variance component analysis using SOLAR revealed that approximately 25% of the relative methylation of CpG sites were significantly heritable. The median heritability was 0.11 and although this is lower than that observed for mRNA (0.32) or miRNA (0.43) expression in the same population, it strongly suggests that methylation is mediated by genetic factors in addition to environmental factors. After removing X-linked sites, we showed significant sex effects upon methylation in 19% of sites with most showing a substantial increase in methylation in females. Linear models (allowing for non-independence amongst family members) were used to identify a number of consistent correlations across multiple dimensions of the metabolic syndrome for a number of CpG sites in highly relevant genes. For example, CpG methylation within *POMC* was found to be correlated with fasting (p=0.031) and 2 hour (p=0.020) insulin, high density lipoprotein cholesterol (HDL-C; p=0.002) and triglycerides (p=0.009). Deficiencies in *POMC* are strongly correlated with obesity and it is possible that methylation reflects another mechanism by which decreased expression influences body weight. Importantly, methylation within *POMC* was also associated with metabolic syndrome (p=0.011), classified as a dichotomous trait. Methylation of CpG sites in other candidate genes such as *RYK*, *RUNX1T1* and *TGFBR3* were also correlated with a number of metabolic syndrome phenotypes including fasting and 2 hour glucose, fasting and 2 hour insulin, body mass index, waist circumference, HDL-C, triglycerides and metabolic syndrome. Of interest is the fact that methylation at each of these sites was associated with a more detrimental profile of phenotypes. We are currently sequencing CpG islands within several genes to further investigate the effect of methylation on metabolic syndrome in a larger cohort.

## 2957/F/Poster Board #889

**Methylation profile of placentas with confined mosaicism for trisomy 16.** *D. Diego-Alvarez[1,2], R. Yuen[1,2], M.S. Penaherrera[1,2], W.P. Robinson[1,2].* 1) Department of Medical Genetics, University of British Columbia, Vancouver, BC., Canada; 2) Child and Family Research Institute, Vancouver, BC, Canada.

Trisomy 16 is estimated to occur in 1% of clinically recognized pregnancies. When confined to the placenta (CPM16), it can contribute to fetal malformations and placental insufficiency. Because of the central role that the placenta has in fetal and maternal physiology, it is presumed that variation in placental gene expression patterns might be linked to important abnormalities in maternal or fetal health. However, the genetic and cellular processes altered in both fetus and placenta in the presence of a trisomy remain poorly understood. We propose the hypothesis that epigenetic differences between trisomic and diploid cells such as DNA methylation can be used to assess the effects of trisomy on tissue-specific genome-wide gene regulation. These differences may be used to identify groups of genes altered in specific trisomies and thus the basic processes affected. To assess differences in methylation profiles in placental samples, the Illumina GoldenGate Methylation Cancer Panel I, which evaluates 1505 CpG sites (807 genes, many of which are important in placental development), was used as a first approach. Samples consisted of trophoblast from 6 CPM16 and 4 euploid placentas, all obtained after delivery. An additional 5 euploid samples from the second trimester of gestation were used for further comparison of the effect of gestational age. Differentially methylated genes were identified using Significance Analysis of Microarrays based on a False Discovery Rate of <10% and an absolute difference of >20%. Analysis of the CPM16 samples and both groups of controls revealed 45 differentially methylated CpG sites from 39 genes, none of them locating to chromosome 16. This suggests that a trans-acting effect on genome methylation may occur in the presence of the trisomy. Furthermore, a greater number of CpG sites (149) appeared differentially methylated in a comparison of second trimester of gestation and term controls, most likely reflecting the difference in cell composition of trophoblast at different times of gestation. The most significant and biologically meaningful candidate genes are being followed up with pyrosequencing using larger samples.

## 2958/F/Poster Board #890

**Effect of X-chromosome inactivation in an autosome: Implication for clinical features in an unbalanced translocation t(X;15) case.** *S. Sakazume[1], R. Sohma[1], N. Harada[2], K. Endo[4], H. Ohashi[3], T. Nagai[1], T. Kubota[4].* 1) Pediatrics, Dokkyo univ, Koshigaya, Saitama, Japan; 2) Kyusyu MNedical science, Nagasaki, Japan; 3) Saitama Children's Medical Center, Saitama, Japan; 4) Epigenetic Medicine, Univ. Yamanashi, Yamanashi, Japan.

(Introduction) X-chromosome inactivation (XCI) is an intrinsic mechanism in females, which compensate genome unbalance between females and males. In an X-autosome translocation case, when XCI extends to the translocated autosome and inactivates genes, this could lead abnormal phenotype. (Clinical features) We report here 5-year-old boy with 46,XY,t(X;15)(p21.2;q11.2). He had similar features to Prader-Willi syndrome patients with 15q deletions, such as hypotonia, hypo-gonadism, and hypo-pigmentation, but he had different appearances in the face and fingers and had severer developmental delay. (Results) FISH demonstrated XIST gene in the translocated X chromosome, and BrdU incorporated-replication study demonstrated that proximal 15q region is late replicated. Bisulfite sequencing and RT-PCR assays showed that OCA2 gene at 15q13 was half-methylated and down-regulated, whereas UBR at 15q15.2 and TRIP15 at 15q21.1 was not either methylated and down-reglulated. Methylation-specific PCR assays revealed that one of two SNRPN alleles at 15q11.2 was methylated, and that the X-translocated chromosome 15 was maternally inherited. (Conclusion and Implication) All these results suggest that functional border of XCI lies between 15q13 and 15q15.2, and that aberrant methylation and suppression of OCA2 leads to the hypo-pigmentation in skin and hairs. Further studies using genome microarray will reveal XCI border at the molecular level and may reveal sequence-based structure of the border.

## 2959/F/Poster Board #891

**Epigenetic associations with triglyceride response to a high-fat meal: A Pilot Study of The Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study.** *M.K. Wojczynski[1], D.M. Absher[2], H.K. Tiwari[1], E.K. Kabagambe[1], D.K. Arnett[1].* 1) University of Alabama at Birmingham, Birmingham, AL 35294, USA; 2) HudsonAlpha Institute for Biotechnology, Huntsville, AL 35806, USA.

Postprandial lipemia (PPL) is a physiological response occurring 2-12 hours after consuming a meal. PPL is implicated as a cause of atherogenesis as an elevated or elongated response precipitates increased production of atherogenic cholesterol enriched remnant lipoprotein particles, formation of small, dense LDL particles, reduction in HDL, and upregulation of thrombotic factors. PPL exhibits a large between person variance suggesting an interaction between dietary and genetic factors regulating PPL. Additionally, diet is known to modify an individuals' epigenetic profile. Similar to the more traditional genotype-environment interactions, epigenetic mechanisms may interact with environmental factors to influence phenotypes. We hypothesize that epigenetic modification, specifically DNA methylation, mediates the influence of dietary fat on gene expression and modulates PPL. We selected three-generational families displaying large average triglyceride area under the curve (TG AUC) values during PPL and the largest corresponding within family standard deviation from the GOLDN study (n=23 individuals). Participants ingested a standardized high-fat meal containing 83% fat and 700 calories/$m^2$. We measured PPL using blood drawn from participants to obtain lipid profiles before eating the meal and at 3.5 and 6 hours after ingestion. We processed stored DNA on the Illumina Methylation27 arrays to examine 20,692 CpGs in ~14,000 genes. We calculated the methylation proportion for each CpG probe per individual to distinguish methylation states from the CpG probe raw signal intensity. We analyzed the methylation proportions using a hierarchical clustering algorithm and identified CpGs near genes that had statistically significant methylation proportions associated with TG AUC values after correction for age and familial effect by modeling the kinship matrix in a mixed model. Based on the pedigrees, the observed methylation patterns are suggestive of maternal transmission. Fourty-eight CpGs demonstrated p-values<$10^{-3}$, and many have biologic plausibility as contributors to lipid metabolism and metabolic syndrome. Notable associated genes were SORBS1, INS, PTPN9, PLA2R1, CHST8, and GMDS as these are involved in carbohydrate metabolism, inflammation, and HDL catabolism. We conclude that specific methylation patterns may play a role in the regulation of PPL in response to a high-fat meal.

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## 2960/F/Poster Board #892

**Extensive Parent-Of-Origin Genetic Effects on Fetal Growth.** *R. Adkins[1], J. Krushkal[2], G. Somes[2], J. Fain[3], J. Morrison[4], C. Klauser[4], E. Magann[5].* 1) Le Bonheur Child Med Ctr, Univ Tennessee-Hlth Sci Ctr, Memphis, TN; 2) Department of Preventive Medicine, Univ. TN Health Science Center, Memphis, TN; 3) Department of Molecular Sciences, Univ. TN Health Science Center, Memphis, TN; 4) Department of Obstetrics and Gynecology, Univ. MS Medical Center, Jackson, MS; 5) Department of Obstetrics and Gynecology, Naval Medical Center, Portsmouth, VA.

Epigenetic effects have recently been recognized as playing a very significant role in several normal and pathological phenotypes. Imprinting, the silencing of either the paternally or maternally inherited allele, is one of the most pervasive and consistent epigenetic mechanisms across species and individuals. The majority of imprinted loci are involved in fetal growth regulation, and several defects in the epigenetic regulation of these genes are associated with extremes of fetal growth. We surveyed patterns of SNP variation in imprinted loci in a cohort of African-American mother-newborn pairs selected using stringent inclusion/exclusion criteria intended to enrich for the genetic component of fetal growth regulation. All association analyses were adjusted for admixture using a suite of ancestry informative SNP markers. By comparing maternal and fetal genotypes, we could unambiguously infer the parental origin of associated alleles in the majority of newborns. We found significant parent-of-origin effects in the insulin, H19 and GNAS genes that were completely consistent with their known patterns of imprinting.

## 2961/F/Poster Board #893

**Whole Genome DNA Methylation Profiling of Major Depression.** *F. Haghighi[1], A. O'Donnell[3], Y. Xin[1], B. Chanrion[4], Q. Yuan[2], Z. Zhou[2], P. Graham[1], V. Arango[1], A. Dwork[1], D. Goldman[2], J. J Mann[1], T. Bestor[3].* 1) Dept Psychiatry, Columbia Univ, New York, NY; 2) Laboratory of Neurogenetics, NIAAA, NIH, Bethesda, MD; 3) Department of Genetics and Development, Columbia University, New York, NY; 4) Columbia Genome Center, Columbia Univ, New York, NY.

Epigenetics may play a role in the etiology of neuropsychiatric disorders, possibly through abnormal genomic DNA and histone methylation patterns that regulate genes involved in brain development or physiology. The aim of the present study is to explore the epigenetic profile of major depressive disorder (MDD). In order to better understand both the wild type genomic DNA methylation patterns and aberrant methylation events that occur in disease states, we have developed a cost-effective, unbiased, whole-genome methylation profiling technique that can assay the methylation state of more than 80% of the CpG sites in the human genome. Using our methodology, which couples advances in next generation sequencing with enzymatic fractionation of DNA by methylation state, we are mapping the methylation at high coverage of 4 controls and 4 MDD cases. We also mapped the histone methylation, H3K4me3 patterns in these same samples using ChIP-seq. We focused on the prefrontal cortex (PFC) due to converging evidence from neuroimaging and functional studies implicating this region in MDD. In the first genome-wide DNA and histone methylation profiling study of major depression, we identified aberrant methylation at several genes including those related to serotonergic and cholinergic pathways, neurotransmitter release, receptor functioning, and synaptogenesis. Subsequent to validation of these differentially methylated regions on the Sequenom platform, we are expanding our analysis of these regions to a large sample of PFC tissue from 60 controls and MDD cases with comprehensive clinical and toxicological profiles. These DNA methylation abnormalities may have clinical utility as biomarkers, and evaluation of the frequency of these alterations may help identify etiologic factors involved in MDD.

## 2962/F/Poster Board #894

**Molecular Testing for Beckwith-Wiedemann and Russell-Silver Syndrome: Growth Disorders at the Opposite Ends of Phenotypic Spectrum.** *B. Coffee, B. Billotte, M. Lee, S. Dharamrup, M. Tayeh.* Emory Genetics Laboratory, Department of Human Genetics, Emory University, Atlanta, GA.

Beckwith-Wiedemann Syndrome (BWS) is characterized by macrosomia, macroglossia, growth asymmetry, omphalacele and tumor predisposition. Conversely, Russell-Sliver Syndrome (RSS) is characterized by growth retardation (both prenatal and postnatal), dysmorphic facial features and growth asymmetry. BWS and RSS represent opposite ends of the phenotypic spectrum. Both disorders are caused by defects in imprinted gene expression at 11p15.5 due to alterations in DNA methylation at Lit1 and H19. In addition, approximately 10% of RSS is caused by maternal uniparental disomy of chromosome 7 (matUPD7). We have developed two novel assays to detect and measure DNA methylation at these imprinted loci. For Lit1 and H19 methylation analyses, we use Taqman methylation sensitive PCR to quantify DNA methylation. For matUPD7 analysis, we use conventional methylation sensitive PCR targeting two different differentially methylated regions (DMRs) in the PEG1/MEST and GRB10 genes. Unlike traditional testing for uniparental disomy, parental samples are not required for methylation analysis making testing available to individuals when one or both parents are not available. Combined, we have tested approximately 1000 individuals for BWS and RSS. We will present our overall experience in testing for these disorders and several interesting cases that include maternally inherited duplications, variation of methylation within an individual and discordance of methylation among twins.

## 2963/F/Poster Board #895

**Genome wide methylation analysis in Beckwith Wiedemann syndrome reveals CpG methylation alterations at several imprinted loci.** *S. Choufani[1], K. Millar[2], JC. Ferreira[1,3], P. Lapunzina[4], C. Shuman[1,2], R. Weksberg[1,2,3].* 1) Dept Genetics & Genomic Biol, Hosp Sick Children, Toronto, ON, Canada; 2) Div Clinical & Metabolic Genetics, Hosp Sick Children, Toronto, ON, Canada; 3) Ins Medical Sciences, Univ Toronto, Toronto, On, Canada; 4) Ins Genetica Medica Y Molecular, Hosp Universitario La PAz, Univ Autonoma de Madrid, Spain.

Beckwith Wiedemann syndrome (BWS) is an overgrowth syndrome that is in most cases associated with epigenetic and genetic alterations of chromosome 11p15. The most common epimutation is a loss of methylation at the imprinting control center 2 (IC2) present in 50% of BWS cases. A subset of BWS cases with loss of methylation (LOM) at IC2 were described to have loss of maternal methylation at other differentially methylated regions (DMRs) such as PLAGL1 on chromosome 6 and GNAS on chromosome 20. However, the status of many imprinted DMRs remains unknown. We hypothesize that LOM at IC2 in BWS patients could be associated with one or more epigenetic aberrations at imprinted DMRs that have not yet been investigated. Therefore, we undertook a genome-wide approach to analyze DNA methylation in BWS patients with LOM at IC2. We used bisulfite modified genomic DNA from 20 BWS patients and 20 controls and hybridized it to the Illumina methylation 27 array. This array covers ~27,600 CpG sites (promoter of ~14000 genes). Comparison of whole genome CpG methylation patterns between BWS patients and controls revealed a similar bimodal distribution of CpG methylation in the 2 groups. Methylation analysis of the CpG sites according to their genomic location within or outside CpG islands and across the different autosomes also showed a parallel distribution between BWS and controls. We then performed a detailed analysis of all CpG sites that overlap known imprinted DMRs. We identified a subset of BWS patients demonstrating CpG methylation alterations at several imprinted loci including PLAGL1 and GNAS. Using a targeted pyrosequencing assays, we found the frequency of PLAGL1 and GNAS epimutations in a larger cohort (n=70) of BWS (with IC2 LOM) to be 31 and 13% respectively. Interestingly, the analysis of imprinted loci identified 2 cases with pUPDs not involving chromosome 11p15. One case was confirmed by microsatellite markers to have pUPD20. Our approach provides the most comprehensive analysis to date for characterizing genome wide deregulation of imprinted loci in BWS. Furthermore, we report here the first case of pUPD20 for the entire chromosome. Further analysis is needed to understand how the interaction among the different imprinted genes can impact the clinical manifestations of human disorders caused by imprinted gene deregulation.

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## 2964/F/Poster Board #896

**High reslolution microarray analysis of DNA methylation.** *A. Wong[1], R. Straussman[2], Z. Yakhini[1], I. Steinfeld[1], A. Ashutosh[1], H. Cedar[2], D. Roberts[1].* 1) Agilent Technologies, Inc. Santa Clara, CA; 2) Dept. of Cellular Biochemistry and Human Genetics, Hebrew University, Jerusalem, Israel.

CpG islands are stretches of high GC content DNA containing multiple CpG dinucleotides. When CpG dinucleotides within these islands are methylated, especially in promoter regions, expression of the corresponding downstream genes is most often repressed. Aberrant CpG island methylation is implicated in genetic diseases and especially cancer. We have developed a microarray based solution for determination of methylated and unmethylated CpG islands in the human genome. This microarray contains ~244,000 oligo probes tiling the 21 megabases of ~25,000 unique CpG islands, with an average spacing between probes of ~100 base pairs. We also tile ~5000 additional regions outside of canonical CpG islands. Methylated DNA is immunoprecipitated (mDIP), fluorescently labeled, and competitively hybridized to the array against differentially labeled pre-immunoprecipitated DNA. We have developed a methylation calling algorithm to accurately determine methylated and unmethylated regions. We describe the application of this method and algorithm in a normal adult liver DNA model system. We demonstrate the ability of the assay to discriminate between regions confirmed to be methylated or unmethylated by bisulfite sequencing. We then apply the whole-genome assay to multiple tissues, including colon and colon carcinoma tissue DNA. We describe differential methylation between these tissues and between normal and cancer tissues.

## 2965/F/Poster Board #897

**MeDIP-chip detection and quantitative DNA methylation analysis of differentially methylated regions in imprinted loci.** *K. Nakabayashi[1], W. Yoshida[1], K. Yamazawa[2], M. Kusumi[1], T. Ogata[2], K. Hata[1].* 1) Dept. of Maternal-Fetal Biol; 2) Dept. of End. & Metab., National Research Institute for Child Health and Development, Tokyo, Japan.

Genomic imprinting, an epigenetic phenomenon whereby genes are differentially expressed according to their parental origin, is known to be crucial for placental development and fetal growth in mammals. Most imprinted loci contain a differentially methylated region (DMR) harboring allelic DNA methylation inherited from the male or the female gamete (germline DMR). Mutations/epimutations affecting DMRs cause rare imprinting disorders such as Silver-Russell and Beckwith-Wiedemann syndromes. Whether imprinted genes also play critical roles in the more common abnormal pregnancy cases such as preeclampsia and intrauterine growth restriction (IUGR) is an important question yet to be fully evaluated. With the aim of addressing this question, we established quantitative COBRA (Combined Bisulfite Restriction Analysis) assay conditions for 30 DMRs from 22 loci that include the majority of known human imprinted DMRs. We first measured DNA methylation levels at these DMRs in twelve blood samples from healthy individuals and twelve placental tissues from normal pregnancies to assess the extent of tissue specificity and individual varieties of methylation levels. Using this dataset as a reference, we are currently screening for epimutations at DMRs of the cord blood and placenta samples derived from 50 IUGR cases. Dnmt3L, a Dnmt3 methyltransferase family member lacking a functional methyltransferase domain, is shown to be a key regulator of maternal imprint establishment. Mouse embryos produced from Dnmt3L$^{-/-}$ females arrest in development by embryonic day 10.5, and completely lack maternal methylation at germline DMRs. As an approach to identify novel maternal DMRs, we obtained DNA methylation profiles for 16,000 CpG islands of these mutant and wild-type embryos (day 9.5) using the methylated-DNA immunoprecipitation (MeDIP) technique followed by array hybridization. Comparison of the mutant/wild-type profiles allowed us to detect decreased levels of methylation at 16 known DMRs as well as 36 novel candidates. Bisulfite sequencing analyses for the top five candidates have revealed that at least three of them are maternally methylated DMRs. The human orthologous regions are being characterized to determine if the regions are differentially methylated. Our COBRA assay system for DMRs is applicable to screen for epimutations in other conditions such as imprinting disorders and infertility, and will become more comprehensive by integrating newly identified DMRs.

## 2966/F/Poster Board #898

**Genome-wide DNA methylation patterns associate with genetic and gene-expression variation.** *J.T. Bell, A. Pai, J.B. Veyrieras, Y. Gilad, J.K. Pritchard.* Department of Human Genetics, University of Chicago, Chicago, IL.

DNA methylation is a critical regulator of transcription that affects phenotypic variation and disease. However, associations between methylation and gene-expression have not been well characterized on the genome scale, and the extent of genetic regulation of DNA methylation itself remains unclear. To this end, we interrogated genome-wide DNA methylation in lymphoblastoid cell lines from 58 Yoruba HapMap individuals, by assaying the methylation status of 27,211 CpG sites near the transcriptional start sites (TSS) of 12,823 Ensembl genes across the genome using the Illumina HumanMethylation27 BeadChip assay. First, we compared variation in DNA methylation and gene-expression using previously published gene-expression data in these samples (Stranger et al. 2007 Science). We observed a strong negative correlation between methylation and gene-expression across 9,500 genes (mean r = -0.4). Further analyses estimated the effect of location of the CpG-sites within CpG islands, within sequence motifs, and relative to the TSS, on the association between methylation and gene-expression. Second, we assessed the evidence that genetic variation contributes to DNA methylation variation. We performed genome-wide association analyses with the methylation data as phenotypes and 2.6M autosomal SNP genotypes from the HapMap consortium with the aim of identifying and characterizing methylation-associated QTLs (metQTLs). Significant associations for cis metQTLs were identified for SNPs within 500kb of approximately 70 genes at a false discovery rate of 1%. We are currently extending these analyses by comparing metQTLs to previously identified eQTLs, and revisiting eQTL results by considering both DNA methylation and gene-expression data in the analyses. These findings help elucidate the functional relationship between DNA methylation and gene-expression and provide a first detailed insight into the genetic control of DNA methylation.

## 2967/F/Poster Board #899

**Epigenetic changes characterized by DNA methylation profiling are associated with risk and phenotypic variation in multiple sclerosis.** *F.B.S. Briggs[1], S. May[1], J.R. Oksenberg[2], A. Bernstein[3], C. Schaefer[3], L.F. Barcellos[1,3].* 1) Division of Epidemiology, School of Public Health, University of California, Berkeley; Berkeley, CA; 2) Department of Neurology, University of California, San Francisco; San Francisco, CA; 3) Division of Research, Kaiser Permanente; Oakland, CA.

Multiple Sclerosis (MS) is a clinically heterogeneous autoimmune disease (AD) of the central nervous system characterized by incomplete concordance in monozygotic twins and greater female preponderance. Both genetic and environmental factors are known to be involved in the pathogenesis of MS. A role for epigenetic mechanisms has also been strongly implicated in autoimmunity. These mechanisms include DNA methylation, which occurs on CpG sequences in DNA and is involved in regulating many cellular processes including gene expression and genomic stability. Maintaining DNA methylation homeostasis is critical for the development and function of immune cells. Aberrant DNA methylation has been associated with several autoimmune diseases, including rheumatoid arthritis, scleroderma, psoriasis and systemic lupus erythematosus. Given the dynamic and heritable nature of the epigenome, epigenetic changes provide a mechanism by which environmental effects can have long-term effects on gene expression and disease manifestation. We performed a large and comprehensive epigenetic investigation in MS cases and controls (total N~200 individuals). Both site specific methylation profiles and global methylation measures in DNA samples derived from peripheral blood mononuclear cells were characterized. DNA methylation patterns were investigated using two approaches: 1) a total of 1,505 CpG loci from 807 (mostly immune related) genes were studied in 146 MS female cases (20 PPMS, 126 RRMS) and 40 healthy controls; 15 MS cases had mild disease (EDSS<2), 15 had severe disease (EDSS>8), and a total of 30 also had comorbid ADs), and 2) 27,578 CpG loci across the genome were investigated in 13 MS cases (4 PPMS females, 5 RRMS females, 4 RRMS males). The LTA locus on 6p21.33 was significantly more methylated (p=4 x 10-4) in RRMS cases compared to healthy controls. TJP2 on 9q21.11 was significantly more methylated (p=7 x 10-5) in PPMS cases compared to controls, while LYN on 8q12.1 was less methylated (p=6 x 10-4). In a case-only analysis of females with either RRMS or PPMS, PPMS cases were significantly less methylated at the EDNRB locus on 13q22.3 (p=1.3 x 10-6) and EGFL4 on 19q13.2 (p=4.2 x 10-5) and more methylated at CLN5 on 13q22.3 (p=1.9 x 10-4). These results demonstrate a prominent role for epigenetic mechanisms in the complex etiology of MS, and highlight the need to incorporate the evaluation of genetic, environmental and epigenetic factors in future studies of MS.

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## 2968/F/Poster Board #900

**Testing Candidate Proteins for their role in DNA demethylation.** *A. Burant, A. Beaudet.* Molecular and Human Genetics, Baylor College Med, Houston, TX.

DNA methylation is a major regulator of gene expression and plays a role in many processes including differentiation, X inactivation, and imprinting. Aberrant methylation during these processes can lead to many diseases including cancer, imprinting disorders such as Prader-Willi and Angelman syndrome, and autism spectrum disorders such as Rett and Fragile X syndromes. Much is known about how DNA becomes methylated, however very little is known about the mechanism and proteins involved in DNA demethylation. Three hypotheses have been proposed for the mechanism of demethylation: removal of the methyl group, removal of the methyl-cytotsine, followed by base excision repair (BER), or removal of the methyl-cytosine along with neighboring nucleotides, followed by nucleotide excision repair (NER). In previous studies, three proteins have been identified as potential demethylases, Thymine DNA Glycosylase (TDG), Growth Arrest and DNA Damage inducible (Gadd45A), and Methyl-binding domain 4 (MBD4). TDG and MBD4 are members of the BER pathway and may work with Activation Induced Cytidine Deaminase (AID) to perform demethylation. Gadd45a has been shown to function along with Xeroderma Pigmentosa G (XPG), a member of the NER pathway. To determine the role of these proteins in demethylation, we expressed the various candidate proteins along with an in-vitro methylated promoter driving luciferase in HEK293 cells. Using the SV-40 promoter as well as the pluripotency Oct-4 promoter, MBD4, Gadd45a and XPG were able to increase the expression of luciferase indicating a possible demethylating role for these proteins. Similar studies are also underway with ES cells containing the endogenous, imprinted SNRPN promoter driving GFP. We have also performed studies on mice knockouts of the demethylase candidate proteins by staining one cell stage embryos with methyl-C antibodies to determine if the genome wide wave of demethylation still occurs in these knockout mice. Thus far, we have found that AID and Gadd45a knockout mice have normal demethylation indicating these proteins are not required for demethylation. A TDG conditional knockout is currently being constructed for these studies using recombineering. We hope that these studies will lead to a better understanding of DNA demethylation.

## 2969/F/Poster Board #901

**Genome-wide analysis reveals overrepresentation of intragenic CpG islands in developmental genes.** *Z.X. Chen[1], X. Wu[2], A.D. Riggs[1].* 1) Department of Cancer Biology, Beckman Research Institute of the City Hope, Duarte, CA 91010; 2) Department of Molecular Medicine, Beckman Research Institute of the City Hope, Duarte, CA 91010.

In mammalian genomes, CpG dinucleotides are globally depleted with the exception of regions called CpG islands (CGIs), which show a relatively high frequency of CpG sites. CGIs located in promoter regions are well studied and known to be important for regulating transcription. In particular, methylation of promoter CGIs has been shown to play critical roles in fundamental physiological processes such as X-chromosome inactivation and genomic imprinting, as well as in silencing tumor suppressor genes during carcinogenesis. In addition to those associated with promoters, many CGIs located in intragenic and intergenic regions have been identified, but so far little is known about their biological roles. In this study, we analyzed all mouse RefSeq genes containing intragenic CGIs to explore the functional significance of the presence of CGIs in gene bodies. We found that intragenic CGIs are overrepresented in genes carrying H3K4me3/H3K27me3 bivalent marks at their promoters in mouse embryonic stem cells. This suggests a possible link between intragenic CGIs and developmental genes, which have been reported to be associated with bivalent promoters in embryonic stem cells. Consistent with this, gene ontology analysis of genes with intragenic CGIs revealed a significant enrichment for genes involved in developmental processes, such as ectoderm development, embryogenesis, and neurogenesis. Together, our results suggest that intragenic CGIs may play a role in the complex regulation of developmental genes.

## 2970/F/Poster Board #902

**Maternal DNA Hypomethylation as a Risk Factor for Congenital Heart Defects.** *S. Chowdhury, M.A. Cleves, S.L. MacLeod, C.A. Hobbs.* Department of Pediatrics, University of Arkansas for Medical Sciences; Arkansas Children's Hospital Research Institute, Little Rock, AR.

**Background:** Congenital heart defects (CHDs) are the most prevalent and serious of recognized birth defects. Multiple factors including genetic susceptibilities, epigenetic mechanisms, and environmental influences are thought to contribute to the development of non-syndromic CHDs. Folic acid deficiency is a risk factor for various birth defects and is associated with increased homocysteine levels. Elevated maternal homocysteine increases the likelihood of having a CHD-affected pregnancy, and is implicated in aberrant DNA methylation patterns. Fluctuations in three additional biomarkers in the homocysteine pathway are also associated with an increased risk of CHDs and altered DNA methylation patterns. These data lead to the hypothesis that maternal DNA hypomethylation contributes to the development of CHDs. We assessed maternal DNA global methylation in 40 women who had CHD-affected pregnancies and 40 women with unaffected pregnancies to test our hypothesis. **Methods:** The proposed study builds on the National Birth Defects Research and Prevention Study (NBDPS), an ongoing multi-site population based case-control study. Blood was collected from Arkansas NBDPS participants who delivered a singleton live birth with a non-syndromic CHD and women who had a live birth without a major defect. Our initial sample population was selected by creating a vector utilizing data from four biomarkers in the homocysteine pathway. Using these data, participants were organized into extreme metabolic phenotype groups and their DNA methylation was assessed. Utilizing methylation of the LINE-1 repetitive element, a Methylight technique was developed to assess global DNA methylation in a total of 80 mothers. The Methylight technique, in conjunction with bisulfite conversion of DNA, allows rapid analysis of methylated and unmethylated alleles. The Mann-Whitney U test was used to compare percent methylation by case and control status. **Results:** Significant DNA hypomethylation was found in case mothers when compared to controls (means 78.9% vs. 86.9% respectively; $p<0.007$). **Conclusions:** Our findings indicate maternal global DNA hypomethylation is associated with an increased risk of CHDs. Aberrations in maternal DNA methylation converge with previous evidence that indicate folate-dependent genetic and metabolic susceptibilities increase the risk of CHDs. A larger sample size to verify associations and multi-factorial analysis are warranted based on our preliminary evidence.

## 2971/F/Poster Board #903

**Loss of paternal methylation affecting the MEG3 locus located on chromosome 14q32.2 imprinted region in a girl with maternal upd-like phenotype.** *B. DEMEER[1], G. MORIN[1], R. GOURON[2], L. RAZAFIMANANTSOA[3], S. KANAFANI[4], J. ANDRIEUX[5], H. COPIN[4], L. CUISSET[6], M. MATHIEU[1].* 1) Dept Gen, Univ Hosp, Amiens, France; 2) Department of Orthopaedics, University Hospital, Amiens, France; 3) Pediatric Department, Beauvais, France; 4) Department of Cytogenetics, University Hospital, Amiens, France; 5) Department of Molecular Genetics, University Hospital, Lille, France; 6) Department of Molecular Genetics, Cochin-Saint Vincent de Paul Hospital, Paris, France.

We report the case of a 8-year old girl seen in the orthopaedic department for scoliosis. She is the third child of non consanguinous healthy parents. She was born after an uneventful pregnancy (BW : 2.330kg, BH : 47.5cm, BHC : 32 cm, Apgar score 10,10). Neonatal feeding difficulties and moderate axial hypotonia were noted. Motor milestones and speech were delayed. From the age of 2 years, she began to put on weight and developed obesity. At 8 years 2/12, parameters are 38.4kg (+4SD), 136.5cm (+2.2SD), BMI 20.6; Tanner stages : S1A1P2. She presents a 40° dorsal scoliosis, rather short hands and feet, no dysmorphic facial features and no mental retardation. Cerebral MRI was normal. Karyotype is 46, XX, ish15q11-13(SNRPNx2), and Array-CGH did not detect any microdeletion or microduplication. As maternal uniparental disomy for chromosome 14 (maternal UPD 14) was suspected, microsatellite studies and methylation analysis of Maternally Expressed Gene 3 (MEG3) located in the 14q32.2 region were performed. Maternal UPD 14 was excluded by showing biparental inheritance of microsatellite markers. Methylation of the MEG3 locus showed loss of paternal methylation with normal profil methylation of both parents. Yet very few patients with exclusively maternal methylation profil of the MEG3 locus have been described, but notably clinical features seemed to be grossly similar in epimutation patients and maternal UPD 14 patients.

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## 2972/F/Poster Board #904

**DNA methylation variation at CpG islands in children with autism spectrum disorders.** *D. Grafodatskaya[1], C. Marshall[2], A. Lionel[2], W. Roberts[3], S.W. Scherer[2], R. Weksberg[1].* 1) Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) The Centre for Applied Genomics,The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Autism Research Unit, The Hospital for Sick Children, Toronto, Ontario, Canada.

Although epigenetic factors have been postulated to play a role in the etiology of autism spectrum disorders (ASD), no specific epigenetic determinants have yet been identified. We compared genome-wide DNA methylation in the white blood cells (WBC) of 5 ASD patients to 7 healthy controls. Methylated DNA was immunoprecipitated and hybridized to Agilent CpG island arrays that contain 97% of USCS genome browser annotated CpG islands. To identify DNA methylation differences, a sample from one control was selected as a reference and a methylation profile for each sample was compared to this reference using Partek Genomic Suite. A fold change in methylation was calculated for each probe and the Hidden Markov Model (HMM) was applied to find genomic regions with methylation variation in consecutive probes. Three sets of HMM parameters were applied, including fold changes in methylation and number of affected consecutive probes: 20-fold in 2 probes, 8-fold in 3 probes and 6-fold in 5 probes. There were no significant differences observed in the number of hypo and hypermethylated regions detected by the 3 sets of parameters between control and ASD samples, indicating that there is no genome-wide loss or gain of DNA methylation at CpG islands in WBCs of ASD patients. Further, we filtered out the genomic regions detected in at least one control sample. ASD-specific methylation variation included: loss of methylation at LHX6 and DUSP22 and gain of methylation in MARCKS, ZNF713 and the 2q12.2 intergenic region. LHX6, DUSP22 and MARCKS are involved in neural pathways, suggesting that these genes are good candidates for further validation. Methylation variation was then compared to copy number variation data for each ASD patient. The gain of methylation in the promoter of ZNF713 was detected in 2 ASD sibs. Both of them had a 6.7 kb deletion inherited from the father that was located ~800 kb downstream of ZNF713. Using bisulphite pyrosequencing, we confirmed ~30% methylation in the ASD patients and their father, hemizygous for the deletion, and ~0% methylation in the mother who did not carry the deletion. We also observed ~0% methylation in this region for 9 unrelated controls. That is, deletions in this genomic region can be associated with DNA methylation changes several 100 kb away. Such epigenetic changes associated with genomic alterations have been previously shown to be important factors in human diseases involving imprinting and trinucleotide repeats.

## 2973/F/Poster Board #905

**Comparison of DNA Methylation Patterns in Three Types of Mouse Tissue.** *B.F. Johnson[1], C.J. Davidson[1], M. Kondo[2], L.K. Joe[1], S.R. Berosik[1], A. Chhibber[1], R.N. Fish[1], S.C. Hung[1], J. Lee[1], R.A. Padilla[1], D. Rodriguez[1], A.A. Pradhan[1], A.C. Felton[1].* 1) Life Technologies, Foster City, CA; 2) Applied Biosystems-Japan, Tokyo, JP.

Enzymatic methylation of the cytosine (C) residues at CpG motifs in genomic DNA (gDNA) has been shown to suppress gene expression and play a role in pathology. New technologies like microarrays and next generation sequencing allow identification of methylation patterns on a genome-wide scale but capillary electrophoresis analysis is ultimately used for detailed information about each CpG site in an amplicon and remains the gold standard in validating DNA methylation results. In this study a specific gene region from three mouse tissues was bisulfite treated and cloned into pGEM-T vector, amplified and sequenced with BigDye® Terminator Cycle Sequencing v3.1 chemistry, and analyzed by capillary electrophoresis. The level of methylation in two possible epigenetic sites of high CpG content of a target gene in mouse epithelial cells from the cornea and conjunctiva was determined and compared to methylation levels in mouse epidermis tissue. A number of factors affect the reliability of methylation patterns revealed by sequencing. In addition to purity of the gDNA and enzymatic bias during PCR, failure to complete bisulfite conversion will yield inaccurate methylation values. The extent of cytosine conversion by bisulfite is affected by the presence of protein associated with gDNA, the denaturation state of the target DNA and well as the quantity of DNA in the conversion reaction. In this study a fragment analysis protocol is described that corroborates the extent of conversion for improved reliability in interpreting methylation profiles. This assay can be used either to verify the conditions used for performing bisulfite conversion or be used to evaluate the ratio of methylated to unmethylated DNA in experimental procedures.

## 2974/F/Poster Board #906

**Genome-Wide Screen of Promoter Methylation Identifies Novel Markers in Melanoma.** *Y. Koga[1,2], M. Pelizzola[3], E. Cheng[4], M. Krauthammer[5], AM. Molinaro[3], K. Miyazaki[2], R. Halaban[4], S. Weissman[1].* 1) Dept Genetics, Yale Med Sch, New Haven, CT; 2) Dept Surgery, Saga Med Sch, Saga, Saga, Japan; 3) Dept Epidemiology and Public Health, Yale Med Sch, New Haven, CT; 4) Dept Dermatology, Yale Med Sch, New Haven, CT; 5) Dept Pathology, Yale Med Sch, New Haven, CT.

DNA methylation is an important component of epigenetic modifications that influences the transcriptional machinery and aberrant in many human diseases. In this study we present the first genome-wide integrative analysis of promoter methylation and gene expression for the identification of methylation markers in melanoma. Genome-wide promoter methylation and gene expression of ten early-passage human melanoma cell strains were compared to newborn and adult melanocytes. We used linear mixed effect models (LME) in combination with a series of filters based on the localization of promoter methylation relative to the transcription start site, overall promoter CpG content, and differential gene expression to discover DNA methylation markers. This approach identified 76 markers, of which 68 were hyper- and 8 hypo-methylated (LME $P < 0.05$). Promoter methylation and differential gene expression of five markers (COL1A2, NPM2, HSPB6, DDIT4L, MT1G) were validated by sequencing of bisulfite modified DNA and real-time reverse transcriptase PCR, respectively. Importantly, the incidence of promoter methylation of the validated markers increased moderately in early- and significantly in advanced-stage melanomas, employing early-passage cell strains and snap frozen tissues ($n = 18$ and $n = 24$, respectively) compared to normal melanocytes and nevi ($n = 11$ and $n = 9$, respectively). Our approach allows robust identification of methylation markers that can be applied to other studies involving genome-wide promoter methylation. In conclusion, this study represents the first unbiased systematic effort to determine methylation markers in melanoma, and revealed several novel genes regulated by promoter methylation that were not described in cancer cells before.

## 2975/F/Poster Board #907

**Epigenomic analysis of discordant monozygotic twins in autism.** *C. Ladd-Acosta[1,2], D. Fradin[1,3], D. Arking[4], K. Cheslack-Postava[3], A. Chakravarti[2,4], D. Fallin[3], W. Kaufmann[5,6], A. Feinberg[1,2].* 1) Center for Epigenetics, Institute for Basic Biomedical Sciences, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 3) Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 4) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 5) Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD; 6) Kennedy Krieger Institute, Baltimore, MD.

Idiopathic autism is a common neurodevelopmental disorder defined by three key features: impairment in reciprocal social interaction and communication as well as a markedly restricted repertoire of activity and interests. Although the molecular basis of autism is elusive, epigenetic aberrations play a central role in several related disorders, namely, Angelman, Fragile X, and Rett syndromes, in which affected individuals share or present clinical attributes of idiopathic autism. One approach to identify epigenetic alterations in autism is to examine DNA methylation (DNAm) in monozygotic twins discordant for the autism phenotype. Discordant monozygotic twins provide a valuable model for studying epigenetic changes because these individuals have the same DNA sequence; thus discordance must be related to changes other than the DNA sequence itself. We used Comprehensive High-throughput Arrays for Relative Methylation (CHARM) to examine DNA methylation (DNAm) across the genome in lymphoblast cells from nine monozygotic twin pairs, four female and five male, discordant for strict autism. Using CHARM, we identified several genomic regions with a gain or loss of DNAm in the individuals with strict autism compared to their twin counterparts with milder autistic features. Examination of three differentially methylated regions (DMRs) using bisulfite pyrosequencing, an independent, highly quantitative approach that measures DNAm at single nucleotide resolution, confirmed the CHARM results both in magnitude and direction. Preliminary analysis also suggests some overlap between parent-of-origin specific SNP data and some DMRs identified by CHARM. This approach provides a pathway to understanding a possible role of epigenetics in the pathogenesis of autism.

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## 2976/F/Poster Board #908

**Comprehensive genetic analysis of Silver-Russell syndrome by methylation-sensitive high resolution melting(MS-HRM) and whole genome strategy.** *SY. Lin[1,2], SP. Lin[3], M. Gorre[4], J. Kim[4], YN. Su[1,2].* 1) National Taiwan University Hospital, Taipei, Taiwan; 2) Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan; 3) Mackay Memorial Hospital, Taipei, Taiwan; 4) CMDX, Irvine, USA.

Background: Silver-Russell syndrome(SRS) is a clinically and genetically heterogenous congenital disorder characterized by severe growth restriction. Hypomethylation of H19 DMR and maternal UPD7 only account for 30 to 75 percent patients. Material and method: We investigated the methylation pattern at H19 DMR by methylation-specific multiplex PCR, MS-MLPA and MS-HRM in 50 patients with SRS and 5 with SRS-like syndrome. We also studied the methylation status in the GRB10 gene, MEST gene, IGFBP1 gene and IGFBP3 gene by MS-HRM. BAC array CGH was applied to identify microdeletion. Results: Nine patients with SRS had hypomethylation at H19 DMR. One patient with SRS-like syndrome had hypermethylated GRB10 gene, MEST gene, IGFBP1 gene and IGFBP3 gene by MS-HRM, which indicated maternal UPD7. The BAC array CGH identified one patient with a 2.6-Mb deletion on chromosome 7p15.3 and a 4.4-Mb deletion on chromosome 15q26.2qter, which were confirmed by multiplex PCR. Discussion: The overall mutation detection rate was 22 percent by MS-HRM and BAC array-CGH. Although epimutation may be the major cause of SRS and can be identified well by MS-HRM, whole genome array-CGH may also provide information in the etiology of SRS.

## 2977/F/Poster Board #909

**Direct Methylation Detection From Cancer Cells, Blood and Tissues: High Speed Conversion Followed By High-Resolution Melting, Methylation Specific PCR and Sequencing.** *C. Liu, S. Dong, N. Chen, J. Wang, A. Tobler, V. Boyd, J. Stevens.* MBS R & D, Applied Biosystems, Foster City, CA.

Aberrant DNA methylation patterns are one of the hallmarks of cancer. DNA methylation changes are detectable not only in tumors, but also in blood, as tumor-derived DNA is released into the bloodstream due to tumor necrosis and apoptosis. DNA methylation patterns are lost when amplifying in a PCR, thus the treatment of DNA with bisulfite, which converts C to U but leaves 5-methyl-C unchanged, forms the basis of many analytical techniques for DNA methylation analysis. The conventional bisulfite conversion usually starts from genomic DNA with longer incubation time which can be time consuming and labor intensive. Therefore, a faster and more stream-lined protocol is desirable. We describe a procedure for the direct bisulfite conversion from cancer cell lines within 2 hours. Direct bisulfite conversion from blood was evaluated along with FFPE samples. Succesful use of FFPE samples opens up large collections of clinical tissue banks, providing more and more value to the studies of epigenomics. All currently available methods require deparaffinization of the specimen prior to DNA extraction. The procedure described is capable of direct bisulfite conversion from the unprocessed FFPE sample, and complete conversion is achieved within 2 hours. Following bisulfite conversion, several methods have been used to measure the level of DNA methylation, including methylation-specific polymerase chain reaction (MSP), methylation sensitive high-resolution melting (MS-HRM), sensitive melting analysis after real time methylation specific PCR (SMART-MSP) as well as sequencing analysis. In cancer research, especially for the early diagnosis, tumor-derived material is hard to detect because of the presence of material from normal cells, and thus highly sensitive methods are needed. The protocol described demonstrates a detection sensitivity of at least 1% or lower methylated template with MS-HRM and 0.1% methylated template with SMART-MSP. The procedure described overcomes many of the common challenges of methylation detection after bisulfite conversion. Very low sample input down to 50pg genomic DNA or 5 cells for each conversion reaction can be used to achieve a 100% conversion rate, avoiding the false-positives inherent to incomplete conversions. In addition, this method allows for single molecule PCR which could greatly facilitate single cell epigenetic studies as well as clinical diagnosis with FFPE, blood cards and tissue LCM samples.

## 2978/F/Poster Board #910

**Studying the extent and function of epigenetic variation in twins.** *R. Lyle[1], G. Gilfillan[1], K. Gervin[1], H.E. Akselsen[1], M. Hammerø[1], R. Moe[1], H. Gjessing[2], J. Harris[2], D.E. Undlien[1,3].* 1) Department of Medical Genetics, Oslo University Hospital Ullevål, Oslo, Norway; 2) Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway; 3) Institute of Medical Genetics, Faculty Division Ullevål University Hospital, University of Oslo, Oslo, Norway.

The phenotypic differences between individuals are an outcome of genetic and epigenetic variation. Whereas variation at the sequence level (SNPs and CNVs) has been studied extensively, much is still unknown regarding the extent and function of epigenetic variation. Disturbance in DNA methylation leading to aberrant gene expression has been shown to be involved in many diseases, and variation in DNA methylation may contribute to the risk of common disease. The aim of this study is to explore variation and patterns of epigenetic variation using twins. Since each cell type has its own epigenome, we have isolated different lymphocyte subpopulations (CD19+, CD8+, CD4+ and CD4+CD25+) from more than 300 twin pairs. The results presented here are from CD4+ cells. We have used both region-specific and genome-wide analyses. First, we studied DNA methylation in the classical human major histocompatibility complex (MHC). The MHC is a gene dense and highly polymorphic region on human chromosome 6p21.3, containing genes with a broad range of functions within the innate and adaptive immune systems. We performed extensive bisulphite sequencing of 1670 individual CpG sites distributed in 176 regions in the classical human Major Histocompatibility Complex (MHC) in 49 monozygotic (MZ) and 40 dizygotic (DZ) healthy Norwegian twin pairs. Regions of interest include CpG islands, the 5'end of genes and non-coding conserved regions. We observed significant variation in DNA methylation both between and within regions. Interestingly, the heritability of this variation is low, ~6% for individual CpGs and ~11% for amplicons, suggesting DNA methylation variation is not under strong genetic control. By measuring gene expression levels in 42 genes in the MHC, the influence of DNA methylation variation on gene expression variation can be seen. Second, we developed methods to perform genome-wide bisulphite sequencing using the Illumina platform. In parallel, covalent histone modifications were examined by chromatin immuno-precipitation (ChIP). Modifications associated with both transcriptionally active chromatin (H3K4me1 and H3K4me3) and those associated with repressive heterochromatin (H3K9me3 and H3K27me3) were examined. Immunoprecipitated DNA was quantified by high throughput sequencing (ChIP-seq). Data collected so far document the variation seen between three normal MZ twins pairs, the link between histone modifications and DNA methylation on a global scale.

## 2979/F/Poster Board #911

**DHPLC screening strategy identifies novel apoptotic gene targets of aberrant promoter hypermethylation in prostate cancer.** *T.M. Murphy, A.S. Perry, C. Lane, L. O'Connor, M. Lawler.* Prostate cancer research group, Institute of Molecular Medicine, Dublin , Leinster, Ireland.

Introduction: Aberrant DNA methylation has been implicated as a key survival mechanism in cancer, whereby promoter hypermethylation silences genes essential for many processes including apoptosis. To date, only limited data exists on the methylation status of apopotic genes in prostate cancer. The aim of this study was to profile methylation of apoptotic-related genes in prostate cancer using a Denaturing High Performance Liquid Chromatography (DHPLC) platform. Methods: A bioinformatics approach was first applied to generate a list of apoptotic genes, which were identified as possible targets of methylation in prostate cancer. PCR assays were designed to amplify whole CpG islands in the gene promoters. Genes were screened for CpG methylation in a panel of prostate cancer cell lines (LNCaP, DU145, PC-3, 22RV1, RC58) and normal prostate cell lines (RWPE1 and PWR1E) using an automated (DHPLC) instrument. Methylation levels were quantified using real time quantitative methylation specific PCR (QMSP). Quantitative real time PCR was performed in our panel of prostate cancer cell lines and PC3 cells treated with a methylation inhibitor (5aza2'deoxycytidine). Results: Our DHPLC methylation screen identified seven targets of CpG promoter methylation, three of which are novel targets of methylation in prostate cancer (BIK, BNIP3 and CFLIP), while our methylation findings for TMS1, DCR1, DCR2 and CDKN2A are consistent with previous methylation studies in prostate cancer. In order to assess the methylation status of individual CG sites QMSP assays were designed to encompass CG sites in the 5'UTR and/or transcriptional elements identified by our promoter CpG island characterisation. Our gene expression analysis for BIK, TMS1, CDKN2A and DCR1/2 shows a significant decrease in gene expression in PC3 cells compared to normal RWPE1. These findings are consistent with our methylation results, showing strong methylation of these genes in PC3 cells. Furthermore, treatment of PC3 cells with a methylation inhibitor causes a moderate to strong increase in expression in each of these genes. Conclusion: Methylation levels are currently being quantified using QMSP in a prostate cancer biorepostitory, representing prostate cancer, normal adjacent prostate and benign prostatic hyperplasia. Determining the methylation profile of a fundamental cellular process such as apoptosis could yield a biomarker signature for early disease and disease progression in prostate cancer.

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## 2980/F/Poster Board #912

**Identification of Differential Methylation in Normal and Cancer Cells.**
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Epigenetic mechanisms, such as DNA methylation and histone modification, play critical roles in the development of normal cellular differentiation, as well as many human diseases including cancer, pediatric syndromes and genetics disorders. Understanding the role epigenetics plays in cellular differentiation and the development of disease will ultimately lead to the development of diagnostics and hopefully preventative and therapeutic options. Recent advances in high density oligo microarray platforms affords the opportunity to examine epigenetic events using methyl-DNA immunoprecipitation followed by microarray (MeDIP-chip) assays at unprecedented scale and resolution. This study was undertaken to measure the sensitivity and reproducibility with which DNA methylation can be detected with MeDIP-chip on samples that demonstrate differential methylation in imprinted regions of the genome and between normal and cancer cells. Each genomic DNA sample was fragmented and immunoprecipitated using an antibody against 5-methyl-cytosine. IP sample and input genomic DNA were labeled with Cy5 and Cy3, respectively, and hybridized to the array, which contains probes tiled across all annotated CpG islands, gene promoters, and miRNA promoters. Data demonstrate that (1) methylated DNA fragments with as few as two methyl groups can be detected and with high reproducibility (R= 0.94) using the reported methods, (2) the positive, negative and non-CG control regions on the array correlate with expected methylation status making them a useful indicator for assessing MeDIP-chip performance, and (3) differential regions of imprinting are sensitively and accurately identifiable between normal male, normal female, and cancer cells.

## 2981/F/Poster Board #913

**The relationship of DNA methylation with age, gender and genotype in twins and healthy controls.**
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Cytosine-5 methylation within CpG dinucleotides is a potentially important mechanism of epigenetic influence on human traits and disease. In addition to influences of age and gender, genetic control of DNA methylation levels has recently been described. We used whole blood genomic DNA in a twin set (23 MZ twins and 23 DZ twins, N=92) as well as healthy controls (N= 96) to investigate heritability and relationship with age and gender of selected DNA methylation profiles using readily commercially available GoldenGate bead array technology. Despite the inability to detect meaningful methylation differences in the majority of CpG loci due to tissue type and locus selection issues, we found replicable significant associations of DNA methylation with age and gender. We identified associations of genetically heritable single nucleotide polymorphisms with large differences in DNA methylation levels near the polymorphism (cis effects) as well as associations with much smaller differences in DNA methylation levels elsewhere in the human genome (trans effects). Our results demonstrate the feasibility of array-based approaches in studies of DNA methylation and highlight the vast differences between individual loci. The identification of CpG loci of which DNA methylation levels are under genetic control or are related to age or gender will facilitate further studies into the role of DNA methylation and disease.

## 2982/F/Poster Board #914

**DNA methylation and the evolution of gene regulation in primates.**
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Heritable DNA methylation is an important epigenetic mechanism, which affects the regulation of gene expression. Specifically, we know that many spatial and temporal gene expression patterns are the result of regulation through DNA methylation. However, while methylation patterns have been described in many contexts within species (for example, across different human tissues), the extent to which these signatures are conserved across primates has not been well characterized. To this end, we assayed genome-wide DNA methylation patterns in liver, heart, and kidney samples from multiple humans and chimpanzees, and combined these data with previously collected gene expression data from the same individual tissue samples. Using the multi-species gene expression and methylation data, we were able to study the evolution of gene regulation in the context of conservation or changes in DNA methylation patterns across species. Specifically, we confirmed that hyper-methylation is correlated with decreased expression levels across tissues and species, and identified individual genes whose regulation across tissues and species can be explained, at least in part, by specific methylation patterns.

## 2983/F/Poster Board #915

**MAOA Promoter Methylation Is Associated with Nicotine Dependence in DNA Prepared from Lymphoblasts and from Whole Blood.**
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Introduction: Nicotine Dependence (ND) results from a dynamic, chronologically dependent, interplay of genetic and environmental, and gene-environment interactions. To date, a handful of small effect sequence variants have been isolated, but the majority of the biological factors, both genetic and epigenetic, that initiate, promote and maintain ND are still undefined. In previously published work using DNA and clinical material from 192 subjects from Iowa Adoption Studies (IAS), we demonstrated that methylation of the two MAOA promoter associated CpG islands was associated with lifetime history of ND and with MAOA genotype. This is of particular interest because the maintenance of ND is thought to be mediated through dopaminergic mechanisms, MAOA is the key enzyme in the catabolism of dopamine, and some agents used to treat ND target MAOA. Objective and Methods: To extend to confirm these prior findings, we re-examined these relationships at MAOA using DNA and clinical material from another 289 subjects using the same methodologies. Results: First, we demonstrate that the methylation signature at the MAOA promoter associated CpG islands is dependent on current smoking status and MAOA genotype. Second, we conduct factor analyses to demonstrate that there appears to be three distinct regions of this CpG enriched region that may function in different ways for males and females. Third, we directly compare methylation signatures in DNA whole blood and from lymphoblasts and provide suggestive evidence favoring the use of lymphoblasts. Conclusion: We conclude that smoking decreases methylation at the MAOA promoter associated CpG islands with the exact extent being dependent on the region being examined, MAOA genotype, smoking history and gender.

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## 2984/F/Poster Board #916

**A human genome-wide study of aging-associated DNA methylation dynamics.** *V.K. Rakyan[1], S. Maslau[2], T. Andrew[3], M. Falchi[4], T.A. Down[2], T.D. Spector[3].* 1) Queen Mary University of London, London, United Kingdom; 2) The Gurdon Institute, University of Cambridge, Cambridge, UK; 3) King's College London St. Thomas' Hospital, Twin Research and Genetic Epidemiology Department, London, UK; 4) Genomic Medicine, Imperial College London, London, United Kingdom.

Aging is a complex multidimensional process associated with biological, psychological, and social changes. In biological terms, aging can be defined as cellular senescence, which results in a diminished ability to respond to stress, increased homeostatic imbalance and risk of disease, and eventually death. Research in a variety of organisms has revealed that many factors impact on the aging process at the molecular level. These include telomere shortening, accumulation of genetic mutations, oxidative stress, and molecular pathways altered by quantitative and qualitative changes in nutrition. More recently, evidence has emerged to suggest that stochastic, or possibly even genetically programmed, events that alter transcriptional and epigenetic programs are involved in the aging process in a variety of organisms including humans. Epigenetic modifications, such as DNA methylation and post-translational modifications of histone proteins, are indispensable for many aspects of genome function including gene expression. The perturbation of epigenetic and transcriptional landscapes during aging could potentially have major implications for cellular function. We have performed a genome-wide study of DNA methylation dynamics during human aging. Methylated DNA Immunoprecipitation (MeDIP) was combined with custom microarrays (MeDIP-chip) to generate high-resolution DNA methylation profiles of >40,000 regulatory features (nearly all known CpG islands and promoters, along with other known elements such as enhancers and CTCF binding sites) in whole blood from 22 monozygotic (MZ) twin pairs spanning a >50 year age range. An important feature of this project was that two time-points (10 years apart) were analyzed for each monozygotic twin pair, allowing elucidation of age-related DNA methylation dynamics both cross-sectionally in unrelated individuals, and longitudinally in the same individual. Key findings include: (i) a genetic influence on age-related DNA methylation dynamics; (ii) identification of the regulatory elements in the human genome that are most susceptible to age-related DNA methylation changes in whole-blood; (iii) identification of the key molecular pathways that display perturbed DNA methylation profiles. Overall, our study represents the first truly genome-wide study of aging-related epigenetic changes in mammals and provides novel insights into the molecular events that underlie the aging process.

## 2985/F/Poster Board #917

**Identification of tissue-specific methylation signatures in the 2nd trimester fetus and placenta.** *W. Robinson, L. Avila, M. Peñaherrera, R. Yuen, D. Diego-Alvarez, D.E. McFadden, M. Kobor.* Dept Med Gen, Univ British Columbia, Vancouver, BC, Canada.

While it is well established that there are tissue-specific methylation patterns, the exact nature and extent of these is not well-known. To investigate this, the Illumina GoldenGate Methylation Cancer Panel I (1,505 CpG loci from 807 genes) was used to interrogate DNA extracted from 2nd trimester fetal tissues (kidney, brain, lung, muscle, skin) and placenta (trophoblast and mesenchyme) samples from 6 chromosomally normal and 8 trisomic pregnancies. Hierarchechal clustering showed that the DNA methylation profile of placental trophoblast was distinct from all other tissues (average r=0.72). Placental mesenchyme clustered more closely with fetal tissues (r=0.87) than placental trophoblast, consistent with its developmental origin from the epiblast. The distribution of DNA methylation values was similar amongst all placental and fetal tissues, with the majority of CpG-island associated CpGs (N=1044) being hypomethylated (<10% methylation) and most non-island associated CpGs (N=461) highly methylated (>75%). However, using Significance Analysis of Microarrays (SAM) with a False Discovery Rate (FDR) of <1% and an absolute difference of >20%, numerous sites were identified as differentially hypomethylated (N=101) or hypermethylated (N=165) in trophoblast as compared to mesenchyme, supporting a role for DNA methylation in gene regulation in the placenta. A number of tumor suppressor genes were specifically hypermethylated in trophoblast, consistent with its invasive properties. Similarly, 429 of 1454 autosomal CpGs showed significant tissue specific differences in methylation by ANOVA (P<0.05) when considering brain, kidney, skin, lung and muscle only. From among these candidates, we were able to identify a subset of "signature CpGs" for which the methylation status is predictive of tissue type. Identification of normal tissue specific patterns of methylation can thus provide useful tools for identification of the tissue-source of biological specimens of unknown origin, as well as providing a framework to understand normal and abnormal development.

## 2986/F/Poster Board #918

**DNA methylation for subtype classification and prediction of treatment outcome in childhood acute lymphoblastic leukemia.** *A-C. Syvänen[1], L. Milani[1], A. Lundmark[1], J. Nordlund[1], A. Kiialainen[1], M. Gustafsson[1,2], G. Lönnerholm[3], Nordic Society of Pediatric Hematology and Oncology.* 1) Dept of Medical sciences, Uppsala University, Uppsala, Sweden; 2) Linneaus Centre for Bioinformatics, Uppsala University, Uppsala, Sweden; 3) Dept of Children's and Women's Health, Uppsala University, Uppsala, Sweden.

Cells from patients with acute lymphoblastic leukemia (ALL) are characterized by complex genomic rearrangements, but the specific genes and molecular mechanisms that cause ALL and affect clinical outcome remain to be elucidated. We have previously used allele-specific gene expression measured by quantitative genotyping of single nucleotide polymorphisms in 8000 genes as a guide for identifying genes with cis-acting regulatory elements in primary ALL cells (Milani et al. 2009 Genome Res.19:1). Based on this analysis we selected 381 genes with putatively strong cis-acting regulatory factors for DNA methylation analysis. We genotyped 1320 CpG sites in the regulatory regions of these genes in bisulphite-treated DNA samples from 400 bone marrow or peripheral blood samples from children with ALL. We found that CpG sites located outside "CpG islands" had higher methylation levels and larger variability in CpG site methylation than CpG sites within "CpG islands". Binary-split hierarchical clustering of 300 CpG sites with the largest inter-individual variation in their methylation levels stratified the patients with high hyperdiploidy (HeH) and t(12;21) ALL into two subgroups with different probability of disease-free survival. The two HeH groups also differed in their *in vitro* sensitivity to doxorubicin and vincristine. Using supervised learning we constructed multivariate classifiers in an external cross-validation procedure. We identified nine genes that consistently contributed to accurate discrimination between T-ALL and BCP ALL, and a corresponding set of 44 genes for discrimination between the main subtypes of BCP ALL. By Kaplan-Meijer analysis of patients grouped according to methylation levels of individual CpG sites, we identified 28 genes that appeared to predict relapse of leukemia. We conclude that DNA methylation analysis should be further explored as a method to improve stratification of ALL patients for different treatment protocols.

## 2987/F/Poster Board #919

**Global DNA methylation in polycystic ovary syndrome.** *N. Xu[1], R. Azziz[2], M.O. Goodarzi[1,2,3].* 1) Div Endocrinology, Cedars Sinai Medical Center, Los Angeles, CA; 2) Department of Obstetrics and Gynecology, Center for Androgen Related Disorders, Cedars-Sinai Medical Center, Los Angeles, California,; 3) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, California.

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, occurring in about 7% of reproductive age women. Although its genetic basis has been established, causative genes for PCOS remain elusive. Prenatally androgenized animal models suggest intrauterine androgen exposure during development may predispose individuals to PCOS. This phenomenon has been formulated as the fetal origins hypothesis of PCOS. However, the underlying mechanism is unknown. Environmental factors during development may lead to permanent changes via epigenetic modification. DNA methylation is an important mechanism of epigenetics, which plays a role in cancer, aging and complex chronic diseases. In the present study, we began to determine the role of epigenetics in the development of PCOS. DNA was extracted from peripheral leukocytes of 20 PCOS patients and 20 age- and BMI-matched control women. The total amount of methylated DNA was measured through an ELISA-like reaction. A dilution series of a 100% methylated control reagent was included in each experiment to generate a standard curve, which was used to calculate absolute global methylation percentage. The global methylation percentage for each subject was obtained by taking an average of triplicate results, and then compared between women with PCOS and controls. A ratio representing the global methylation alteration of each PCOS subject compared to an age- and BMI- matched control was examined for each PCOS subject as well. The median (interquartile range) global methylation percentages were 5.70% (2.40%) for PCOS women and 5.75% (2.75%) for controls. We found no significant difference in the global methylation percentage in PCOS patients compared to matched controls (Mann-Whitney U test: P=0.79; non-parametric one-sample sign test for Log[ratio]: P= 0.82). However, it was observed that five out of 20 PCOS samples (25%) had normal ratios (defined as a ratio 0.8-1.2), and 15 PCOS samples (75%) had abnormal ratios, including 9 (45%) with hypermethylation (ratio >1.2) and 6 (30%) with hypomethylation (ratio <0.8). In conclusion, the global methylation percentage in peripheral leukocyte DNA is not significantly altered in PCOS women compared to matched controls. Our study represents an initial step focusing on DNA methylation in PCOS, which deserves further effort to elucidate the epigenetic process in tissues more relevant to PCOS (e.g. ovary, adrenal, adipose) and/or specific target genes or regions.

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## 2988/F/Poster Board #920

**Identification of epipolymorphisms in the human placenta.** *R. Yuen[1], M. Peñaherrera[1], P. von Dadelszen[2], L. Lefebvre[1], M. Kobor[1], W. Robinson[1].* 1) Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Department of Obstetrics and Gynaecology, University of British Columbia, Vancouver, BC, Canada.

Genomic sequence polymorphisms, such as single nucleotide polymorphisms (SNPs) and copy number variants (CNVs), have been widely investigated in a whole genome fashion. However, little is known of genome-wide epigenetic variation among individuals. To identify genes with a high degree of interindividual DNA methylation variation in the human placenta, we surveyed the human genome using the Illumina GoldenGate Methylation Cancer panel targeting 1505 CpG sites of 807 genes in 62 placental samples. Several placenta-related genes were identified to have a polymorphic bimodal distribution of DNA methylation variation at their promoter region which was confirmed by bisulfite pyrosequencing. The methylation polymorphism at the promoter of these genes is concordant with mRNA allelic expression, suggesting it can be a cis-acting factor affecting gene expression. Its placenta specificity and absence in mouse implies a biological significance to the phenotypic diversity of human placenta. Similar to the epimutation of MLH1 and MSH2 found in cancer patients, epipolymorphism identified here can also silence the genes that are crucial for placental development in an allele-specific manner. Thus further investigation of epipolymorphism in human tissues should improve our understanding of interindividual phenotypic variability and complex disease susceptibility.

## 2989/F/Poster Board #921

**Hypermethylation of *OPRM1* in DNAs Extracted from Peripheral Blood of Alcoholics.** *H. Zhang[1,2], A.I. Herman[1,2], H.R. Kranzler[3], R.F. Anton[4], A.A. Simen[1], J. Gelernter[1,2].* 1) Psychiatry Dept, Yale University School of Medicine, New Haven, CT; 2) VA Connecticut Healthcare System / 116A2, West Haven, CT; 3) Psychiatry Dept, University of Connecticut School of Medicine, Farmington, CT; 4) Psychiatry and Behavioral Sciences Dept, Medical University of South Carolina, Charleston, SC.

The $\mu$-opioid receptor (MOR) mediates the rewarding effect of opioids, non-opioid drugs and alcohol. A recent study demonstrated an increased DNA methylation level in the MOR gene (*OPRM1*) in a sample of former heroin addicts. Based on this finding, we hypothesized that hypermethylation of *OPRM1* also occurred in subjects with alcohol dependence (AD). We examined the methylation status of 16 CpG sites in the potential regulatory region (93 bp upstream and 84 bp downstream of the translation start site) of *OPRM1* in 190 European Americans (EAs) with AD and 133 EA healthy controls. Direct sequencing of bisulfite-treated DNA (extracted from peripheral blood) indicated that the overall methylation level of the 16 CpG sites was elevated in AD cases (7.8%) compared with controls (5.0%) ($\chi^2$=14.36, df=1, $P$=0.0002). Individual CpG site analyses showed that three CpG sites (at -80, -25 and -18) had significantly increased methylation levels in AD cases (11.6%, 15.3% and 8.4%, respectively) compared to controls (3.0%, 7.5% and 2.3%, respectively) (-80CpG: $\chi^2$=7.77, df=1, $P$=0.0005; -25CpG: $\chi^2$=4.42, df=1, $P$=0.032, and -18CpG: $\chi^2$=5.37, df=1, $^P$=0.032). Controlling for sex and age using backward logistic regression analysis, two CpG sites showed significantly elevated methylation in AD cases (-80CpG: $\beta$=1.41, $P$=0.013, odds ratio=4.08; -18CpG: $\beta$=1.40, $P$=0.042, odds ratio=4.06). The findings from this research indicate that *OPRM1* methylation is associated with AD in EAs. Functional studies are needed to clarify whether CpG hypermethylation in the *OPRM1* regulatory region alters *OPRM1* expression levels.

## 2990/F/Poster Board #922

**CpG methylation proximal to the CTG/CAG tract of the DM1 locus in patients and transgenic mice.** *C.E. Pearson[1,2], M. Nakamori[3], D. Chitayat[1,2,4,5], G. Gourdon[6], C.A. Thornton[3], A. López Castel[1].* 1) Genetics and Genome Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 3) Department of Neurology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA; 4) Deparment of Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, Canada; 5) Department of Pediatrics, Hospital for Sick Children, Toronto, Canada; 6) Inserm U871, Hopital Necker-Enfants Malades, Paris, France.

Myotonic dystrophy type 1 (DM1), as well as other trinucleotide repeat diseases, are often characterized by complex patterns of inherited and tissue-specific instability and pathogenesis. Disease progresses as the patient ages and is coincident with increasing instability. Repeat-expansion associated epigenetic effects have been recently described at the DMPK locus, although a link to tissue-specificity had not been assessed. We established a highly detailed DNA methylation profile of the DM1 locus in several human (fetuses and adult autopsies) and transgenic DM1 mouse tissues that included tissues from the central nervous system, different muscles and various organs. Our analysis included the CTCF binding sites upstream and downstream of the DM1 CTG tract, as the methylation-sensitive binding of this insulator protein has been demonstrated to affect chromatin packaging, transcriptional regulation of the DMPK locus, and possibly CTG instability. We studied the effects of CTG repeat size, tissue source and age of patient/mouse upon methylation pattern. CpG methylation profiles showed a strong link between appearance of this DNA modification and long expanded alleles, but evident only upstream (5') from the CTG repeat including that methyl-sensitive CTCF binding site. This enrichment of methylation was evident only in patient or mouse tissues with a CTG expansion, and appeared to be specific for the expanded allele. Tissue-specific differences in methylation and CTG instability were evident, with some tissues consistently showing either high levels or low levels of methylation. In addition, age-dependant changes in methylation levels were apparent with a decrease in degree with age. Our data offer novel insights into the dynamics and regulation of the DM1 disease locus at the epigenetic level.

## 2991/F/Poster Board #923

**Maternal, but not paternal, interstitial duplications of chromosome 15q11.2-q13 are associated with ASD in 7 individuals.** *L. Reiter[1,2], J. Cleary[3], V. Brewer[2], J.T. Jabbour[4], N.C. Schanen[5], N. Urraca[1].* 1) Dept Neurology, Univ Tennessee HSC, Memphis, TN; 2) Dept Pediatrics, Univ Tennessee HSC, Memphis, TN; 3) Dept Audiology Speech Lang Path, University of Memphis, Memphis, TN; 4) Memphis Neurology, Memphis, TN; 5) Dept Biological Sciences, University of Delaware, Newark, DE.

It has been estimated that ~3-5% of all autism cases may be the result of duplications of the 15q11-q13 region. Unfortunately, genotype-phenotype correlations have not consistently demonstrated that these duplications are the sole cause of autism spectrum disorder (ASD) in these cases. Maternally transmitted 15q duplications consistently show autistic features with variable degrees of developmental delay. Only a few cases of paternally transmitted 15q duplication have been reported and are associated with speech delay and behavior problems, but not autism. The aims of the present study were to perform an in depth phenotype analysis of individuals with interstitial 15q duplications and determine if maternal duplication is required for the diagnosis of ASD. We used neuropsychological, language and ASD diagnostic tools for phenotypic analysis in patients recruited through the IDEAS parent support group (www.idic15.org). In addition we performed High Resolution Melting analysis (HRM) of the maternally methylated SNRPN locus to determine the parent of origin of the duplications. Eight pediatric subjects with interstitial duplication 15q have participated in the study. Seven patients scored as ASD upon ADOS/ADI-R analysis and one scored "no ASD" on both tests. In the neuropsychological evaluations 3/8 patients had a low average IQ score, 2/8 were borderline and the others had mental retardation, although the patients were noted to have a higher verbal IQ than performance, they had a low-moderate adaptive functioning score on the Vineland II evaluation. All patients performed below age corrected average for receptive vocabulary (Peabody Picture vocabulary test). HRM analysis was done in 7 patients: 6/7 had a maternal duplication and 1/7 had a paternal duplication. The sizes of the duplications ranged from 5Mb to 12.77Mb and included typical BP1-BP3, BP2-BP3 and duplications including the BP1-BP3 region plus additional regions upstream or downstream. The size of the duplication involved does not appear to correlate with the severity of the phenotype or the ASD diagnosis. In conclusion: in our study only maternally derived interstitial duplications result in ASD, not paternal duplications. It should be noted that several of our patients were adopted so parental DNA was unavailable for genotype analysis. HRM analysis for maternal specific methylation patterns at the SNRPN locus allowed us to determine the parental origin of the duplication in all samples.

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## 2992/F/Poster Board #924

**Allelic mRNA expression imbalance in inflammasome related genes.**
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Multiprotein complexes, termed inflammasomes, are assembled in a stimulus dependent manner for the activation of caspase-1 and ultimate release of the endogenous pyrogen, interleukin-1β (IL-1β) and the interferon inducing factor, IL-18. Because IL-1β and IL-18 are central to the innate host response, dysregulated inflammasome functioning is associated with numerous diseases and disorders including auto inflammatory diseases, autoimmunity, and leukemias. Therefore, naturally occurring genetic variations within any of the components of the inflammasome have the potential to modulate the activation and assemblage of this complex. One important variation that, to our knowledge, has not yet been systematically investigated in the context of inflammasome function is the possibility that regulatory polymorphisms in specific inflammasome genes may result in altered mRNA expression, which is detectable as an allelic mRNA expression imbalance in subjects heterozygous for a marker SNP in the transcribed exonic region of the genes. These so called, “cis acting regulatory polymorphisms” are thought to account for a main portion of genetic variability in humans. To search for these cis acting polymorphisms, we have selected 6 genes of particular importance to inflammasome function: NLRP1, NLRP3, PYCARD, MEFV, CASP1 and CASP12. We have used the public data bases to identify TagSNPs in exonic regions that have high frequency of heterozygosity within these 6 inflammasome genes. We will use a single base extension technique (SNaPshot, Applied Biosystems) to measure allele mRNA expression ratios in primary human blood monocytes and MCSF derived macrophages from heterozygous individuals, in comparison to the corresponding genomic allele ratios measured in the same subjects. A relative deficit in one allele compared to the alternate allele suggests a cis acting regulatory element within the gene locus of interest. Using this approach we show that specific inflammasome genes will be regulated at least in part by cis acting genetic elements. We show differential expression of similar genotypes. Heterozygotes exhibit monoallelic expression of inflammasome gene maybe due to DNA methylation. For those monoalleleic expressions that are not found to be due to DNA methylation, extracellular stimulus (LPS)is able to restore the heterozygous state. This finding has profound implications for interpretations of genetic linkage, association studies and pharmacogenetics.

## 2993/F/Poster Board #925

**MicroRNAs involved in BMP2 mediated osteoblast differentiation.**
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Osteoblast differentiation requires tight regulation of program of gene activation and suppression in response to physiological ques. Bone morphogenic proteins (BMPs) are potent morphogens that activate transcriptional programs of mesenchymal stem cells to commit into specific lineage. BMP2 can activate osteoblastogenesis via activating Runx2 and Osterix, which are osteoblast specific transcription factors. MicroRNAs (miRNAs) have emerged as key negative regulators of various biological and pathological processes, including developmental timing, organogenesis, apoptosis, cell proliferation, and differentiation and in the control of tumorigenesis. How BMP2 mediated osteogenesis is regulated by miRNAs remains unknown. In this study, to identify miRNAs related to the osteoblast differentiation, Exiqon miRNA array was performed using total RNA from 72 hours of BMP2-treated and vehicle treated C2C12 premyoblast mesenchymal cells. Two biological replicates were included for each BMP2 treated and control group. In response to BMP2, Runx2 and Osterix mRNA were induced along with other osteoblast markers indicating the osteoblast lineage was committed by BMP2 treatment. The result showed that the total number of 34 miRNAs were more than 2 fold differentially expressed between control and BMP2 treated groups. Among BMP2 mediated up-regulated miRNAs, miR-140 was enriched which has identified as cartilage specific miRNA previously. Fourteen miRNAs were down-regulated in response to BMP2, among these, myogenic miRNA including miR-206, miR-1, miR-133b and miR-133a were all significantly down-regulated. Our study suggest that BMP2 can promote osteogenic lineage commitment by tightly regulating miRNAs by both suppressing alternative lineages and relieving the repression of the targets of miRNAs specific to bone differentiation. However, the analysis of putative targets of miRNAs relevant to osteoblast lineage is still under investigation to understand the function of miRNA during bone development.

## 2994/F/Poster Board #926

**Role of microRNAs in the Regulation of Telomerase (hTERT) in Ovarian Cancer.**
*B. Herbert, M. Fox, S. Skillman.* Dept Med and Molec Gen, Indiana Univ Sch Medicine, Indianapolis, IN.

A hallmark of cancer is its limitless proliferative potential which is governed in part by elevated levels of human telomerase (hTERT) or telomerase activity. Most normal cells do not have sufficient expression of active telomerase and are mortal, while an estimated 90% of cancer cells express active telomerase. Therefore, the understanding of how telomerase can be regulated in normal cells, and how this regulation can be lost during cancer progression, has led to the search for suppressors of telomerase. MicroRNAs (miRNA or miR) are evolutionarily conserved, small, non-coding, single-stranded, ~19-23 nucleotide RNA molecules that are estimated to negatively regulate protein encoding genes. Recent evidence has shown that alteration of microRNA levels can lead to cellular transformation and cancer development. We aimed to test the hypothesis that microRNAs play a role in telomerase regulation in normal cells and that hTERT microRNA expression is lost during cancer progression leading to active telomerase and sustained growth. Based on *in silico* analyses, microRNAs were predicted to target different regions of the hTERT mRNA, suggesting that telomerase expression in normal cells may be regulated in part by microRNA-mediated repression of hTERT mRNA translation and ultimately protein expression. By using commercial microRNA isolation and detection protocols, we demonstrated a correlation of the predicted hTERT microRNAs with the levels of telomerase in normal and cancerous (of different stages and recurrence) ovarian cells. We identified nine candidate microRNAs that may play a role in telomerase regulation. Next, we observed that precursor microRNAs to these candidate microRNAs reduced telomerase activity in ovarian cancer cells and their cell growth. Telomerase inhibition by microRNA can thus lead to new nonviral gene therapy as well as an understanding of how cancer progresses (from normal, early, then to late stage).

## 2995/F/Poster Board #927

**Systematic gene regulation pathway involving functional small RNAs during neuronal differentiation of mouse embryonic carcinoma cell.**
*H. Hohjoh, A. Eda, Y. Tamura, M. Yoshida.* NCNP, Natl Inst Neuroscience, Tokyo, Japan.

MicroRNAs (miRNAs) are small noncoding RNA, with a length of 19- to 23-nucleotide, which are processed from longer transcripts by digestion with a microprocessor complex in the nucleus and Dicer in the cytoplasm. Matured miRNAs are incorporated into the RNA-induced silencing complex (RISC) and function as mediators in gene silencing; and the gene silencing involving miRNAs appears to play an important role in regulation of gene expression in development, differentiation and proliferation. In this study, we examined a gene regulation system during neuronal differentiation of mouse P19 embryonic carcinoma cells and described a systematic gene regulation pathway involving miRNAs. In the pathway, the upstream Lin-28 gene is suppressed and let-7 is upregulated after induction of the differentiation, thereby triggering suppression of the downstream High Mobility Group A2 (Hmga2) gene expression via activation of gene silencing mediated by the let-7 miRNAs. Our data further suggest that miR-9, as well as miR-125b, participate in the reduction of the Lin-28 gene expression, and that the pathway for the gene regulation is already constructed in undifferentiated P19 cells. Taken together, such a systematic pathway for gene regulation likely contributes to a rapid and programmed change in gene expression under cell differentiation.

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## 2996/F/Poster Board #928

**A Highly Effective and Long-lasting Inhibition of miRNAs with PNA-based antisense oligonucleotides.** *S. Oh, Y. Ju, H. Park.* Panagene, Daejeon, Korea.

MicroRNAs (miRNA) are non-coding RNAs, of approximately 22 nucleotides in length. They play a role in the regulation of such major processes as cellular development, differentiation and apoptosis. The inhibition of miRNA using antisense oligonucleotide (ASO) is a unique and effective technique for characterizing miRNA function and subsequent therapeutic targeting of miRNA. Recently, advances in ASO chemistry are increasingly being utilized to increases nuclease-resistance, target affinity and specificity. PNAs are artificial oligonucleotides constructed on a peptide-like backbone. PNAs have a stronger affinity and greater specificity than DNA or RNA and are resistant to nuclease, which is essential characteristic for a miRNA ASO that will be exposed to abundant serum and cellular nucleases. We evaluated the effect of a PNA-based ASO on anti-miRNA activity. In this experiment, PNAs were conjugated with CPPs at N-terminal for increasing cell penetration. We studied cell penetration of CPP-conjugated PNAs by confocal microscopy. Among the tested 10 types of CPPs, Tat-modified peptide-conjugated PNAs have most effective function for cell penetration. We confirmed that PNA-based ASOs are more effective ASOs than LNA-modified DNA and 2'-O-Methyl-oligonucleotide. Microarray and real time PCR analysis confirmed that the level of miRNA expression was decreased by PNA-based ASO in dose-dependent manner. These miRNA inhibition effects by PNA-based ASO were sustained for 15 days after transfection. We found that inhibition activity of PNA-based ASO can be lasted after 20 week storage at room temperature. PNA-based ASOs showed lower cytotoxicity than other nucleic acid-based miRNA ASOs. Especially, we tested PNA-based ASOs for over 100 miRNAs and found that all of the PNA-based ASO could act as powerful miRNA ASOs. These results demonstrated that PNA-based ASO was demonstrated to be a more effective inhibitor of miRNA than other miRNA ASOs.

## 2997/F/Poster Board #929

**Transcription Control and Myogenesis: Mapping Genome-Wide Changes In DNaseI-Hypersensitive Sites in Chromatin.** *M. Ehrlich[1], K. Tsumagari[1], L. Song[2], T.S. Fuery[2], D. London[2], A.P. Boyle[2], G.E. Crawford[2].* 1) Hayward Genetics Program, SL31, Tulane Med. Sch., New Orleans, LA; 2) Inst. for Genome Sciences & Policy, Duke Univ., Durham, NC.

Induction of the formation of multinucleated myotubes from cultured human myoblasts is one of the best *in vitro* models for human cell differentiation. Nonetheless, high-resolution genome-wide studies of human myoblast epigenetics have been lacking. We have begun mapping DNaseI-hypersensitive sites (DH sites) throughout the genomes of human myoblast cell strains before, during, or after differentiation to myotubes. The analysis used next-generation DNA sequencing (DNase-seq). DH sites represent nucleosome-free regions that are usually associated with transcription control elements actively engaged in or poised for transcription. Our comparison of myoblasts and myotubes to other cell types indicated the expected muscle lineage-specificity for many DH sites in or near muscle-related genes. Some DH sites that were located 5' to a myogenesis-associated gene, inside it, or downstream increased in signal intensity in myotubes *vs.* myoblasts. Such genes included *MB* and *DES*, which encode myoglobin and desmin. Their increase in DH character is consistent with increased production of the gene product during differentiation of myoblasts to myotubes. Other genes had decreases in intensity of DH sites in myotubes *vs.* myoblasts. For example, *SMARCD3* had muscle lineage-specific DH sites near the 3' end that might indicate the presence of an enhancer. They were much reduced in myotubes relative to myoblasts. As a subunit of a chromatin remodeling protein, SMARCD3 was reported to be critically involved in positively regulating the onset of zebrafish myogenesis; therefore, downregulation of SMARCD3 production might be necessary once myogenesis is underway. Not only can DNase-seq reveal new transcription control elements associated with differentiation, but also it can point to unanticipated associations of genes with myogenesis. For example, *PLEKHA7*, a provisional gene that encodes a protein with actin cytoskeleton-like structure, had a greatly reduced DH site at its 5' end in myotubes compared with myoblasts, suggesting that the dramatic changes in cellular morphology during myotube formation require a decrease in PLEKHA7 production. We have demonstrated that DNase-seq is a powerful tool for analysis of myoblast differentiation and therefore should also be very valuable for elucidating gene dysfunction in muscular dystrophies. (Supported by NIH Grants NS048859 and HG003169, the FSH Global Research Foundation, and the National Human Genome Research Institute).

## 2998/F/Poster Board #930

**Widespread reprogramming of human DNA replication across cell lineages.** *R. Hansen.* Med/Div Med Gen, Univ Washington, Seattle, WA.

Faithful transmission of the genetic material to daughter cells involves a characteristic temporal order of DNA replication, which may play a significant role in the inheritance of epigenetic states. We developed a high-resolution; genome-scale approach to map newly replicated DNA using next-generation sequencing and applied it to study regional variation in human DNA replication time in multiple human cell types and embryonic stem cells. We find that different cell types exhibit characteristic replication signatures that comprise both regional variations in replication time during S-phase and also distinct patterns of replication fork movement. We observed striking plasticity in regional DNA replication patterns between cell types covering nearly 50% of the genome. Additionally, we identified hundreds of autosomal regions with marked biphasic replication timing, including some known imprinted regions as well as many previously uncharacterized domains. High-resolution genome-wide profiling of chromatin accessibility revealed that DNA replication stereotypically initiates in accessible chromatin domains comprising clustered DNaseI hypersensitive sites. The data collectively provide an unprecedented view of chromosomal replication patterns and a unique genome-wide picture of the epigenetic compartmentalization of the human genome.

## 2999/F/Poster Board #931

**Risk of tuberculosis (TB) among close contacts to TB patients: the role of Interleukin- 12 receptor, $\beta$1 gene polymorphisms and environmental factors.** *C. Luo[1], M. Reichler[1], B. Chen[1], E. Sigman[1], F. Maruri[2], T. Sterling[3].* 1) Division of TB Elimination, CDC, Atlanta, GA; 2) Tennessee Dept of Health, Nashville, TN; 3) Vanderbilt University, Nashville, TN.

**Background**: Single nucleotide polymorphisms (SNP) in the Interleukin-12 $\beta$1 receptor gene (IL12RB1) have been implicated in susceptibility to TB, but reports have been inconsistent and associated with low attributable risk.

**Methods**: We enrolled U.S.-born contacts with latent TB infection or active TB disease with >180 hours of exposure to smear + TB patients at 9 sites. Blood was collected and genotyped for IL12RB1 gene SNPs G378R and M365T and epidemiologic data collected by contact interview. Pair-wise analysis was conducted using $\chi^2$ tests with homozygous wild genotype as the referent group. This analysis is limited to contacts of non-Black race/ethnicity (White, Hispanic, American Indian, and Asian).

**Results**: Among 132 contacts, 7 had active TB and 125 had a positive tuberculin skin test (TST+). Compared with TST+ contacts, those with active TB were more likely to have the homozygous mutant genotype for IL12RB1 G378R (OR=3.78, P=.12) but the difference did not reach statistical significance. The association between mutant genotype for the G378R gene SNP and TB was increased for contacts that smoked (SM), used alcohol (ALC), and used street drugs (ST). (OR=7.33, P=.10 among SM; OR=12.00, P=.05 among ALC; and OR=11.00, P=.08 among ST). TST+ contacts and contacts with active TB were equally likely to have the homozygous mutant genotype for IL12RB1 M365T (OR=1.34, P=.79).

**Conclusions**: In this U.S. population, there was a trend towards a greater risk for active TB among contacts having a homozygous mutant genotype for IL12RB1 G378R, with stronger associations observed for contacts with SM, ALC, or ST. These findings suggest that the risk of active TB after exposure may be modulated by both genes and environmental factors such as SM, ALC, and ST.

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## 3000/F/Poster Board #932

**Derivation of live Angelman syndrome neurons from induced pluripotent stem (iPS) cells.** *S.J. Chamberlain[1,3], F. Lemtiri-Chlieh[2], E.S. Levine[2], D. Rosenblatt[4], M. Lalande[1,3].* 1) Department of Genetics and Developmental Biology, University of Connecticut, Farmington, CT; 2) Department of Neuroscience, University of Connecticut, Farmington, CT; 3) Stem Cell Institute, University of Connecticut, Farmington, CT; 4) Department of Human Genetics, McGill University, Montreal, Quebec, Canada.

The recent discovery of reprogramming human somatic cells into induced pluripotent stem cells (iPS) offers an innovative approach to the study of human genetic disorders. Like human embryonic stem cells, iPS cells self-renew indefinitely and have unlimited developmental potential. The creation of patient-specific iPS cells holds great promise for understanding genetic disease mechanisms and for *in vitro* testing of potential therapies.

We have generated an *in vitro* model of Angelman syndrome (AS) using iPS technology. AS is a neurogenetic disorder characterized by severe mental retardation, lack of speech, seizures and ataxia. About 70% of all AS cases result from deletion of chromosome 15q11-13, a region that is subject to genomic imprinting. The gene encoding the E3 ubiquitin ligase, *UBE3A*, maps to 15q11-q13, displays maternal allele-specific expression, and is inactivated by mutation in some AS cases. How the loss of UBE3A function leads to AS has not been completely elucidated. Moreover, the timing and tissue-specificity of *UBE3A* imprinting during human neuronal development has not previously been studied due to the lack of a suitable model system.

iPS cell lines have been created from AS deletion and normal fibroblasts. The iPS cell lines express NANOG and OCT4, two markers that characterize the undifferentiated stem cell state. The iPS cell lines can be differentiated into mature neurons, using a technique that mimics neural development in the embryo. During this process of *in vitro* neurogenesis, there is upregulation of PAX6, a marker of neuronal precursors, at early stages, followed by expression of MAP2 and TUJ1 in neurons. A marked decrease in *UBE3A* mRNA is observed in neurons derived from AS iPS cells, indicating that silencing of the paternal *UBE3A* allele occurs in our in vitro model of AS. These AS neurons have also been subjected to single-cell recordings to measure action potentials (APs)and spontaneous synaptic activity. Some cells with mature APs showed robust spontaneous excitatory post-synaptic potentials (ePSPs), while some did not, suggesting a developmental delay of excitatory neuronal circuits, consistent with recent findings in the mouse model of AS. Our human *in vitro* model of AS may prove invaluable for examining the developmental timing and regulation of *UBE3A* imprinting and for characterizing the functional abnormalities of human AS neurons.

## 3001/F/Poster Board #933

**Chromatin Structure and Regulatory Elements of the Imprinted Mouse *Snrpn* Locus: Evidence the Prader-Willi Syndrome Imprinting Center Consists of Multiple Functional Components.** *S. Rodriguez-Jato[1], J. Khadake[1], K.A. Johnstone[2], J.L. Resnick[2,3], T.P. Yang[1,3].* 1) Dept Biochemistry & Molecular Biology, Univ. Florida, Gainesville, FL; 2) Dept Molecular Genetics and Microbiology, Univ. Florida, Gainesville, FL; 3) Center for Epigenetics, Univ. Florida, Gainesville, FL.

Genes in the imprinted Angelman/Prader-Willi syndrome (AS/PWS) domain are controlled by a bipartite imprinting center (IC) associated with the *SNRPN* gene. The human PWS-IC, which spans 4.3 kb and includes the *SNRPN* 5' flanking region and 1st intron, acts to establish and maintain the paternal epigenotype within the domain, whereas the ~0.8 kb human AS-IC is located ~35 kb upstream of the PWS-IC and establishes the maternal epigenotype. The syntenic region of mouse chromosome 7 contains the same genes organized in the same order and exhibiting the same pattern of genomic imprinting. The mouse PWS-IC is defined by a 35 kb deletion of the *Snrpn* locus spanning the 5' flanking region and 1st intron that when paternally-inherited disrupts the paternal epigenotype across the AS/PWS domain and yields offspring with features of PWS. We now have identified 5 DNase I hypersensitive (DH) sites in primary mouse brain cells that are all located within the 35 kb PWS-IC/Snrpn deletion and are specific to the paternal chromosome. We hypothesize that these 5 DH sites comprise functional components of the murine PWS-IC. Similar analysis of the PWS-IC in mouse spleen cells, which express the paternal genes in the domain at very low levels, yielded no DH sites in this region, suggesting these DH sites may play a role in tissue-specific activation of paternal genes. The location of these hypersensitive sites in brain cells relative to previous targeted ES cell deletions of the murine PWS-IC also may explain the varying effects of these deletions on imprinting and suggests that the PWS-IC contains functionally redundant components. To identify regulatory factors within these DH sites that may mediate PWS-IC function, in vivo footprinting and/or chromatin immunoprecipitation assays were performed on primary mouse brain cells. Binding of both nuclear respiratory factor-1 (NRF-1) and YY1 was detected within multiple hypersensitive sites specifically on the paternal chromosome. Both NRF-1 and YY1 were previously identified to bind within the human PWS-IC region on the paternal chromosome. Binding of nuclear respiratory factors and YY1 have also been detected in the promoter region of the paternal allele of other imprinted genes (*Mkrn3*, *Ndn*) within the AS/PWS domain. Thus, these transcription factors may play a role in PWS-IC function and in the coordinate regulation of gene expression and imprinting across the AS/PWS domain on the paternal chromosome.

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## 3002/F/Poster Board #934

**Regulation of the Imprinted Mouse Mkrn3 Locus.** *J.X. Shan[1], A.D. Heggestad[1], S. Rodriguez-Jato[1], C. Mione-Kiefer[1], K.A. Johnstone[2], J. Dostie[5], J. Dekker[5], J.L. Resnick[2,3], T.P. Yang[1,3,4].* 1) Department of Biochemistry and Molecular Biology, University of Florida College of Medicine, Gainesville, FL 32610; 2) Department of Molecular Genetics and Microbiology, University of Florida College of Medicine, Gainesville, FL 32610; 3) Center for Epigenetics, University of Florida College of Medicine, Gainesville, FL 32610; 4) Division of Pediatric Genetics, University of Florida College of Medicine, Gainesville, FL 32610; 5) Program in Gene Function and Expression, Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA, 01605.

The Angelman/Prader-Willi syndrome (AS/PWS) domain contains at least 8 imprinted genes governed by an imprinting center (IC) that regulates imprinting across the domain. The PWS-IC is required to establish and maintain the paternal epigenotype and is postulated to act as a positive regulator of genes expressed specifically from the paternally-inherited chromosome, whereas the AS-IC establishes the maternal epigenotype and is postulated to function as a negative regulator of the PWS-IC. Genes in the syntenic region of mouse chromosome 7 are organized and imprinted similarly to the human domain. The Mkrn3 gene is located within the AS/PWS domain, expressed only from the paternal chromosome, and located ~2.4 Mb upstream of the murine PWS-IC. The mechanism by which imprinting and transcription of genes within the domain, such as Mkrn3, is regulated by the IC is not well-understood. Analysis of long-rang interactions between Mkrn3 and the murine PWS-IC by chromosome conformation capture (3C) demonstrates a close physical association specifically on the paternally-inherited chromosome. This is consistent with a mechanism of PWS-IC function in which the PWS-IC locates itself in close proximity to its target genes via long-range loop formation to activate transcription of these genes on the paternal chromosome. To identify potential regulatory factors that may mediate PWS-IC function and long-range interactions, we mapped DNase I hypersensitive (DH) sites in both the murine PWS-IC and the Mkrn3 locus and performed in vivo footprinting and/or chromatin immunoprecipitation (ChIP) analyses of these DH sites. Previous analysis of DH sites in the human and mouse PWS-IC detected binding of nuclear respiratory factor-1 (NRF-1) and YY1 specifically on the paternal chromosome. Analysis of the differentially methylated DH site in the Mkrn3 promoter detected binding of nuclear respiratory factor-2 (NRF-2), YY1, Sp1, and STAT1 exclusively on the paternal allele. ChIP analysis of the promoter region of the imprinted mouse Necdin locus, located adjacent to Mkrn3 and expressed only from the paternal chromosome, also detected binding of YY1 and NRF-1 to the paternal allele. Based on these results, we hypothesize that the PWS-IC functions as an active chromatin hub that facilitates localization of its target genes on the paternal chromosome to a transcription factory enriched in nuclear respiratory factors and YY1.

## 3003/F/Poster Board #935

**Epigenetically Altered Wound Healing in Keloid Fibroblasts.** *S.B. Russell, S.R. Opalenik, L.B. Nanney, A.H. Broquist, L. Raju, S.M. Williams.* Vanderbilt University School of Medicine, Nashville, TN.

Keloids are benign fibrotic tumors of the dermis that form during a prolonged wound healing process in genetically susceptible individuals. The predisposition to form keloids is found predominantly in people of African and Asian descent. The key alteration(s) responsible for the pathological process has not been identified and there is no satisfactory treatment for this disorder. We have reported phenotypic differences in growth and matrix synthesis between fibroblasts cultured from normal scars and keloid lesions, including glucocorticoid resistance of keloid fibroblasts to downregulation of collagen, elastin and CTGF gene expression. Gene profiling studies revealed altered regulation of multiple genes in several signaling pathways associated with fibrosis, including IGF/IGFBP and Wnt. As previously observed for the pattern of glucocorticoid resistance we now find that the differential expression of fibrosis-associated genes, including decreased expression of Wnt inhibitors SFRP1 and SFRP2 and overexpression of IGFBP5, is seen only in fibroblasts cultured from the keloid nodule; fibroblasts from perilesional and normal dermis of keloids behave like fibroblasts from normal skin and scar. In agreement with these in vitro findings, immunohistochemical examination of lesional and perilesional sites of keloid relative to normal scar tissue reveals differential expression of SFRP1, SFRP2 and IGFBP5 only in lesional sites. In addition, the pattern of differential gene expression in keloid cells is maintained throughout their in vitro lifetime. Preliminary studies using Chip-chip analysis and inhibitors of DNA methylation and histone deacetylation support a differential pattern of DNA methylation and histone acetylation in keloid relative to normal fibroblasts. These findings support the hypothesis that abnormal regulation of growth and matrix synthesis in keloid fibroblasts is due to an epigenetically altered wound healing program. Characterization of this program may give clues to the mechanism(s) that initiates the pathological process. Epigenetically altered wound healing in keloids suggests therapeutic strategies to treat or prevent keloids and possibly other fibrotic disorders, including asthma, hypertension, nephrosclerosis, sarcoidosis, scleroderma, and uterine fibroma, that disproportionately affect individuals of African ancestry. Supported by NIH grants F33AR052241, R03AR048938, P50DE10595, P30AR041943 and UL1RR024975.

## 3004/F/Poster Board #936

**Epigenetic silencing in Friedreich ataxia is associated with depletion of CTCF (CCCTC-binding factor) and antisense transcription at the *FXN* locus.** *S.I. Bidichandani, Y.K. Chutake, I. De Biase.* Dept of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Friedreich ataxia (FRDA) patients are homozygous for an expanded GAA triplet-repeat sequence in intron 1 of the *FXN* gene. The expanded GAA triplet-repeat results in severe deficiency of *FXN* gene transcription, which is reversed via administration of histone deacetylase inhibitors indicating that the transcriptional silencing is due to an epigenetic abnormality. We found a severe depletion of the chromatin insulator protein CTCF (CCCTC-binding factor) in the 5'UTR of the *FXN* gene in FRDA, and coincident heterochromatin formation via enrichment of H3K9me3 and recruitment of heterochromatin protein 1. We identified FAST-1 (Frataxin Antisense Transcript - 1), a novel antisense transcript that overlaps the CTCF binding site in the 5'UTR, which was expressed at higher levels in FRDA. The reciprocal relationship of deficient *FXN* transcript and higher levels of FAST-1 seen in FRDA was reproduced in normal cells via knockdown of CTCF. CTCF depletion therefore constitutes an epigenetic switch that results in increased antisense transcription, heterochromatin formation and transcriptional deficiency in FRDA. These findings provide a mechanistic basis for the transcriptional silencing of the *FXN* gene in FRDA, and broaden our understanding of disease pathogenesis in triplet-repeat diseases.

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## 3005/F/Poster Board #937

**Deciphering the early stages of human X-chromosome inactivation.** *J. Minks, J.C. Chow, N.P. Thorogood, S.E.L. Baldry, C.J. Brown.* Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada.

X inactivation in female mammals is achieved by epigenetic silencing of one of the two X's to ensure equal dosage of X-linked genes in both sexes. This silencing, driven by the non-coding XIST/Xist RNA, occurs early in development and has been most extensively studied in mouse. To learn more about human X inactivation, our lab has developed a HT1080 male fibrosarcoma cell line harboring an *XIST* cDNA transgene which, when induced with doxycycline, rapidly silences an *EGFP* reporter.

**Chromatin changes in XIST-induced silencing:** Chromatin immunoprecipitation-qPCR shows that ~2/3 of H3K9ac is lost after 90 days of XIST expression; however this deacetylation does not occur within the first 5 days when *EGFP* becomes ~90% silenced. Moreover histone deacetylase inhibitors do not prevent the XIST induced silencing, suggesting that deacetylation is a consequence rather than cause of silencing in our system. Thus we propose that XIST accomplishes *EGFP* silencing either by recruiting other repressive chromatin remodeling complexes or by a more direct inhibition of transcription.

**Defining the minimal functional XIST:** Using RT-qPCR, we have found that *XIST* transcription does not vary substantially among female somatic cells and, although alternatively spliced, most XIST transcripts extend to the end of the 19 kb transcript. The level of expression induced from the transgene is equivalent to normal female *XIST* expression and we have assessed the ability of XIST deletion constructs to localize and silence the reporter. Truncation of multiple *XIST* regions has limited impact on the localization of the XIST RNA and *EGFP* silencing. However deleting the 5' repeat A sequence abolishes the capacity of XIST to silence, although we have shown that the RNA is still retained in the nucleus. Intriguingly, we have also observed *EGFP* silencing in the absence of a localized XIST signal when the 5' repeat A is repositioned to the very 3' end of XIST.

We thus hypothesize that the XIST-induced silencing of the proximally located *EGFP* reporter is achieved by the repeat A region and may be independent of chromatin changes, suggesting that XIST is involved in multiple pathways driving X-chromosome inactivation. Further analysis of XIST fragments in the well-defined transgenic system will provide insights into the mechanisms of XIST action. *The work is supported by CIHR-13690, IWRH and UGF.*.

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## 3006/F/Poster Board #938

**Combinatorial Use of Gene Therapy and Nitric Oxide Donors to Rescue a Mouse Model of Argininosuccinic Aciduria.** *O.A. Shchelochkov[1], A. Erez[1], N. Brunetti-Pierri[1], S.C.S. Nagamani[1], Y. Chen[1], D. Palmer[1], N.S. Bryan[2], B. Lee[1, 3].* 1) Molecular & Human Genetics, Baylor Col Medicine, Houston, TX, USA; 2) Brown Foundation Institute of Molecular Medicine, UT Health Science Center at Houston, TX, USA; 3) Howard Hughes Medical Institute.

Argininosuccinic aciduria (ASA) is characterized by elevated plasma argininosuccinic acid and depletion of plasma arginine. Despite the diligent control of hyperammonemia with a low-protein diet and arginine supplementation, many patients still develop mental retardation and hepatic dysfunction. It has been suggested that decreased intracellular arginine, the substrate for nitric oxide (NO), results in NO dysregulation and could be in part responsible for these findings. In order to dissect out the NO contribution to ASA, we conducted experiments on human $Asl^{-/-}$ fibroblasts, performed in vivo stable isotope studies in ASA patients, and pursued NO rescue experiments in a novel hypomorphic ASA mouse model. In vitro studies using $Asl^{-/-}$ fibroblasts and in vivo stable isotope infusion showed that normalization of plasma arginine in ASA patients did not result in increased NO production. In contrast, classic citrullinemia patients were able to use arginine for NO generation emphasizing the importance of ASL in NO synthesis. The utility of restoring NO biology was supported by improved survival and optimized NO production in ASA mice treated with arginine and sodium nitrite (an NO donor). To assess the utility of liver transplantation in the treatment of ASA, we conducted a liver-targeted *Asl* gene transfer in hypomorphic ASA mice. Such animals were predicted to recapitulate physiological consequences of liver transplantation after treatment with a helper-dependent adenoviral vector carrying mouse *Asl* under control of a liver-specific promoter. ASA mice were supplemented with low-dose arginine, sodium benzoate, and sodium nitrite from birth until 4 weeks of life, when they were randomized to receive either the *Asl* vector (n=6) or saline (n=7). Mice treated with the *Asl* vector showed dramatic improvement in their physical growth, had a statistically significant weight difference already 10 days after injection (p = 0.001) and 100% survival at age 165 days. In contrast, ASA mice, which received saline only, expired by age 103 days. Plasma amino acids from ASA mice treated with *Asl* vector showed a normalization of ASA, citrulline, glutamine compared to the wild type. In summary, we demonstrate that NO dysregulation is one of the possible mechanisms contributing to ASA phenotype. The restoration of normal ASA and NO biology by combining NO donors and *Asl* gene transfer offers a new approach to the treatment of this disorder.

## 3007/F/Poster Board #939

**Identification of proteins interacting with the OCTN2 transporter responsible for primary carnitine deficiency.** *O. Ardon[1], N. Longo[1,2].* 1) ARUP Inst Clin Exp Path, ARUP Laboratories, Salt Lake City, UT; 2) Med Genet/Pediatrics and Pathology, Univ Utah.

Carnitine is essential for the transfer of long chain fatty acids into the mitochondrial matrix for subsequent beta oxidation. Defects in the OCTN2 carnitine transporter cause primary carnitine deficiency (OMIM 212140) resulting in increased loss of carnitine in the urine, low plasma carnitine levels and decreased uptake of carnitine in tissues. Patients can present with hypoketotic hypoglycemia, hepatic encephalopathy and/or cardiomyopathy. Primary carnitine deficiency is autosomal recessive with an overall population frequency of 1:40,000. The diagnosis is confirmed by finding markedly reduced carnitine transport in fibroblasts of affected patients. DNA studies shave identified heterogeneous mutations in the *SLC22A5* gene encoding the OCTN2 carnitine transporter in patients with carnitine deficiency, although there is no definite genotype/phenotype correlation. In a few patients, no abnormalities in this gene have been found, despite the lack of carnitine transport in fibroblasts. We have hypothesized that these patients could have a defect in proteins that interact and regulate the activity of the OCTN2 carnitine transporter. Co-immunoprecipitation was used to identify OCTN2 interacting proteins from HEK293 cell lysates. Mass spectrometry analysis identified several candidate proteins that co-immunopreciptated with the OCTN2 carnitine transporter. Some of the proteins identified with this technology were adaptor proteins, linking the cytoskeletal system to membrane proteins. Several of these proteins contain PDZ domains and others were cytoskeletal proteins. These include actin crosslinking and molecular scaffold proteins (spectrin, PDZK1, Shrm), structural proteins (nonmuscle myosins), and proteins associated with intracellular trafficking. The potential interaction of some of these proteins with OCTN2 was confirmed by additional specific co-immunoprecipitation and immunofluorescence co-localization. Additional studies are aimed to confirm an effect of these proteins on the transport activity of the OCTN2 transporter. This study shows that OCTN2 interacts with the cytoskeleton trough specific adaptor proteins and may lead to the identification of new gene(s) responsible for primary carnitine deficiency.

## 3008/F/Poster Board #940

**Characterization of Gaucher Disease Bone Marrow Mesenchymal Stromal Cells Reveals an Altered Inflammatory Secretome.** *P.M. Campeau[1, 2], M. Rafei[2], M.-N. Boivin[2], Y. Sun[3], G.A. Grabowski[3], J. Galipeau[2, 4].* 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) The Montreal Center for Experimental Therapeutics in Cancer, Lady Davis Institute for Medical Research, Montreal, QC, Canada; 3) Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 4) Division of Hematology/Oncology, Jewish General Hospital, McGill University, Montreal, QC, Canada.

Gaucher disease causes pathological skeletal changes which are not fully explained. Considering the important role of mesenchymal stromal cells (MSCs) in bone structural development and maintenance, we analyzed the cellular biochemistry of MSCs from an adult patient with Gaucher disease type 1 (N370S/L444P mutations). Gaucher MSCs possessed a low glucocerebrosidase activity and consequently had a 3-fold increase in cellular glucosylceramide. Gaucher MSCs have a typical MSC marker phenotype, normal osteocytic and adipocytic differentiation, growth, exogenous lactosylceramide trafficking, cholesterol content, lysosomal morphology and total lysosomal content, and a marked increase in COX-2, prostaglandin E2, IL-8 and CCL2 production when compared to normal controls. Transcriptome analysis on normal MSCs and cells treated with the glucocerebrosidase inhibitor conduritol B epoxide revealed an upregulation of an array of inflammatory mediators, including *CCL2*, and other differentially regulated pathways. These cells also demonstrated a decrease in sphingosine-1-phosphate. In conclusion, Gaucher disease MSCs display an altered secretome which could contribute to skeletal disease and immune disease manifestations in a manner distinct and additive to Gaucher macrophages themselves.

## 3009/F/Poster Board #941

**Oxidized Cholesterol: Therapeutic and Diagnostic Biomarkers for Niemann-Pick Disease, type C.** *D.S. Ory[1], V. Molugu[1], M.H. Lanier[1], D.E. Scherrer[1], S.J. Langmade[1], N.M. Yanjanin[2], J.E. Schaffer[1], F.D. Porter[2].* 1) Department of Medicine, Washington University, St. Louis, MO; 2) PDGEN, NICHD, NIH, DHHS Bethesda, MD.

Niemann-Pick disease, type C (NPC) is an autosomal recessive disorder that results in progressive neurodegeneration due to the intracellular accumulation of cholesterol and other lipids. The lack of well defined outcome measures has prevented the testing of potential therapeutic interventions. In addition, although therapeutic interventions are likely to be of greater benefit prior to the development of significant neurological impairment, diagnostic delay is on the order of five years. This diagnostic delay underscores the critical need for a blood-based screening/diagnostic test. At the cellular level, mutations in NPC1 gene profoundly affect the intracellular trafficking of cholesterol and, as a consequence, lead to multiple sterol homeostatic defects. Evidence is emerging that the sequestration of unesterified cholesterol in endolysosomes is accompanied by cellular oxidative stress. NPC1-deficient cells exhibit elevated reactive oxygen species (ROS) and harbor signs of chronic oxidative damage. Cellular levels of cholesterol oxidation products (i.e. oxysterols), which are produced non-enzymatically through interaction of cholesterol with ROS, are markedly elevated in NPC1-deficient cells. We show that this finding is recapitulated in the mouse model of NPC1 disease, in which there are similar elevations of these oxysterols both in tissues and in plasma, and the level of plasma oxysterols associated with disease progression. To study whether plasma oxysterol levels are associated with human NPC disease, we measured by mass spectrometry a panel of oxysterols in plasma samples from 25 NPC subjects (1-51 yrs; mean age 11.3 yrs), 25 age-matched controls and 17 obligate heterozygotes. We identified several oxysterol species, which were strikingly elevated in NPC subjects but not in the heterozygotes or the matched controls. To evaluate the specificity of the elevated plasma oxysterols for NPC disease, we measured circulating oxysterol levels in several other lysosomal storage diseases, including those with known CNS involvement, and found that the oxysterol profile was able to differentiate NPC subjects from subjects with other lysosomal storage diseases. Taken together, these findings suggest that plasma oxysterols may serve as novel diagnostic test for NPC, have the potential to be used in a newborn screen, and may have utility as an outcome measure for therapeutic trials.

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### 3010/F/Poster Board #942

**The association of primary iron overload, chronic liver diseases and common mutations in HFE gene (C282Y & H63D) in India.** *P.M. Tamhankar[1], D. Tewari[1], R. Sharma[1], G. Chaudhari[2], S. Agarwal[1].* 1) Medical Genetics, Sanjay Gandhi Postgraduate Institute of Med Sci, Lucknow, Uttar Pradesh, India; 2) Gastroenterology, Sanjay Gandhi Postgraduate Institute of Med Sci, Lucknow, Uttar Pradesh, India.

Background and Aim: Present research indicates conflicting data on the association of Histone family E gene (HFE) mutations responsible for hereditary hemochromatosis (primary iron overload (PIL)) and PIL in chronic liver diseases. We aimed to find out the association of the common HFE mutations (viz., C282Y & H63D) with primary iron overload in chronic liver disease (CLD) patients in India. Materials and Methods: Polymerase chain reaction-restriction fragment length polymorphism method was used for screening C282Y and H63D mutation in a total of 496 various CLDs (Hepatitis B virus associated (HBV) cirrhosis = 74, Hepatitis C virus associated cirrhosis (HCV) = 50, alcoholic cirrhosis with hepatitis = 38, alcoholic cirrhosis without hepatitis = 92 & cryptogenic cirrhosis = 242) and 502 randomly selected healthy controls. A transferrin saturation of >45% or serum ferritin of >1000 ng/mL with normal total exogenous iron intake (sum of dietary, supplementary and transfused blood iron) was suggestive of PIL. Liver biopsy was performed for confirmation of parenchymal iron overload by haematoxylin and eosin stain and Perls Prussian blue stain. Results: Out of 496 patients with cirrhosis, 13 (2.6%) were found to have PIL. Among these thirteen patients, 9 (69.2%) had cryptogenic cirrhosis, 2 (15.3%) had alcoholic cirrhosis without hepatitis, 1 (7.6%) had HBV-cirrhosis and 1 had alcoholic cirrhosis with hepatitis. However, only two (15.3%) of the 13 patients (1 with cryptogenic cirrhosis and other with HBV cirrhosis) were found positive for H63D heterozygous mutation. All the subjects of the present study were found to be C282Y wild type, except single compound heterozygote (C282Y/H63D) who however did not have PIL. The overall frequency of H63D allele in patients and controls was not significantly different (5.95 and 4.58 respectively, p = 0.22). A highly significant H63D allele frequency (p value<0.005) was observed in two patients subgroups i.e. HBV cirrhosis (10.82) and alcoholic hepatitis with cirrhosis (11.84) but there was no associated iron overload. Conclusion: Primary iron overload is not a significant problem in Indian patients with chronic liver disease. The allele C282Y is rare in India. Primary iron overload in chronic liver disease patients in India is not associated with the common HFE gene mutations.

### 3011/F/Poster Board #943

**SUCLG1 mutations: 3 new patients expanding the phenotypic spectrum of this rare cause of mild methylmalonic aciduria.** *A.S Lebre[1], C. Haudry[1], V. Serre[1], M. Barth[2], N. Boddaert[1], J.B. Arnoux[1], M. Rio[1], D. Rabier[1], A. Vassault[1], V. Cormier-Daire[1], A. Munnich[1], P. de Lonlay[1], A. Rötig[1], J.P. Bonnefont[1], V. Valayannopoulos[1].* 1) Université Paris Descartes, Hôpital Necker-Enfants Malades et Inserm U781 et U797, Départements de Génétique, de Radiologie pédiatrique, des Maladies du développement et de Biochimie B, Paris F-75015 France; 2) Université d'Angers, Hôpital d'Angers et Inserm U694, Département de Génétique, Angers F-49000 France.

Background: Deficiencies in two subunits of the succinate-Coenzyme A synthetase (SUCL) have been involved in patients with encephalomyopathy and mild methylmalonic aciduria (MMA). SUCL is the enzyme complex that catalyzes the conversion of succinyl-CoA to succinate in the Krebs cycle. The patients reported so far with SUCLG1 mutations displayed a very severe antenatal phenotype with early mortality within a few days of life. The phenotypes of patients with SUCLA2 mutations were milder with hypotonia, muscle weakness, a Leigh-like disorder with hearing impairment and dystonia, and they had a life span of up to 21 years. Results: We describe the clinical, radiologic, biochemical and genetic features of 3 SUCLG1 patients from European families presenting with early onset encephalopathy, hypotonia, muscle atrophy and dystonia. Recurrent attacks of lactic acidosis, epilepsy or liver impairment were not constant. One patient was deceased at age 12 months while the other 2 patients are alive at age 5 and 10 years. The constant diagnostic hallmarks were mild MMA and characteristic brain MRI findings with basal ganglia lesions. High propionylcarnitine (C3) and methylmalonyl carnitine (C4-DC) in urine were observed when searched. All three patients presented with combined respiratory chain deficiencies in muscle but mtDNA depletion was not constant (25% to 128%). Conclusion: This report enlarges the phenotypic spectrum of SUCLG1 mutations and confirms that mild MMA and brain MRI lesions are the hallmarks of the disease. Moreover, our findings may suggest alternative physiopathological mechanisms than mtDNA depletion to explain the combined respiratory chain deficiency observed in SUCLG1 patients.

### 3012/F/Poster Board #944

**Impaired cellular $Ca^{2+}$ homeostasis and endoplasmic reticulum (ER) stress in Smith-Lemli-Opitz Syndrome (SLOS).** *J.-B. Roullet[1], G. Ren[2], L. Merkens[1], R. Steiner[1], T. Tulenko[2].* 1) Pediatrics, Oregon Hlth & Sci Univ, Portland, OR; 2) Surgery, Thomas Jefferson Univ., Philadelphia, PA.

Smith Lemli Opitz (SLOS) is an autosomal recessive multiple malformation/mental retardation syndrome caused by deficiency in 7-dehydrocholesterol reductase (DHCR7), the last enzyme in cholesterol synthesis. The pathophysiology of SLOS is incompletely understood and there is no proven treatment. In SLOS, cholesterol is reduced while 7DHC accumulates. Since sterols are key structural components of cell membranes, we postulate that altered membrane properties play a role in the pathogenesis of SLOS. Earlier studies by our group demonstrated increased plasma-membrane dependent $Ca^{2+}$ permeability and fluidity in SLOS skin fibroblasts as well as impaired caveolae-mediated signaling (Tulenko, 2006). We now have preliminary data showing that intracellular membranes may be affected as well. In a first series of experiments, we determined intracellular $Ca^{2+}$ concentration in SLOS and control skin fibroblasts, using fura-2. Basal free cytosolic $Ca^{2+}$ concentration was reduced (~30%) and the response to thapsigargin, an inhibitor of endoplasmic reticulum (ER)-bound $Ca^{2+}$ pump (SERCA) was completely abolished in SLOS cells suggesting impaired ER-membrane dependent signaling and depleted ER $Ca^{2+}$ stores in SLOS. Cell proteins are transported to the ER for folding, where, if folding is not fully realized, activation of the unfolded protein response (UPR or "ER stress response") occurs. If the UPR is sustained, apoptosis is triggered. Since 7DHC accumulates in SLOS cell membranes, UPR may be activated in SLOS cells and result in further cell alterations. Supporting this idea, we now demonstrate UPR activation in SLOS fibroblasts as reflected in the increase (~4 fold) in BiP (GRP78), the signature protein marking UPR activation. Significant ($p<0.05$) UPR activation is also demonstrated in rat brain-derived astrocytes incubated with 7DHC and AY9944, a selective inhibitor of DHCR7, and in human neuroblastoma cells (BE-2 cells) incubated with oxidized sterols generated by UV exposure of the cells in the presence of 7DHC. Lastly, we demonstrate that tauroursodeoxycholic acid, a bile acid and small molecule chaperone, inhibits the increase in BiP observed in SLOS fibroblasts. These studies point to a significant impairment of ER-mediated signaling in SLOS and suggest new molecular targets for restoring compromised cell function and perhaps for treating patients with SLOS.

### 3013/F/Poster Board #945

**Peripheral immune status in patients with Juvenile Neuronal Ceroid Lipofuscinosis.** *M. Velinov, N. Dolzhanskaya.* Dept Human Genetics, Institute for Basic Research in Dev. Disabilities, Staten Island, NY.

Juvenile Neuronal Ceroid Lipofuscinosis (JNCL) is the most prevalent type of Batten disease. Evidence of immune dysregulation was previously shown in mouse model and JNCL patients. We studied total gene expression in peripheral native mononuclear cells in three JNCL patients and two controls. Three immunity-associated genes shown in the table below were found to be most significantly down-regulated in affected individuals:

| Gene symbol | affected/non-affected mRNA signal ratio | Gene description summary |
|---|---|---|
| CXCL12 | 1: 5.35 | chemokine ligand 12 |
| HLA-DQA1 | 1: 25.77 | MHC-class II gene |
| HLA-DQA2 | 1: 4.7 | MHC-class II gene |

Additional 12 genes: LILRA3, HLA-DOB, IGHD, SERPINB4, CCL7, CD244, CNR2, CCL19, FCGR2B, CCL2, LILRB1 and CMKLR1 were significantly down-regulated and 8 genes: PXDN, MICA, IL1R2, OSM, CXCL1, CCR2, CNR2, HLA-DQB1, were significantly up-regulated in JNCL. Down regulation of MHC class II gene expression was previously shown to be associated with repeat exposure to bacterial endotoxin in cases of burns and septic shock. Such down regulation may be associated with abnormal antigen presentation, immune suppression and autoimmunity. It may be speculated that chronic exposure to toxic storage metabolite(s) in JNCL leads to immune dysfunction by similar mechanisms, as in cases of repeat endotoxin exposure. CXCL12 (chemokine stromal cell-derived factor 1 (SDF-1) participates in B-lymphocyte proliferation, migration and differentiation, and possibly in immune surveillance. Our data supports the presence of immune abnormalities that may play an important role in the JNCL pathogenesis.

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## 3014/F/Poster Board #946

**Metformin sensitizes insulin signaling through AMP-mediated PTEN down regulation in adipocyte cells.** *S. Lee, J. Lee, J. Kim, J. Jung, S. Park, H. Kim.* KOREA University, Seoul, Korea.

PTEN acts as a tumor suppressor gene through the action of its phosphatase protein product. This phosphatase is involved in the various cellular events, such as cell cycle, proliferation, dividing, and migration. Until now, however, the role of PTEN in insulin-related signaling is still unclear. To gain insights into the role of PTEN in insulin-mediated signaling, we investigated the effect of metformin, a well known insulin signal sensitizer, on PTEN expression. In results, we found that metformin suppresses PTEN expression in adipocyte 3T3L1 cells. Metformin also stimulates JNK and its downstream molecule c-Jun, a direct substrate of JNK. Phosphorylation of both JNK and c-Jun was not observed in the presence of Compound C, a specific AMPK inhibitor, suggesting the involvement of AMPK in metformin-induced JNK signaling. Moreover, chemical inhibition of JNK blocked metformin-induced PTEN suppression. Knock-down of AMPK blocked metformin-induced PTEN suppression. JNK phosphorylation was also impaired in the AMPK knock-down cells. In conclusion, our results suggest that metformin may have insulin signal sensitizing effect through PTEN suppression via AMPK-mediated JNKdependent pathway.

## 3015/F/Poster Board #947

**Metformin modulates GLUT4 translocation through AMPK-AS160-Rab4-PKC ζ pathways in both skeletal muscle and adipocyte cells.** *J. Lee, S. Lee, J. Jung, J. Kim, S. Park, H. Kim.* Korea univesity college of medicine, seoul, Korea.

Metformin is one of the major components of oral anti-diabetic drug. In the diabetes, GLUT4 trafficking to the plasma membrane is key cellular event for regulating glucose homeostasis. Till now, however, the mechanism of GLUT4 trafficking is not completely understood. To understand the signal transduction mechanism of GLUT4 trafficking, we examined the effect of metformin, a current drug for diabetes on Rab4, a vesicle related protein GTPase. In results, we found that metformin induces mRNA and protein expression of Rab4 with reverse transcriptase-polymerase chain reaction (RT-PCR) and western immune-blotting, in both skeletal muscle C2C12 cells and adipocyte 3T3L1 cells, respectively. Metformin stimulates AMP-activated protein kinase (AMPK) activity and also triggers GLUT4 translocation from cytosol to the plasma membrane. In addition, metformin increases the phosphorylation of AS160 Rab GTPase-activating protein, a direct substrate of Akt, and PKC zeta. Knock-down of Rab4 blocked the metformin-induced AS160 and PKC zeta phosphorylation. Moreover, GLUT4 translocation due to metformin treatment was disappeared in Rab4 knock-down cells. Furthermore, GLUT4 translocation was impaired in the AMPK knock-down cells, suggesting the involvement of AMPK in metformin-induced GLUT4 vesicle trafficking. In summary, our results demonstrates that metformin modulates GLUT4 translocation in skeletal muscle and adipocyte cells via AMPK-Rab4-AS160-PKC zeta pathway and provide novel role of AMPK as a key regulator of GLUT4 trafficking.

## 3016/F/Poster Board #948

**Heterodimerization of the Sialidase NEU1 with the Chaperone PPCA Prevents its Premature Oligomerization.** *E. Bonten[1], Y. Campos[1], V. Zaitsev[2], A. Nourse[1], B. Waddell[1], G. Taylor[2], A. d'Azzo[1].* 1) Dept of Genetics and the Hartwell Center for Bioinformatics and Biotechnology , St Jude Children's Research Hospital, Memphis, TN, USA; 2) Centre for Biomolecular Sciences, University of St Andrews, St Andrews, Scotland.

Lysosomal neuraminidase 1 (NEU1) initiates the intralysosomal hydrolysis of sialo-oligosaccharides, and -glycoproteins by removing terminal sialic acid residues. NEU1 forms a high molecular weight complex with β-galactosidase (β-gal) and protective protein/cathepsin A (PPCA). By virtue of its association with PPCA, which acts as a molecular chaperone, NEU1 is transported to the lysosome, catalytically activated and stabilized. Deficiency of NEU1 is associated with 2 neurodegenerative diseases of glycoprotein metabolism: sialidosis, caused by structural lesions in the lysosomal NEU1 locus, and galactosialidosis (GS), a combined deficiency of NEU1 and β-gal, due to a primary defect of PPCA. Patients with sialidosis and those with GS share clinical and biochemical features, and are classified according to the age of onset and severity of the symptoms. The understanding of the interaction between NEU1 and PPCA, and the determinants that control their association, will provide important insights into their regulation and function, both in physiologic and pathologic conditions. We have analyzed the biochemical, analytical and hydrodynamic properties of PPCA, selected PPCA mutants, NEU1, and a complex of the 2 proteins. We measured by surface plasmon resonance the binding between NEU1 and PPCA, or with a series of synthetic overlapping 15-mer peptides spanning the entire PPCA or NEU1 sequence, and identified key binding sites on both proteins. We found that one of these sites on NEU1 is needed for its binding to PPCA but also to other NEU1 molecules, albeit with lower affinity. Therefore, NEU1 tends to self-assemble into chain-like oligomers in absence of PPCA. Using analytical ultracentrifugation we demonstrated that self-association of NEU1 could be reversed by addition of PPCA, which resulted in the disassembly of NEU1-oligomers and the formation of a new PPCA-NEU1 heterodimeric complex with different hydrodynamic properties compared to PPCA and NEU1 homodimers. The identification of binding sites on both proteins has allowed us to generate alternative structural models of the NEU1 oligomer and the PPCA-NEU1 heterodimeric complex. We propose a novel mechanism of interaction between NEU1 and its accessory protein PPCA, which may provide a rationale for the secondary deficiency of NEU1 in galactosialidosis. (Supported in part by NIH grants GM60905 and DK52025, the Assisi Foundation of Memphis, and the American Lebanese Syrian Associated Charities (ALSAC)).

## 3017/F/Poster Board #949

**Delineating the spatio-temporal nature of neurochemical and pathological change in the MPS IIIA mouse brain.** *K.M. Hemsley, H. Beard, S. Hassiotis, A.L. Luck, J.J. Hopwood.* Lysosomal Diseases Research Un, SA Pathology (WCH Campus), North Adelaide, South Australia, Australia.

Mucopolysaccharidosis type IIIA (MPS IIIA) is a neurodegenerative lysosomal storage disorder resulting from a lack of the lysosomal enzyme sulphamidase (SGSH). Heparan sulphate [HS]-derived oligosaccharides accumulate intracellularly and subsequent to this primary insult, a cascade of neuropathological changes occurs, including sequestration of GM2 and GM3 gangliosides and cholesterol and neuroinflammation and neurodegeneration. The spatio-temporal nature of these changes within discrete brain regions, how they impact upon the function of neuronal circuits, ultimately leading to appearance of clinical symptoms has yet to be elucidated. We have begun to examine the time-course of neuropathological changes in the MPS IIIA mouse brain. The mouse is a good model of a human disease, with biochemical, pathological and clinical deficits analogous to the human condition. In tests applied so far, clinical changes are apparent from 12 weeks of age. In the present study, we have undertaken quantitative immunohistochemical analyses on brain tissue from mice in the pre-symptomatic, symptomatic and advanced disease stages (4 to 21 weeks of age). We examined the lysosomal integral membrane protein-II (LIMP-II), a marker of microgliosis (isolectin B4) and astrogliosis (glial fibrillary acidic protein; GFAP). The spatio-temporal pattern of accumulation of GM3 and cholesterol is being assessed. From our data thus far, it is apparent that one of the earliest pathological changes is microglial activation. Mice aged four weeks exhibited widespread, statistically significant increases in LIMP-II and large numbers of activated microglia in all brain areas assessed. The number of activated microglia was not different in 4 week-old and advanced disease mice. Astrocytosis was apparent in some but not all brain regions at 4 weeks of age, but progressed as mice aged. These data (and those from our published studies in symptomatic mice) enable us to devise a hypothesised schema outlining the cascade of neurochemical and neuropathological changes occurring within the MPS IIIA mouse brain during disease development. In order to devise and effectively target therapies for this untreatable condition, it is vital that we elucidate the cascade of neuropathological change that occurs within the brain. Understanding these processes will also lead to the development of biomarkers of disease and disease amelioration.

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### 3018/F/Poster Board #950

**Correlation between thrombospondin-1, vascular endothelial growth factor, and adiponectin in kidney of mouse model of fabry disease, a sphingolipidosis.** *M.H. Lee, E.N. Choi, J.-O. Choi, J.-W. Park, H.-Y. Park, S.-C. Jung.* Depart of Biochemistry, School of Medicine, Ewha Wonans University, Seoul, Korea.

Fabry disease is a lysosomal storage disease caused by a deficiency of α-galactosidase A, which results in abnormality of glycosphingolipid metabolism and accumulation of globotriaosylceramide (Gb3). As correlations between the level of Gb3 and clinical manifestations of Fabry disease have not been developed, we investigated altered gene expression. Hepatic and renal gene expression of male α-galactosidase A-deficient mice (Fabry mice) was compared with that of wild type mice. Microarray analysis was performed using samples taken before and after intravenous infusion of α-galactosidase A. The identified genes were validated using quantitative real-time PCR and Western blot assay. Expression of hepatic Saa1 (Serum Amyloid A1), S100a8 (S100 Calcium-binding protein A8), S100a9 (S100 Calcium-binding protein A9) and Lcn2 (Lipocalin 2) and renal Npy (Neuropeptide Y), Tsp2 (Thrombospondin 2) and Tsp4 (Thrombospondin 4) was significantly upregulated in Fabry mice compared with wild type mice and was normalized by enzyme replacement therapy. Upregulation of Saa1, S100a8, S100a9 and Lcn2 may modulate inflammation and Lcn2, Npy and Tsp may be associated with vascular and renal involvement in Fabry disease. The expression of Tsp-1 protein and adiponectin was increased in the kidney of the Fabry mice, but VEGF expression is decreased. Therefore their expression might play a role in the kidney dysfunction, especially in mouse model of fabry disease. This study was supported by a research grant of the Life Insurance Philanthropy Foundation. Keywords: Fabry disease, Gene expression, Tsp-1, VEGF.

### 3019/F/Poster Board #951

**Mice doubly-deficient in lysosomal hexosaminidase A and neuraminidase 4 show epileptic crises accompanied by rapid loss of cortical and hippocampal neurons.** *A. Pshezhetsky[1,5], V. Seyrantepe[1], A. Caqueret[1], J. Michaud[1], L. Carmant[2], T. Levade[3], R. Gravel[4], C. Morales[5].* 1) Dep. of Medical Genetics, CHU Ste-Justine, University of Montreal , Montreal, Quebec, Canada; 2) Dep. of Neurology, CHU Ste-Justine, University of Montreal, Montreal, Quebec, Canada; 3) INSERM U858, Laboratoire de Biochimie “Maladies Metaboliques”, CHU Toulouse, France; 4) Dep. of Biochemistry & Molecular Biology, University of Calgary, Calgary, Alberta, Canada; 5) Dep. of Anatomy and Cell Biology, Faculty of Medicine, McGill University, Montreal, Quebec, Canada.

Tay-Sachs disease is a severe lysosomal disorder caused by mutations in the *HEXA* gene coding for the α-subunit of lysosomal β-hexosaminidase A, which converts $G_{M2}$ to $G_{M3}$ ganglioside. HexA-/- mice, depleted of β-hexosaminidase A, remain asymptomatic to at least 1 year of age, owing to the ability of these mice to catabolize stored $G_{M2}$ ganglioside via a lysosomal sialidase into glycolipid $G_{A2}$. $G_{A2}$ is further processed by β-hexosaminidase B, to yield lactosyl-ceramide, thereby completely bypassing the β-hexosaminidase A defect. This bypass is not effective in humans, with the outcome that infantile Tay-Sachs disease is fatal in the first years of life. Our data provide an explanation for the difference in Tay-Sachs disease severity in humans and mice and suggest a novel therapeutic approach for this disease. Previously we have identified a novel lysosomal sialidase/neuraminidase 4 (Neu4) abundantly expressed in brain neurons and showed that Neu4 is a functional component of the ganglioside metabolizing system (Seyrantepe et al., Hum. Mol. Genet. 2008). Now we demonstrate that mice with targeted disruption of both Neu4 and HexA genes show seizures characterized by myoclonic jerks which correlate with polyspike discharges on electroencephalograms originating from the cortical electrodes. Siblings with a single HexA or Neu4 deficiency do not show any of the above symptoms. Microscopic and electromicroscopic examination of brain tissues show that double-knockout, but not single knockout mice have multiple degenerating neurons in the hippocampus and cortex. They also show multiple layers of cortical and hippocampal neurons accumulating $G_{M2}$-ganglioside. Altogether our data suggest that Neu4 plays a role of a modifier gene in the mouse model of Tay-Sachs disease, effectively “treating” the disease through the metabolic bypass.

### 3020/F/Poster Board #952

**Self-complementary Adeno-associated virus mediated delivery of Pro-opiomelanocortin to the ventral tegmental area ameliorates dietary obesity in rats.** *L.M. Andino, M. Judge, Y. Zhang, A. Shapiro, N. Tumer, P.J. Scarpace.* Pharmacology and Therapeutics, University of Florida, Gainesville, FL.

The activation of Pro-opiomelanocortin (POMC) neurons in different regions of the brain, including the arcuate nucleus of the hypothalamus (Arc) and the nucleus of the solitary tract have been demonstrated to curtail feeding. The region believed to be most responsive to POMC activation is considered to be the Arc. Because we were able to identify corresponding POMC melanocortin 4 receptors in brain punches of the ventral tegmental area (VTA) of rats, we hypothesized that this region of the brain would be responsive to POMC activation. Here we compare the effects of delivery of an Adeno-associated viral (AAV) construct encoding POMC to the Arc and the VTA. Our experimental design consisted of three groups of four month old F344×Brown Norway rats. The animals were all high fat fed for 14 days after which unilateral stereotaxic injections were performed with coordinates targeting either the VTA or the Arc. Self-complementary AAV (scAAV) constructs expressing either green fluorescent protein (GFP) or the POMC gene were administered. The control group received scAAV expressing GFP either in the Arc or the VTA and the experimental groups received scAAV expressing POMC into the VTA or the Arc. Food intake and body weight were measured over the course of 10 weeks. About four weeks post injection, the groups receiving POMC into the VTA and Arc were beginning to display a change in body weight that was significantly different from the control treated group. Interestingly, the group injected into the Arc was transiently significantly different for about 3 weeks time after which their delta body weight was comparable to control treated animals. At ten weeks post-injection, the GFP injected control group had gained over 78 grams, the group injected into the Arc had gained greater than 60 grams while the VTA injected group had only gained 40 grams ($p<0.005$). Additionally, caloric intake was unaltered amongst the 3 groups, suggestive of increased energy expenditure. Consistent with this was a significant increase in oxygen consumption with the VTA treated group consuming 25% more oxygen than controls ($p<0.0001$). Our results are the first to show direct activation of POMC neurons in the VTA via expression of a POMC vector. In addition, it appears that activation of POMC neurons in the VTA are more effective at curtailing high fat feeding than those in the Arc although, the Arc is the putative primary site of POMC anorectic function in the brain.

### 3021/F/Poster Board #953

**A systematic cell-based analysis for the patients with cytochrome *c* oxidase deficiency.** *H. Hatakeyama, K. Sawa, Y. Goto.* Mental Retard & Birth Def Res, Natl Inst Neurosci, NCNP, Kodaira, Tokyo, Japan.

Cytochrome *c* oxidase (COX) is a terminal protein in mitochondrial electron transport system with oxidative phosphorylation and is composed of 13 structural COX subunits: The largest 3 are encoded in mitochondrial DNA (mtDNA), and the remaining 10 are encoded in nuclear DNA (nDNA). Recently, some pathogenic mutations have been reported in nDNA-encoded COX assembly factors as well as in genes of mtDNA- and even nDNA-encoded structural COX subunits. However, the relationship between the biochemical abnormality in COX and its contributions to aberrant mitochondrial biogenesis and cellular activity is to be extensively studied because the phenotypic differences in molecular and cellular levels in relation to the clinical symptoms have been barely clarified so far. Here, we introduced a systematic analysis to characterize the phenotypic differences in mitochondrial biogenesis and cellular activity for the patients exhibiting mitochondrial diseases with infantile-onset COX deficiency ($n$=8). The established myoblasts from each patient revealed the decreased activity in COX with different severities confirmed by enzymatic analysis and cytochemical COX staining. Protein analysis obviously indicated the structural abnormality in mature COX only in the 3 patients with enzymatically severe COX deficiency (less than 20% activity of controls; $n$=10). Furthermore, we demonstrated that COX-specific abnormality actually triggers the critical defects in mitochondrial biogenesis; the decreased ATP level, the increased ROS level, and the abnormal membrane potential, all of which may lead to drastically decreased cellular viability, metabolisms, and dynamics. In this case, however, mutation analysis in genes of all mtDNA-encoded COX subunits, entire mitochondrial tRNAs, and typically known nDNA-encoded COX assembly factors showed all negative. Protein analysis for the detection of all 13 structural COX subunits revealed almost normal level. Our results may suggest that there still remain some candidate genetic determinants hidden in other unidentified nDNA-encoded COX-associating factors. In conclusion, our cell-based functional analysis would be advantageous as powerful tools to effectively examine the molecular pathogenesis in COX deficiency. Additionally, our method seems comprehensive and systematic enough to be applicable in functional diagnosis and pathogenic study of various mitochondrial diseases.

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### 3022/F/Poster Board #954

**Evaluation of a Unique Metabolomic Signature for Monitoring Therapeutic Efficacy and Disease Progression in Cystinosis.** *F.F. Snyder[1,2], D.S. Sinasac[2], E. Fung[2], K.B. Mantik[2], A. Parenta[2], K.S. Reid[2], D.M. Scheuchner[2], J.P. Midgley[3].* 1) Dept. of Medical Genetics, University of Calgary, Calgary, AB, Canada; 2) Biochemical Genetics Lab, Alberta Children's Hospital, Calgary, AB, Canada; 3) Dept. of Pediatrics, University of Calgary, Calgary, AB, Canada.

Nephrotic cystinosis is an autosomal recessive storage disorder resulting from mutations in the *CTNS* gene (OMIM 606272) encoding cystinosin, the lysosomal membrane transporter responsible for exporting disulfide cystine into the cytoplasm. Here we apply a restricted metabolomic survey of cystinosis patient samples to define a metabolite pattern which may be an informative biomarker in monitoring the efficacy of therapy and/or disease status. Metabolomics utilizes multivariate statistical methodologies to identify patterns of metabolites that can function as unique signatures in differentiating between physiological states. Oral cysteamine is known to be an effective therapy in converting cystine to cysteine and the mixed disulfide cysteine-cysteamine, both of which can exit the cystinotic lysosome and deplete accumulating cystine. Here we describe a unique urine metabolomic cystinosis profile and compare it with the conventional measurement of leukocyte half-cystine content. We serially sampled nine patients with nephrotic cystinosis, ranging from one to 20 years of age, and monitored their leukocyte half-cystine levels (measured by an amino acid analyzer) and their urine organic acids (measured by gas chromatography-mass spectrometry) over a three year period. Score plots derived from a principal component analysis of their urine organic acids showed substantial although incomplete segregation of cystinosis patients from controls. Loading plots did reveal a subset of 6 urine metabolites which contribute to this distinction, with the greatest change being found in pyroglutamic acid (mean value of 2400 mmol/mol creatinine in cystinosis versus <300 in controls; p=<0.05). This suggests that flux through the gamma-glutamyl cycle in cystinosis may be impacted by reduced cytoplasmic cystine and ATP levels; a perspective supported by a report of pyroglutamic aciduria resolving after cysteamine therapy (J. Inher. Metab. Dis. 22; 224, 1999). Finally using individual patient-class membership probability scores to compare the mean leukocyte half-cystine levels with the urine metabolomic signature in order to evaluate its utility as an alternate or supplementary biomarker, only a weak correlation was found (R=0.53). Based on our initial evaluation, our metabolomic signature although unique for cystinosis, requires further refinement to be of utility in evaluating the therapeutic status of cystinosis patients on cysteamine therapy.

### 3023/F/Poster Board #955

**Study on feto-placental outcomes affected by different onset of preeclampsia-like symptoms in wild-type and apoE-/- mouse model.** *Z. Yang, R. Ma, M. Sun.* Dept Ob & Gyn, Peking University Third Hospital, Beijing, China.

Background and aims: This study aimed to explore the effects of onset at multiple gestational stages of preeclampsia-like on feto-placental changes in mouse model. Method: preeclampsia-like model were established in C57 wild-type (WT) and apoE-/- pregnant mice at early, mid and late gestational stages by injecting nitric oxide synthase (NOS) inhibitor L-arginine methyl ester (L-NAME) subcutaneously. Control groups were received normal saline (NS) simultaneously. Groups were subdivided into subgroups at early, mid and late gestational stages in L-NAME and NS groups in both WT and apoE-/- mice respectively. Blood pressure was measured until day 14 and 18 of gestation when the fetuses and placentas were removed under anesthesia. Blood pressure and urine protein were measured for identification of mice model, fetus and placenta histological changes were analyzed and compared among each groups. Data were analyzed statistically. Results: Blood pressure and urine protein in all the L-NAME subgroups in WT and apoE-/- mice were significantly higher than those in all the gestational age matched NS control groups (p<0.05). A lower live fetus rate and fetal weights as well as placental weights, and a higher absorbed fetus rate were found in early L-NAME subgroups in both WT and apoE-/- groups than those in the NS controls at day 14 and day 18 of pregnancy, whereas no significant difference among the late L-NAME subgroups and the NS controls in both WT and apoE-/- mice (p>0.05). The early L-NAME subgroups had lower living fetus rate and fetal and placental weights and higher absorbed fetus rate than those in the mid, late L-NAME subgroups in both WT and apoE-/- mice (p <0.05). Placenta and fetal weights were lower in apoE-/- L-NAME subgroups than in each WT L-NAME control subgroups (p<0.05). Morphological examination of placental showed that varying degrees of fibrinoid necrosis. Villi interstitial edema were found in the early and mid L-NAME groups in both WT and apoE-/- mice, but there were no significant pathological changes found in the placenta in late L-NAME subgroups and NS control subgroups. Conclusions: Preeclampsia-like symptoms occurred on earlier stage in pregnant mice were more likely to have remarkable feto-placental impacts especially in apoE-/- mouse model.

### 3024/F/Poster Board #956

**Tandem mass spectrometry analysis of whole blood glutathione in mitochondrial disorders and organic acidemias.** *G.M. Enns[1], K.R. Atkuri[2], T. Moore[3], L.A. Herzenberg[2], L.A. Herzenberg[2], T.M. Cowan[3].* 1) Dept Ped, Div Med Gen, Stanford Univ, Stanford, CA; 2) Dept Genetics, Stanford Univ, Stanford, CA; 3) Dept Pathology, Stanford Univ, Stanford, CA.

Primary mitochondrial disorders and organic acidemias are associated with respiratory chain dysfunction and resultant impaired redox balance. Intracellular reduced glutathione (iGSH) is the major defense mechanism for combating oxidative damage. Previous studies in our lab using high-dimensional flow cytometry (Hi-D FACS) and biochemical analysis provided evidence for the existence of chronic oxidative stress in patients who have disorders characterized by mitochondrial dysfunction; iGSH levels in T-lymphocyte subsets, monocytes and neutrophils were low and plasma protein carbonyls were elevated. We further analyzed blood samples from patients with primary mitochondrial disorders (n=13) and organic acidemias (n=7) using both Hi-D FACS and a novel tandem mass spectrometry (MS/MS) assay. The MS/MS assay was performed after derivitization of sulfhydryl groups with *N*-ethylmaleimide (NEM) in order to prevent glutathione oxidation and GSH-$^{13}$C -$^{15}$N-NEM was used as an internal standard. Whole blood GSH concentration, as determined by MS/MS, correlated with Hi-D FACS measurement of cellular iGSH levels. Compared to normal controls, whole blood GSH as determined by MS/MS was significantly lower in both mitochondrial disease patients (p<0.0058) and those with organic acidemias (p<0.0001). Although GSH levels were low in mitochondrial disease patients as a whole, a strikingly low level of GSH was detected in one patient who had associated oxoprolinuria. Overall, GSH levels were lower in organic acidemia patients when compared to mitochondrial patients, but this may have been related to poor clinical status (samples were more frequently collected during hospitalization for acute metabolic crisis in the organic acidemia group). Whole blood GSH and iGSH are promising biomarkers for disorders characterized by mitochondrial respiratory chain dysfunction, with potential to make relatively non-invasive monitoring of disease status and response to therapies possible.

### 3025/F/Poster Board #957

**Role of saposin C in CNS impairment and in vivo function of acid β-glucosidase (GCase).** *Y. Sun[1,4], H. Ran[1,4], M. Zamzow[1,4], K. Kitatani[6], M.R. Skelton[2,4], M.T. Williams[2,4], C.V. Vorhees[2,4], D.P. Witte[3,5], Y.A. Hannun[6], Y.H. Xu[1,4], G.A. Grabowski[1,4].* 1) Div Human Gen; 2) Div Neurology; 3) Div Pediatric Path, Cincinnati Children's Hosp Med Center; 4) Dept Pediatric; 5) Dept Pathology, U of Cincinnati, Cincinnati, OH; 6) Dept Biochem and Mol Biology, Medical U of SC, Charleston, SC.

Saposins A, B, C and D are derived from a common precursor, prosaposin (*psap*). Saposin C enhances the activity of the Gaucher enzyme, GCase. Saposin C deficiency leads to a Gaucher-like central nervous system (CNS) disease attributed to diminished glucosylceramide (GC) cleavage activity by GCase. A saposin C deficient mouse was made by knocking-in a Cys-to-Pro in exon 11 on *psap*. C-/- mice were selectively deficient in saposin C, but prosaposin and saposins A, B and D proteins were present at near wild-type levels. By 1 year, C-/- mice had weakness of the hind limbs and progressive ataxia with foamy cells in the dorsal root ganglion, and progressive loss of cerebellar Purkinje cells and atrophy of cerebellar granular cells. EM revealed inclusions in axonal processes in the spinal cord, sciatic nerve and brain. Activated microglial cells and astrocytes were diffusely present in the CNS. No storage cells were in visceral organs. Moderate increases in GC and lactosylceramide and their deacylated analogues were in the CNS. Backcrossing C-/- into V394L or D409H GCase mutant mice produced a nearly pure neuronal (V394L) disease and a visceral (D409H) storage disease. The nature of the storage lipids depended upon the kinetic properties of the mutant enzyme with primarily glucosylsphingosine in the CNS V394L disease and GC in the visceral D409H disease. These results support the concept that saposin C has a prominent role in CNS function and GCase substrate preference, as well as in axonal integrity and as an optimizer/stabilizer of GCase.

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## 3026/F/Poster Board #958

**A murine model of mut (0) methylmalonic acidemia (MMA) treated by orthotopic liver transplantation.** *I. Manoli[1], R.J. Chandler[1], P. Zerfas[2], V. Hoffmann[2], C.P. Venditti[1]*. 1) Genetics and Molecular Biology Branch, NHGRI, NIH, Bethesda, MD; 2) Diagnostic and Research Services Branch, Division of Veterinary Resources, NIH, Bethesda, MD.

To study hepatocyte targeted gene expression as a therapy for methylmalonic acidemia (MMA) and create a mouse model of MMA treated by orthotopic liver transplantion, we generated mice that express the methylmalonyl-CoA mutase (*Mut*) gene in a liver specific fashion on the $Mut^{-/-}$ background. A transgene, $Tg^{INS\text{-}Alb\text{-}}$*Mut*, was engineered to express the *Mut* gene from the murine albumin promoter and then insulated with chicken HS4 globin locus barrier elements. $Mut^{-/-}$;$Tg^{INS\text{-}Alb\text{-}}$*Mut* animals were present in expected Mendelian proportions at weaning. Analysis of various tissues from the $Mut^{-/-}$;$Tg^{INS\text{-}Alb\text{-}}$*Mut* animals between 2-7 months of age by qPCR revealed liver restricted *Mut* mRNA expression. To assess the *in vivo* function of the transgene, metabolism of 1-$^{13}$C propionate into $^{13}CO_2$ was measured. The $Mut^{-/-}$;$Tg^{INS\text{-}Alb\text{-}}$*Mut* mice metabolized 54.7±9.2% of administered label in 25 minutes, compared to 76.5±4.5 in the $Mut^{+/-}$ and 10±2 in the $Mut^{-/-}$ mice. Challenge with a high-protein diet resulted in significant weight loss at one and 6 months in the $Mut^{-/-}$;$Tg^{INS\text{-}Alb\text{-}}$*Mut* mice, compared to a large weight gain in control $Mut^{+/-}$ littermates on the same diet. Plasma methylmalonic acid levels (μM) in the $Mut^{-/-}$;$Tg^{INS\text{-}Alb\text{-}}$*Mut* mice were elevated at 500±177 at baseline and substantially increased to 1500±620 after 2 months on the high-protein feeds. After 6 months, $Mut^{-/-}$;$Tg^{INS\text{-}Alb\text{-}}$*Mut* mice developed tubulointerstitial nephritis with microvesicular tubular degeneration, accompanied by increased BUN levels. The proximal tubular epithelial cells were full of megamitochondria, while the liver showed no abnormalities. This mouse model proves that selective hepatic expression of the Mut enzyme by transgenesis in hepatocytes can result in complete rescue from the neonatal lethality of the $Mut^{-/-}$ state. Furthermore, the lack of hepatic pathology in the $Mut^{-/-}$;$Tg^{INS\text{-}Alb\text{-}}$*Mut* mice suggests that cell or mitochondrial autonomous effects are responsible for disease manifestations. As in patients who have received liver transplantation, circulating metabolites remain elevated, despite the presence of a large reservoir of functional hepatic enzyme. A high-protein diet induced metabolic changes and the development of kidney disease very similar to that described in humans with MMA. These animals will be useful to study the renal, pancreatic and CNS pathophysiology of MMA, and test therapies aimed at treating these manifestations.

## 3027/F/Poster Board #959

**Evaluation of nonsense suppressor therapies in cells from patients with peroxisome biogenesis disorders.** *P. Dranchak[1], A. Snowden[2], E. Di Pietro[3], W.Y. Yik[1], N. Braverman[3], S. Steinberg[2], J. Hacia[1]*. 1) Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA, USA; 2) Peroxisomal Diseases Laboratory, Kennedy Krieger Institute, Baltimore, MD, USA; 3) Department of Human Genetics, McGill University, Montreal, Quebec, CA.

Peroxisomal biogenesis disorders (PBDs) are a group of autosomal recessive neurodegenerative disorders caused by mutations in *PEX* genes. Our goal is to evaluate the efficacy of a set of newly developed drugs capable of selectively promoting the read-through of premature stop codons (nonsense suppressor compounds) in *PEX* genes as therapeutic agents for a subset of individuals with PBDs. One such nonsense suppressor drug, PTC-124 is in clinical trials for the treatment of inherited disorders caused by nonsense mutations. Other drugs, including the aminoglycoside G418, have shown nonsense suppressor activity in cell culture assays. Here, we conducted a series of functional assays on drug-treated cultured skin fibroblasts derived from PBD patients with previously identified nonsense mutations in *PEX* genes (*PEX1* R183X / S1246fs, *PEX2* R119X / R119X and R125X / R125X, *PEX7* L292X / Y40X, *PEX10* E298X / Nt4delG, *PEX12* R180X / Nt887_888delTC, and *PEX26* W99X / W99X). This includes the biochemical, immunolocalization, immunoblot, and reporter gene-based characterization of peroxisome protein function and assembly in treated cells. Although the aminoglycoside G418 shows promising activity in cultured fibroblasts derived from a subset of PBD patients (*PEX2* R119X / R119X and *PEX12* R180X / Nt887_888delTC), PTC-124 treatment has not resulted in appreciable rescue of peroxisome biochemical function or peroxisome assembly. Furthermore, co-treatment of patient fibroblasts with nonsense suppressor drugs and an inhibitor of the nonsense-mediated mRNA decay (NMD) pathway did not lead to measurable improvements in peroxisomal functions. Using a mammalian two-hybrid system, we screened these compounds for their ability to read-through all reported nonsense mutations in *PEX7*. In preliminary experiments, PTC-124 appeared to cause an increase in luciferase activity with wild-type *PEX7* which could reflect the luciferase stabilizing ability of PTC-124. However, after correction, PTC-124 also promoted read-through of some of the nonsense codons. Overall, our studies provide an initial evaluation of the potential efficacy of nonsense mutation suppressor therapies for PBDs. This represents an important step towards our longer-term goal of identifying novel treatments for PBDs.

## 3028/F/Poster Board #960

**Effects of growth differentiation factor 3 signaling on Adiposity.** *J.C. Bournat[1], J.J. Shen[4], L. Huang[1], L. Li[3], C. Jorgez[2], M.M. Matzuk[1], C.W. Brown[1]*. 1) Molecular and Human Genetics, Baylor College Medicine, Houston, TX; 2) Urology, Baylor College Medicine, Houston, TX; 3) Pediatrics, MD Anderson Cancer Center, Houston, TX; 4) Children's Hospital of Center California, Madera, CA.

Growth differentiation factor 3 (GDF3) is a member of the TGF-beta superfamily located on human chromosome 12p13.1, a region genetically linked to obesity and that has positive associations with obesity-related phenotypes, including BMI and fat mass. GDF3 is expressed in white adipose tissues where it is induced by high fat diet (HFD). Body weight and adipose tissue mass normally increase under HFD conditions, and these processes are augmented when GDF3 is overexpressed systemically. In contrast, GDF3 knockout mice under HFD conditions have less adipose tissue mass than wild type mice and exhibit higher basal metabolic rates. These results suggest that GDF3 may act as an adipogenic factor under conditions of caloric excess, and that GDF3 deficiency results in the dysregulation of energy expenditure, creating a relative hypermetabolic state under HFD conditions. However, the signaling pathway(s) that mediate these effects are unknown. Recent reports suggest that GDF3 signaling can occur through the TGF-beta superfamily type I receptor, Alk7, which is located on human chromosome 2q24.1, another locus with linkage to obesity-associated traits. Alk7 is highly expressed in normal human adult adipose tissues, whereas its expression is reduced in obese individuals, and correlates with factors implicated in the metabolic syndrome. Alk7 knockout mice have reduced fat accumulation and resistance to diet-induced obesity. In addition, GDF3 can inhibit bone morphogenetic protein (BMP) signaling. Several BMPs play important roles in adipogenesis, and BMP7 is sufficient to initiate and sustain brown adipose differentiation in vitro and in vivo with consequential effects on energy expenditure. Interestingly, the brown adipose tissue markers, PGC1-alpha, CPT1b and UCP1 are overexpressed selectively in the white adipose tissues of GDF3 knockout mice on HFD, suggesting that GDF3 may modulate the activity of BMP signaling on fate decisions during adipocyte differentiation. We are studying the potential molecular mechanisms underlying the effects of GDF3 on adipogenesis and energy expenditure using pre-adipocyte cell lines. In addition, we are developing transgenic mouse models of GDF3 expression under spatiotemporal control. Ultimately, these in vivo and in vitro models will help us to better understand the roles of TGF-beta superfamily signaling in human obesity and energy expenditure.

## 3029/F/Poster Board #961

**Salivary Methylmalonate and Salivary Microbiome Profiling in Methylmalonic Acidemia.** *C.W. Bassim[1], P.P. Sulima[1], J. Sloan[2], C.P. Venditti[2], T.C. Hart[1]*. 1) NIDCR, NIH, Bethesda, MD; 2) NHGRI, NIH, Bethesda, MD.

**Background:** The methylmalonic acidemias (MMA) are autosomal recessive enzymopathies with massive accumulation of bodily fluid methylmalonate and propionate-derived metabolites. Isolated MMA is caused by mutations in methylmalonylCoA mutase (MUT), a cobalamin-dependent enzyme in the mitochondrial matrix, or by defects that impair cofactor metabolism (*cblA*, *cblB*). MMA is clinically and biochemically heterogeneous but many patients exhibit failure-to-thrive, strokes, kidney disease, and early mortality. Based on our previous studies, we hypothesized that elevated methylmalonate salivary levels may affect oral health. **Objectives:** 1) To analyze salivary and plasma methylmalonate in affecteds and controls. 2) To compare caries index in affecteds and controls. 3) To characterize dental plaque microbial flora in affecteds and controls. **Methods:** Blood and saliva were collected from 14 *mut* and 1 *cblA* MMA patients and from 9 controls. Metabolites were measured using mass spectrometry. Caries index was calculated as decayed/missing/filled teeth (DMFT) from clinical and radiographic analysis of 35 affecteds and 70 controls. Dental plaque samples were collected from 5 *mut* MMA patients. Microbial profiling followed the amplification, cloning and bioinformatic analysis of 16SrDNAs from the dental plaque of 5 mut MMA patients. **Results:** MMA patients had significantly higher salivary methylmalonate levels than controls (geometric mean 4549 ± multiplicative SD 5 vs. 195 ± 2 nmol/l, p<0.0001) that correlated with serum methylmalonate concentration (p=0.0003). MMA patients had nonsignificantly lower DMFT than controls (mean 1.23 ± SD 2.67 vs. 1.81 ± 2.55 teeth, p=0.281). A reduced oral microbiome was seen in the MMA patients, with limited caries-associated oral flora and a complete absence of *Lactobacillus*. **Conclusions:** The novel finding of elevated salivary methylmalonate in MMA patients raises the possibility of the use of saliva as a non-invasive diagnostic medium for metabolic monitoring. Clinically, the MMA patients show limited dental caries. Salivary methylmalonate may alter the oral microflora, possibly impacting oral and gastro-intestinal health. Genomic analysis of the oral microbial flora showed a reduction in bacterial diversity, a trend towards less cariogenic species, and the striking observation of absent cariogenic species such as *Lactobacillus* in the MMA plaque samples. These findings may correlate with clinical severity in MMA in addition to oral health.

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## 3030/F/Poster Board #962

**ACYLCARNITINE PROFILING AND FATTY ACID TOXICITY IN FIBROBLASTS FROM PATIENTS WITH FATTY ACID OXIDATION DEFECTS.**
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Inherited disorders of the carnitine cycle and mitochondrial beta oxidation can impair the oxidation of fatty acids and result in hypoglycemia, liver failure, cardiomyopathy, cardiac arrest and death. Unfortunately, establishing a diagnosis in affected patients is not always straightforward since biochemical abnormalities might disappear while the patient is well and DNA analysis might not identify all possible mutations/deletions/rearrangements. Functional assays, such as fatty acid fluxes and acylcarnitine profiling can be performed in cells derived from affected patients. Some of these methods, however, cannot distinguish among different fatty acid oxidation defects and some of them cannot differentiate well carriers from affected patients. We have developed a method for the evaluation of fibroblasts from patients with fatty acid oxidation defects. These cells were incubated with a mixture of fatty acids that more closely resembled that of human serum. The acylcarnitines produced by the cells after 3 days were analyzed by tandem mass spectrometry and had a profile very similar to that observed in affected patients while acutely stressed for most fatty acid oxidation defects. Specifically, this method detected increased levels of C14:1 carnitine in cells from patients with Very Long Chain Acyl CoA dehydrogenase deficiency and other characteristic metabolites in long-chain fatty acid oxidation defects. We also noted that, in some glucose-free media, cells from patients with fatty acid oxidation defects were more sensitive than normal cells to high concentrations of fatty acids, while albumin had a protective effect. We are currently testing whether nutritional supplements can protect fibroblasts of patients with fatty acid oxidation defects from fatty acid toxicity. These results indicate that the use of a more physiological mixture of fatty acids allows the *in vitro* differentiation of fatty acid oxidation defects. Free fatty acids seem to have increased toxicity towards cells from patients with fatty acid oxidation defects.

## 3031/F/Poster Board #963

**Early detection of neuropsychological deficits in adult non-compliant patients with PKU.** *M. Bik-Multanowski, J.J. Pietrzyk.* Chair of Pediatrics, Jagiellonian Univ, Krakow, Poland.

Adult patients with phenylketonuria (PKU) demonstrate serious compliance problems often leading to discontinuation of dietary treatment. In some of such patients serious neurological or psychiatric symptoms are reported. Prefrontal cortex-dependent deficits are considered to be early symptoms of brain dysfunction in these patients. However, no standardized tests for reliable, early detection of such deficits are available. The aim of this pilot study was to evaluate usefulness of computerized neuropsychological tests for detection of prefrontal cortex dysfunction in adults with PKU. Methods: Young, early treated adults (treatment for at least the first 12 years of life) with classical PKU and normal mental development were tested by means of computerized CANTABeclipse system (Cambridge Cognition Ltd), which includes normative database for assessment of results of the tests used. Neuropsychological tests assessing prefrontal cortex-dependent functions such as attention, working memory and executive functions were applied. Results: A group of 15 patients (homozygotes or compound heterozygotes for null mutations of the phenylalanine hydroxylase gene: p.R408W and p.P281L) aged 17-24 years participated in the study. At the study time 10 patients were off-diet (mean phenylalanine levels > 1.2 mmol/L) and 5 of them followed dietary recommendations (mean phenylalanine levels < 0.8 mmol/L). Attention deficits were detected in 9 out of 10 non-compliant patients (mean SD of the results: -2.53; Rapid Visual Information Processing Test) accompanied by a tendency to impaired working memory capacity (mean SD: -1.86; Spatial Working Memory Test). Lower speed of choice response (Choice Reaction Time Test) positively correlated with the above findings. On the contrary, the results achieved by all compliant patients were within normal range. Conclusion: CANTABeclipse system allows for reliable detection of neuropsychological deficits in non-compliant adults with PKU. Study was sponsored by government research grants NN402329233 and N40708032/3085.

## 3032/F/Poster Board #964

**A Custom Microarray for Fatty Acid Oxidation Disorders.** *T. Lewis[1], N. Longo[2,3], E. Lyon[2,3].* 1) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; 2) ARUP Laboratories, Salt Lake City, UT; 3) Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, Utah.

**Introduction:** Deletions and duplications involving DNA regions are known to cause many genetic disorders by leading to under-expression, over-expression, or disruption of the gene. Recently, high-resolution comparative genomic hybridization microarrays have been shown to be a valuable tool for examining genomic regions for deletions and duplications. An advantage of high-resolution CGH microarrays over other methods is ability to interrogate many probes per exon, include intronic regions, and cover a large number of genomic target regions. Here we describe the development, design, and validation of a high-resolution CGH microarray for the detection of both deletions and duplication in thirteen fatty acid genes. **Methods and Results:** A custom microarray was developed to analyze 13 genes involved in fatty acid metabolism. The array was constructed on a NimbleGen 4x72K platform using unique oligomers with an average length of 53 base pairs. Probe coverage extended across all exons and introns of each gene and an additional 5kb both upstream and downstream of each genomic region. The median probe spacing was nine base pairs, providing a resolution of 100 base pairs. Self-self, within run, and between run hybridizations were performed to validate the array design. Two self-self hybridizations were performed to confirm probe validity, estimate a false positive rate, and to verify the parameters used for data analysis. Seven samples were hybridized in duplicate on the same slide to confirm reproducibility of results. Four of the samples were additionally repeated on different arrays to examine between run reproducibility. In total, 6 control samples were examined and 11 clinical samples were analyzed on thirty-two arrays. The clinical samples had established biochemical diagnosis of fatty acid oxidation disorders but gene sequencing had not been able to confirm the presence of two mutations. In two of the clinical samples, deletions of a single exon were detected using the high-resolution fatty acid oxidation CGH array. **Conclusions:** A high-resolution custom microarray for fatty acid oxidation disorders is useful in cases where sequencing does not detect mutational changes. The tightly spaced, tiled probe design of our array allow for detection of deletions as small as 100 base pairs. These findings indicate that high-resolution microarrays provide a highly sensitive approach for the detection of single and multi-exon deletions or duplications.

## 3033/F/Poster Board #965

**A New Tandem Mass Spectrometry (MS/MS) Method For Detecting Pompe Disease In Dried Blood Spots.** *K. Cusmano-Ozog, A. Le, G.M. Enns, T.M. Cowan.* Stanford University, Stanford, CA.

Pompe disease is caused by a deficiency of acid alpha-glucosidase (GAA). Typical findings in the infantile-form include hypotonia, weakness and cardiomyopathy. Treatment with enzyme replacement therapy (ERT) has improved survival. Early diagnosis by expanded newborn screening (NBS) will allow for optimal treatment that may minimize morbidity and mortality in Pompe disease and other lysosomal storage diseases (LSDs). Recently, a laboratory approach was developed for identification of LSDs based on enzymatic assays of dried blood spots (DBS) using synthetic substrates, stable-isotope standards (IS) with detection of products by tandem mass spectrometry (MS/MS). The reaction occurs in a solution of salt and detergent, which must then be removed by a lengthy and complex process of liquid/liquid and solid phase extractions prior to MS/MS analysis. We have developed a novel, simplified method for MS/MS detection of GAA product. In brief, DBS are rehydrated and incubated with GAA substrate and IS (provided by Genzyme through the CDC) in 2% dimethyl sulfoxide. Acarbose is added to inhibit an alpha-glucosidase isomer present in neutrophils. Salting-out assisted liquid/liquid extraction (SALLE) with acetonitrile is used to stop the reaction and prepare the sample for MS/MS analysis. We have been able to evaluate the performance of this new technique in an infant with classic Pompe disease. A four-month-old was evaluated following the death of her monozygotic twin from cardiomyopathy. She appeared critically ill and had respiratory distress, severe hypotonia, weakness, macroglossia and elevated CK level. GAA activity was low using our novel method for blood spot analysis and confirmed by standard blood enzymology. She was started on ERT and has shown dramatic improvement; she now has spontaneous movements against gravity and is weaning off CPAP. This case reinforces the importance of NBS for LSDs. By detecting infants before the onset of symptoms, appropriate therapy can be initiated early, with subsequent decreases in morbidity and mortality. Our novel, straightforward method can detect individuals with Pompe disease and is appropriate for NBS. Ongoing studies include validating this method on a larger number of individuals with Pompe disease and expanding this approach to other LSDs in order to streamline high-throughput analysis.

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## 3034/F/Poster Board #966

**Erythropoietic Protoporphyria: Molecular Analysis Reveals Patients with X-Linked Causative Mutations in the Erythroid-Specific 5'-Aminolevulinate Synthase 2 Gene.** *D. Doheny[1], I. Nazarenko[1], M. Balwani[1], L. Liu[2], R.J. Desnick[1].* 1) Dept Gen & Genomic Scienc, Mount Sinai Sch Med, New York, NY; 2) Dept Medicine, Liver Diseases, Mount Sinai Sch Med, New York, NY.

Erythropoietic Protoporphyria (EPP), the most common erythropoietic porphyria, is an inherited disorder characterized by acute photosensitivity and painful cutaneous lesions. Autosomal recessive EPP is biochemically diagnosed by elevated levels of ertythrocyte protoporphyrins (PROTO) with free-PROTO greater than zinc-PROTO. Most cases result from mutations in the ferrochelatase (FECH) gene that cause the markedly deficient activity of ferrochelatase, the last enzyme in the heme biosynthetic pathway. To date, 119 FECH mutations have been described (Human Gene Mutation Database, 2009), as well as the common hypomorphic IVS3-48T>C allele. Recently, an X-linked variant of EPP was described with similar symptoms, elevated erythrocyte PROTO with equal proportions of free-PROTO and Zn-PROTO, and no FECH mutations; however, causative mutations were identified in the C-terminus of the erythroid-specific 5'-aminolevulinate synthase 2 (ALAS2) gene at Xp11.21 (Whatley et al, 2008). We performed FECH mutation analysis on 22 unrelated patients with biochemical and/or clinical evidence suggestive of EPP, identifying FECH mutations in 15 patients. Among these, six had novel mutations, including one deletion (c.1052delA), one insertion (c.215-216insT), two splice-site (c.1077-1G>T, c.314+1T>G), two nonsense (c.832C>T (p.Q278X), c.181C>T (p.Q61X)), Of the seven cases with no detectable FECH mutation, we identified three patients with ALAS2 lesions. Of these, a 19 year old male had photosensitivity since childhood, elevated PROTO with equal proportions of free-PROTO and Zn-PROTO, and a fluorescence peak at 633nm consistent with EPP. The patient's mother reported mild photosensitivity. Molecular studies revealed a novel ALAS2 frame-shift mutation, c.1734delG, in exon 11. The two other patients, a 7 year old male and 3 month old female, had the previously reported ALAS2 c.1706-1709delAGTG mutation. These findings indicate that X-linked EPP is not rare among EPP patients (14% of index cases; 43% of FECH-mutation negative cases) and that ALAS2 mutation analysis should be undertaken in patients with EPP symptoms of acute photosensitivity who have elevated erythrocyte PROTO, high Zn-PROTO, and no detectable FECH lesion.

## 3035/F/Poster Board #967

**Defects of pyruvate metabolisim in cultured lymphoblastoid cells from 70 Japanese patients with Leigh syndrome.** *E. Naito[1,2], T. Hashimoto[1], Y. Kotani[2], S. Kagami[2].* 1) Division of Pediatrics, Japanese Red Cross Tokushima Hinomine Rehabilitat , Komatsushima City, Tokushima, Japan; 2) Department of Pediatrics, Institute of Health Biosciences, The University of Tokushima, Graduate School, Tokushima, Japan.

Leigh syndrome is a neurodegenerative disease caused by defects in mitochondrial energy generation, including pyruvate dehydrogenase complex (PDHC) and components of the respiratory chain. It is characterized by progressive neurological disease with motor and intellectual regression, with onset typically in infancy, and by characteristic lesions of the basal ganglia and brainstem. Elevated lactate levels in both blood and cerebrospinal fluid (CSF) are often present. We investigated defects of pyruvate metabolisim in 70 Japanese patients with Leigh syndrome. Biochemical and molecular defects were studied using cultured lymphoblastoid cells obtained from 41 boys and 29 girls with this clinical diagnosis. All patients had a progressive neurological disease with developmental delay; raised lactate levels in blood and/ or CSF; and the abnormalities in bilateral basal ganglia on computed tomography or magnetic resonance imaging. Defects identified in 35 patients (50 per cent), i.e., PDHC deficiency in 5, NADH-cytochrome c reductase (complex I) deficiency in 4, cytochrome c oxidase (COX) deficiency in 4, and a point mutation of mitochondrial DNA in 22 (A3243G in 1, A8344G in 1, T8993C in 2, T8993G in 16, and G13513A in 2). Five patients with PDHC deficiency had mutations in the X-chromosomal gene encoding the E1$\alpha$ subunit of PDHC. Three patients with COX deficiency had mutations of the SURF-1 gene. Cultured lymphoblastoid cells were useful for elucidating the etiologies of Leigh syndrome. T8993G mutation was the most common cause in Japanese patients with Leigh syndrome. In 4 of 5 patients with PDHC deficiency, PDHC showed reduced affinity for thiamine pyrophosphate. Treatment with high doses of thiamine resulted in decreased lactate and clinical improvement in these four patients, suggesting that some patients with Leigh syndrome may have a thiamine-responsive PDHC deficiency.

## 3036/F/Poster Board #968

**Screening for Fabry disease using dried blood spots on filter paper: an improved alpha-galactosidase A assay.** *E. CAUDRON[1], P. PROGNON[2], F. JABBOUR[3], L. LACASTAIGNERATTE[3], C. BOUCLY[3], D.P. GERMAIN[4].* 1) Groupe de Chimie Analytique, EA 4041, Faculté de Pharmacie Paris XI, Châtenay-Malabry, France; 2) Département de Pharmacie, Hôpital Européen Georges Pompidou, 75015 Paris, France; 3) Laboratoire de Biochimie. Hôpital Raymond Poincaré (AP-HP), Garches, France; 4) Unité de Génétique Médicale et Centre de Référence de la maladie de Fabry. Hôpital Raymond Poincaré (AP-HP), Garches, France. dominique.germain@rpc.aphp.fr.

Background: Fabry disease (FD, OMIM 301500) is an X-linked glycosphingolipid storage disorder caused by the deficiency of the lysosomal enzyme $\alpha$-galactosidase A. A fluorimetric method that uses filter paper cards containing dried blood spots (DBS) instead of peripheral white blood cells pellets as the enzyme source was recently introduced for diagnosis, allowing storage of the samples for up to 6 months due to stability of the enzyme on the filter paper [1]. However, the original DBS fluorimetric reference method is impaired by fluorescence quenching due to hemoglobin interference. In this study, we describe an improved assay based on protein precipitation by zinc sulfate. Methods: A simple and sensitive fluorimetric microassay was designed for an improved determination of $\alpha$-galactosidase A activity on DBS and tested in 60 hemizygotes, 68 heterozygotes, and 81 controls. Fluorogenic 4-methylumbelliferyl-$\alpha$-D-galactose was used as a substrate. The enzymatic reaction was stopped and proteins were precipitated by the addition of 300 $\mu$L of zinc hydroxide suspension (aqueous zinc sulphate heptahydrate (2%, w/v): 0.1 mol.L-1 sodium hydroxide) to eliminate interfering species (hemoglobin). In addition, a comparison with the originally published DBS assay was performed on 33 individuals (8 hemizygotes, 12 heterozygotes and 13 controls). Results : A cut-off level of 2.1 $\mu$mol.h-1.L-1 (control values: 5.6 ±2.0 $\mu$mol.h-1.L-1, mean ± SD) was chosen corresponding to 40% of median control value. For the 60 hemizygotes, all alpha-gal A activities were below 1.1 $\mu$mol.h-1 .L-1 (0.11 ± 0.2 $\mu$mol.h-1 .L-1). For the 68 heterozygotes, $\alpha$-gal A activities ranged from 0 to 7.8 $\mu$mol.h-1 .L-1 (2.2 ± 1.7 $\mu$mol.h-1 .L-1). Two third of the heterozygous females were correctly identified using this assay. Conclusion: This modified alpha-gal A assay on DBS proved sensitive, robust and decreased the frequency of false-positive results when compared to the originally published procedure [1] where hemoglobin was responsible for quenching of fluorescence. The new assay can be applied for the screening of patients undergoing haemodialysis or presenting with left ventricular hypertrophy or cryptogenic stroke who may have unrecognized FD. However, screening studies using enzymatic assays fail to detect 1/3 of heterozygotes due to random X-linked inactivation. Chamoles NA et al Fabry disease: enzymatic diagnosis in dried blood spots on filter paper. Clin Chim Acta 2001;308:195-6.

## 3037/F/Poster Board #969

**Evaluation of mutant $\alpha$-galactosidase A activity - a rapid screening method to determine disease relevant defects.** *J. Lukas[1], U. Goelnitz[2], A. Wree[3], R. Köhling[4], A. Rolfs[1,2].* 1) Albrecht-Kossel-Institute, University of Rostock, Rostock, Mecklenburg-Vorpommern, Germany; 2) Centogene GmbH, Rostock, Germany; 3) Department of Anatomy, University of Rostock, Rostock, Germany; 4) Department of Physiology, University of Rostock, Rostock, Germany.

Fabry disease is a lysosomal storage disorder caused by the deficiency of $\alpha$-galactosidase A (AGLA) activity. So far, about 300 AGLA missense mutations have been described according to the Human Gene Mutation Database (HGMD). However, numerous amino acid exchanges appear to have only mild effects on enzyme activity reduction. Bioinformatic tools cannot reliably predict the effects of these changes and inevitably need to be supported by enzymatic measurements. Therefore, a rapid screening procedure for all AGLA mutations will be invaluable for this purpose. To solve the limitations of all routine methods we developed an in vitro model based on the overexpression of AGLA mutants using a HEK293 cell system. Using fluorescence enzyme activity assays we are able to determine the activity of mutant enzymes. This procedure has shown that the following mutations, which have been found in our screening programs for Fabry have to be interpreted as SNPs and not clinically relevant AGLA mutations: D83N, S102L, S126G, R220Q and R252T. Tools for the systematic in-vitro evaluation of newly detected mutations are also of high value for the evaluation of the consequences of new therapeutic strategies, e.g. using pharmacological chaperones (PCs) such as 1-Deoxygalactonojirimycin (DGJ). Thus, we started a substance screen in order to find mutation-specific PCs performing better on chosen mutations with regard to a higher efficacy, potency and therapeutic index.

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## 3038/F/Poster Board #970

**Standard Protocols to Investigate Brain Structure and Function for the Lysosomal Disease Network.** *E. Shapiro[1], I. Nestrasil[2], A. Ahmed[1], K. Bjoraker[1], K. Delaney[1], R. Ziegler[1], L. Charnas[1], C. Whitley[1], Lysosomal Disease Network.* 1) Dept Pediatrics, Univ Minnesota, Minneapolis, MN; 2) Dept Neurology, Univ Minnesota, Minneaoplis, MN.

Background: The Lysosomal Disease Network (NIH 1U54NS065768-01) will use quantitative and qualitative standardized MRI and neuropsychological protocols to longitudinally track brain structure and function in the course of lysosomal disease, seek markers of change, and evaluate treatment response. This multi-center multi-disease endeavor requires repeatable standardized protocol sets that will serve both research and clinical needs including diffusion tensor imaging (DTI) and volumetric analysis. Quantitative methods may yield addition disease-specific localization of pathology, correlated with disease stage and rate. Standard repeatable neuropsychological protocols include tests that are age- specific and measure specific deficits associated with brain regions. Tests of cognitive ability, memory, attention/executive function, sensory and motor function, spatial ability, language, adaptive functions, emotion/behavior, and quality-of-life for each age group have been selected for their sensitivity, specificity, reliability, validity, and repeatability. Methods: Pilot studies are being done on patients with MPS I (n=20), MPS II (n=10), and MPS VI (n=10) using various MRI analysis methods and associations with neuropsychological tests. Pilot studies of the reliability of the volumetric MRI data have been carried out comparing automated (FreeSurfer) and manual programs (Brains2). Results: Measurement of hippocampus and amygdala volumes are not reliable on automated programs but can be reliably measured with manual programs such as Brains2. Other calculations such as white matter and gray matter volumes, ventricular volume, as well as volumes of larger and more discrete structures can be reliably carried out using FreeSurfer. DTI Studio has yielded positive results. Pilot studies indicate strong associations of neuropsychological tests with neuroimaging. Examples: 1) hippocampal size and memory in attenuated forms of MPS I; 2) DTI - fractional anisotropy with attention/reaction time measures in Hurler syndrome treated with HCT. Conclusions: Reliable methods of quantification of brain structure have been developed which can be correlated with functional capacity on neuropsychological tests. These methods identify late treatment effects and quantify disease status for the Lysosomal Disease Network. The next step is validating these measures as markers of change in brain disease both at diagnosis and in the late effects of treatment.

## 3039/F/Poster Board #971

**The spectrum of Lysosomal Storage Disorders (LSDs) in children with neuroregression in India.** *J. Sheth[1], F. Sheth[1], P. Gambhir[2], U. Dave[3], N. Oza[1], M. Mistri[1].* 1) Biochem & Molec Bio, Inst Human Gen, Ahmedabad, India; 2) Birth Right Clinic, Pune, India; 3) Preventine Life Care, Mumbai, India.

Background: Neurodegenerative disorders are commonly observed in pediatric population with multiple frequencies due to different causes. This includes polygenic condition in 50 % and chromosomal abnormality in 30 % of the cases. Other rare causes are injury in developing brain [10%], multiple congenital anomalies (MCA) [4-5%] and endocrine-metabolic disorders in 3-5% of the cases. Nonetheless, neuroregression affecting large number of children are commonly seen with Lysosomal Storage Disorders [LSDs]. However due to its rarity and lack of investigational facility in India, they remain under diagnosed. Phenotypic heterogeneity and central nervous system (CNS) involvement further complicates the diagnosis at an early stage. Therefore present study is aimed to know the spectrum of LSDs associated with neuroregression in Indian children. Objective: To know the prevalence of different LSDs associated with progressive neuroregression. Material and Method: The study consisted of 500 children in the age range of newborn to 12 years referred for various LSDs. Of these, 161children were found to have neuroregression and lysosomal enzyme study from leucocytes, fibroblasts and/or plasma were analyzed for β-Hexosaminidase, β-Galctosidase, Sphingomyelinase, various enzyme for MPS (I to VII), β-Galactocerebrosidase, β-Glucocerebrosidase and Neuraminic acid (Free & Total). Results: Of 161 children having neuroregression or neurodegentration, 119(73.9%) were normal and remaining 42 (26.1%) have shown different types of LSDs associated with neuroregression. This includes, GM2 gangliosidosis in 15 (9.3%), Metachromatic leucodystrophy in 8 (4.96%), GM1 gangliosidosis in 3 (1.86%), Gaucher disease in 3 (1.86%), Sialic acid storage disorder in 3 (1.86%), Morquio-B in 3 (1.86%), Krabbe disease in 2 (1.24%), Morteoux-lamy syndrome (MPSVI) in 2 (1.24%), Sanfilippo disease (MPS III B) in 1 (0.62%), Sly syndrome (MPS VII) in 1 (0.62%) and Niemann-Pick disease A/B in 1 (0.62%) cases. Conclusion: LSDS are highly prevalent in children with neuroregression and common neurology screen for LSDs can help to identify the disease at an early age for better therapeutic or prognostication advise in addition to prenatal diagnosis in the subsequent pregnancy.

## 3040/F/Poster Board #972

**Fabry disease: Identification of large deletions in α-galactosidase gene.** *R. Dobrovolny, I. Nazarenko, D. Doheny, D. Bishop, R. Desnick.* Genetics & Genomic Sciences, Mount Sinai School Medicine, New York, NY.

Fabry disease is an X-linked inborn error of glycosphingolipid metabolism caused by the deficient activity of lysosomal α-galactosidase A (α-Gal A) leading to the progressive accumulation of glycosphingolipids with terminal α-galactosyl residues. In the patients with classical phenotype who have little or no enzymatic activity, the disease presents in the childhood and progresses to renal failure, cardiac involvement, cerebrovascular disease and early demise. Patients with the later-onset phenotype present in adulthood with heart or kidney disease, and are often misdiagnosed. In males with either phenotype, the diagnosis is reliably made by the markedly deficient α-Gal A enzyme activity. In female heterozygotes, accurate diagnosis requires mutation analysis. However, suspect heterozygotes can be missed by the occurrence of deletions that include entire exons, or by mutations that result in PCR failures due to alterations involving the PCR primers. Therefore, we developed a simple semi-quantitative method for PCR detection of gene dosage which can detect the copy number of the amplified α-Gal A genomic regions. Absence of the mutated region was determined by comparison with the signal heights of normal male and female α-Gal A gene sequences. For a deletion, the exact breakpoints can be identified by finer mapping and direct sequencing. To date, this method has been used for deletion screening of 27 unrelated females with decreased α-Gal A activity suggesting heterozygosity for Fabry disease, but no detectable α-Gal A mutation. Two novel deletions were identified in these heterozygotes: c.369+3_c.547+954del4096bp which was low for exons 2-4 by gene dosage and c.194+2051_c.369+787del2623insCG which deleted the entire exon 2. The latter deletion was confirmed by RT-PCR. This method simplifies the detection of α-Gal A deletions and is useful for deletions that cannot be detected by standard sequencing protocols and when RNA sources are not available.

## 3041/F/Poster Board #973

**Creatine Deficiency Syndromes: Clinical experience of a diagnostic laboratory.** *J. Goldstein, S. Young, A. Vaisnins-Carroll, E. Winchester, D. Koeberl, D. Millington.* Biochemical Genetics Laboratory, Dept. of Pediatrics, Duke University Medical Center, Durham, NC.

Creatine deficiency syndromes (CDS) are caused by defects in creatine synthesis and transport. Clinical symptoms include expressive speech and language delay, mental retardation (MR), seizures, autistic features, and movement disorder. The full phenotypic spectrum is unknown but emerging evidence suggests that these disorders vary widely in presentation. Some patients have presented with intractable seizures, while others have presented only with speech delay. From previous published studies, X-linked creatine transporter deficiency is found in about 0.3-3.5% of males with developmental delay/MR. Initial diagnosis of CDS can be made by measuring creatine and guanidinoacetate in urine and plasma. The diagnosis may be confirmed by creatine uptake studies, DNA analysis, and proton magnetic resonance spectroscopy. Prompt diagnosis is important because dietary treatment can ameliorate the symptoms of creatine synthesis disorders. The goal of this study is to describe the experience of our clinical diagnostic laboratory in measuring urine and plasma creatine and guanidinoacetate by stable isotope dilution liquid chromatography electrospray-tandem mass spectrometry (LC-MS/MS). In total, 402 patients were referred for testing urine and 159 for testing in plasma. The majority of patients had two or more of the classical clinical features for this disorder. Two male patients (0.5%), ages 6 and 7 years, had persistent marked elevations of urinary creatine suggestive of X-linked creatine transporter deficiency (mean 3860 mmol/mol creatinine; control range <823). Both patients had features typical of this disorder. Thirty-six patients had marked elevation of creatine in one urine sample and no follow-up sample was received. Ten patients (2.5%) had elevated creatine in an initial urine sample that normalized upon repeat, suggesting that the first result was a false positive, caused by another factor such as diet. Autistic features are a characteristic of CDS. Detailed review of medical records from 49 patients referred for testing revealed 39 with autism spectrum disorder (ASD). All of them had additional clinical features such as developmental delay or seizures. Our results indicate that testing for CDS should be considered for patients with speech delay, developmental delay/MR and seizures. Further studies are required to fully delineate the clinical spectrum, including larger studies to determine the prevalence of CDS in individuals with ASD.

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## 3042/F/Poster Board #974

**Spectrum of Mutations Identified in Saudi Arabian Patients with Various Inherited Metabolic Disorders.** 
*F. Imtiaz[1], Z. Al-Hassnan[2], M. Al-Owain[2], M. Al-Hamed[1], B. Al-Mubarak[1], A. Mustafa[1], R. Allam[1], D. Trabzuni[1], M. Rashed[1], H. Khalak[1], B.F. Meyer[1], M. Al-Sayed[2].* 1) Department of Genetics, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 2) Department of Medical Genetics, KFSH & RC, Riyadh, Saudi Arabia.

The number, complexity and varied clinical presentation of inherited metabolic diseases (IMDs) present a formidable challenge simultaneously for clinicians and for the laboratory in the pursuit of a rapid and precise molecular genetic diagnosis. Single gene defects result in abnormalities in the synthesis of various carbohydrates, proteins or fats. Most IMDs are caused by a defect in an enzyme or a transport protein that results in a block in a metabolic pathway. As a consequence of extensive consanguinity, IMDs are relatively common in Saudi Arabia. In our laboratory we are committed to characterizing the molecular defects underlying a large number of IMDs. Recently, we have characterized a large number of such disorders and have identified between 85-95% of the disease-causing mutations (novel and previously published) that are specifically responsible for the occurrence of Argininosuccinic Lyase Deficiency, HMG-CoA Lyase Deficiency, Medium Chain Acyl-CoA Dehydrogenase Deficiency and Very-Long Chain Acyl-CoA Dehydrogenase Deficiency in the Saudi population. We have also made considerable progress with respect to the molecular detection of pathogenic mutations in Saudi patients with Methylmalonic Aciduria, Propionic Acidemia and Tyrosinemia Type 1. From a diagnostic standpoint, we have also performed and reported mutation analysis on a further 33 metabolic diseases. To date, in total we have identified 127 mutations, 55 of which are novel in 40 different metabolic diseases. As a direct result of this effort, efficient molecular diagnosis of these diseases in Saudi Arabia is now possible and lays the foundation for preventative measures including inductive screening of extended families, counseling, prenatal diagnosis, pre-implantation genetic diagnosis and regional pre-marital screening.

## 3043/F/Poster Board #975

**Random screening for late onset Pompe disease using GAA activity on dried blood spot assays in a Brazilian rehabilitation center (ABBR-RJ/Brazil).** 
*J.C. Llerena[1], C. Pimentel[2], S.M.M.Q. Martins[2], A.C.B. Franzói[2], K.B. Müller[3], V. D'Almeida[3], A.M. Martins[3], D. Horovitz[1], REBREPOM/BRAZIL.* 1) Medical Genetics, IFF/FIOCRUZ, Rio de Janeiro, Rio de Janeiro, Brazil; 2) Associação Brasileira Beneficente de Reabilitação (ABBR) - Rio de Janeiro - Brazil; 3) Centro de Referência em Erros Inatos do Metabolismo-IGEIM/UNIFESP - São Paulo - Brazil.

Late-Onset Pompe Disease (LOPD) is a metabolic neuromuscular disorder caused by deficiency of the lysosomal enzyme, acid alpha-glucosidase (GAA). The disease manifests at various ages and is characterized by muscle weakness and respiratory insufficiency. Respiratory failure causes the major morbidity and mortality of this form of the disease. Diagnosis of LOPD is made through the measurement of GAA enzyme activity in fibroblasts, muscle, peripheral leukocytes, and lately, on dried blood spots (DBS); thus allowing rapid and sensitive analysis. Considering that the clinical spectrum of PD is manifested predominantly in the skeletal muscular system, with muscular weakness and/or respiratory symptoms, the investigation of GAA enzyme activity in patients with no presumptive diagnosis under treatment in a motor rehabilitation and/or respiratory physiotherapy clinic could uncover new undiagnosed cases. We conducted a random search for LOPD in a physiatrist rehabilitation center using DBS GAA activity. Four hundred and thirty seven patients attending different sectors at ABBR (Rio de Janeiro, Brazil) were enrolled in 2008. A cut-off of 87% maltose inhibition was used and a NaG/AaGIA ratio below 40 was considered as an unaffected case. No cases of LOPD were detected, and a 1% (4 patients) false positive rate was found taking into consideration the maltose inhibition fraction only in discordance with NaG/AaGIA ratio. These patients were clinically reevaluated and none had LOPD (one case each of neonatal hypoxia, HTLV-1 positive child, post-surgery for cervical vertebral disc hernia, and arthrosis). Such discrepancy was interpreted as related to the sample conditions once preparing the DBS. Twenty nine cases (6.6%) had a second DBS collected due to technical reasons. Our series showed a median value for maltose inhibition and NaG/AaGIA ratio of 70.72±11.72 (range 8.60-86.76) and 14.20±6.09 (range 5.17-34.47), respectively; compared to our lab series of 18 LOPD cases of 90.56±2.17 (range 88.39-92.73) and 60.04±17.75 (range 44.0-75.79), respectively. Fourteen cases of neuromuscular disorders, 17 of stroke, 25 of flaccid paraplegia, 48 of developmental delay, and 193 cases of cerebral palsy were of interest and remained with no etiological diagnosis. GAA activity screening through DBS proved to be successful in our series; however, technical pitfalls and drawbacks related to the DBS as a screening method should not be underestimated.

## 3044/F/Poster Board #976

**Compound Heterozygosity of the POLG1 Gene Presenting with Liver Failure and Hypotonia.** 
*M.F. Walsh[1,2], T. Wang[1,2], K. Schwartz[2], T. Crawford[2], M. Hirano[3], L. Wong[4], R.D. Cohn[1,2].* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins Hospital, Baltimore, MD; 2) Department of Pediatrics, Johns Hopkins Hospital, Baltimore, MD; 3) Department of Neurology, Columbia University, New York, NY; 4) Department of Molecular and Human Genetics, Baylor Colege of Medicine, Houston, TX.

The association of psychomotor retardation, seizures and hepatic failure was described previously in two non-related teenagers at a British Hospital as Alpers Syndrome. Mitochondrial DNA mutations, specifically in the POLG1 gene have been implicated in Alpers Syndrome. A study of nine infants with Alpers Syndrome revealed different allelic mutations in the POLG1 gene which were associated with multiple deletions, depletion or point mutations of mitochondrial DNA (mtDNA). Here we describe an 8 month-old female who presented with hypoglycemia and liver failure but otherwise normal motor and cognitive development. Over the course of two months she developed significant hypotonia and developed seizures associated with loss of cognitive function. Laboratory investigations demonstrated elevated transaminases, elevated alpha fetal protein, decreased cholesterol and hypoglycemia. A brain MRI bared no structural abnormalities. A gastric emptying study was positive for delayed motility and an EEG was positive for diffuse electrical disturbance. A liver biopsy was performed and electron microscopy revealed abnormal architecture and depletion of the mitochondria. A biopsy of intestinal cells did not manifest the same findings. Interestingly, a muscle biopsy was completed and initial structural evaluation did not expose any abnormalities. However, analysis of the mitochondrial DNA content as well as respiratory chain function of the muscle exhibited significant abnormalities of mitochondrial DNA content, respiratory chain function and abnormalities of mitochondrial metabolism, thus supporting the diagnosis of a systemic mitochondrial DNA depletion syndrome. Genetic analysis of various genes associated with mitochondrial DNA depletion syndromes unveiled an abnormality in the POLG1 gene. Our patient is heterozygous for a known G737R mutation previously associated with Alpers syndrome and an unclassified variant of V855L. The V855L mutation has previously not been described, however, this is a highly conserved residue that is invariant from yeast to man. Moreover it is in the thumb domain of the polymerase active site and biochemical studies of this areas show decreased POLG enzyme activity. We believe that it is highly likely that the compound heterozygous state of the patient is indeed disease causing and therefore represents a novel mutation in a patient with an unusual clinical presentation of Alpers syndrome.

## 3045/F/Poster Board #977

**A Novel LCR- and *Alu*- Mediated Rearrangement of the Iduronate-2-Sulfatase (*IDS*) Gene in a Patient with Mucopolysaccharidosis Type II.** 
*D. del Gaudio[1], J. Oshima[1], A.M. Breman[1], P.H. Fernandes[1], D. Babovic-Vuksanovic[2], P.A. Ward[1], C.M. Eng[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030; 2) Department of Medical Genetics, Mayo Clinic, Rochester, Minnesota 55905.

Mucopolysaccharidosis type II (MPSII; Hunter syndrome) is a rare X-linked recessive inborn error of metabolism caused by mutations in the iduronate-2-sulfatase (*IDS*) gene that spans approximately 24 kb on Xq28.2. The deficiency of iduronate-2-sulfatase leads to accumulation of heparan sulfate and dermatan sulfate within the lysosomes ultimately leading to the development of Hunter syndrome that occurs in both severe and mild forms. While the majority of *IDS* mutations are missense, nonsense, splicing and small deletions, in about 20% of MPSII patients the disorder is caused by gross *IDS* structural rearrangements. In some cases, highly homologous sequences promote nonallelic recombination events between the *IDS* gene and its nearby pseudogene (*IDSP1*) that has been annotated as a low copy repeat (LCR). Here we report the identification of a novel complex rearrangement involving *IDS* and the *IDSP1* pseudogene in a 3 years old male with MPS II. Standard *IDS* mutation analysis failed to amplify exon 8 suggesting the presence of a hemizygous deletion spanning exon 8. This finding was confirmed by MLPA analysis. Sequence analysis of the breakpoints, facilitated by oligonucleotide array-CGH testing, revealed that the mutant allele consisted of a 6 kb deletion spanning IDS exon 8 and a ~50 kb insertion in the deleted region. The 50 kb insert was derived through an inverted duplication of a telomeric region of *IDS* and it was flanked by the *IDSP1* (i.e. LCR) on one side and a combination of repetitive elements, L1-*Alu*-L1, on the other side. This duplicated fragment was inserted between the highly homologous LCR in *IDS* intron 7 and the L1-*Alu*-L1 elements in *IDS* intron 8 most likely through a nonallelic recombination mechanism. RT-PCR analysis confirmed the presence of aberrant mRNA transcripts. This rearrangement was detected in this patient's mother, indicating that the mutation is not a de novo event. There has been series of reports describing rearrangements involving *IDS* and *IDSP1*, suggesting that these events may not be infrequent. However, detailed analyses of these rearrangements have often been difficult because the extent of the recombination events could not be easily estimated. Given the involvement of LCRs and repetitive elements, this particular complex rearrangement may represent one of the common aberrations involving *IDS*.

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## 3046/F/Poster Board #978

**ETFDH gene mutations in Italian patients presenting Multiple Acyl-CoA Dehydrogenase Deficiency.** *C. Gellera[1], B. Castellotti[1], V. Pensato[1], C. Antozzi[2], B. Garavaglia[1], G. Uziel[3], M. Rimoldi[1], F. Taroni[1].* 1) Genetics of Neurodegeneration, IRCCS - Neurologico C. Besta, Milano, Italy; 2) Department of Neurology, IRCCS - Neurologico C. Besta, Milano, Italy; 3) Department of Child Neurology, IRCCS - Neurologico C Besta, Milano, Italy.

Mutations in genes encoding for Electron Transfer Flavoprotein (ETF) and Electron Transfer Flavoprotein Dehydrogenase (ETFDH) are the pathogenic cause of Multiple Acyl-CoA Dehydrogenation Deficiency (MADD), a metabolic disorder of fatty acid catabolism also known as glutaric aciduria type II (GAII). ETF is a mitochondrial-matrix heterodimer protein composed by a 30kD alpha subunit (ETFA) and a 28kD beta subunit (ETFB) and contains one FAD and one AMP per heterodimer. ETFDH is a 64kD monomer integrated in the inner mitochondrial membrane and contains one FAD and an iron-sulfur cluster. Both enzymes are required for electron transfer from at least 9 mitochondrial flavin-containing dehydrogenases to the main respiratory chain. The clinical phenotypes of GAII and MADD can have variable presentation ranging from a severe encephalopathy of childhood (more frequently associated with ETFA or ETFB mutations) to the mild myopathic form of adults, (more frequently associated with ETFDH mutations). We have studied 22 index cases, in which the biochemical spectrum and the clinical presentation were compatible with the diagnosis of MADD. Six patients had a positive familial history for the presence of an affected sibling, the onset was variable from childhood to adult age. AcylCoA-dehydrogenase activities of short, medium and long chain fatty acids were marked reduced in the analyzed cases (10/22) showing a residual activity of less than 10% in two cases, and about 50% in the remaining 8 patients. Analysis of Acyl-Carnitines showed a pathological increase of medium and long chain esterified carnitines in plasma along with a pathological pattern of urinary organic acids. Molecular analysis of ETFDH was performed in all index cases. We have identified 13 different missense mutations either in homozygous or in compound heterozygous form in 14/22 patients, thus confirming in these cases the clinical diagnosis. Eleven mutations were newly described. In three patients we identified mutations on one allele only. This latter result needs to improve molecular analysis by RT-PCR in order to search for the possible presence of intra-genic deletions. The remaining 8 patients resulted negative for mutations in ETFA and ETFB genes. Our study indicates that mutations in ETFDH gene are the major cause of MADD and support the hypothesis that other genes can be implicated in the pathological mechanism of this rare metabolic disease.

## 3047/F/Poster Board #979

**Mutation screening of the PAH gene causing phenylalanine hydroxylase deficiency in Greek patients with phenylketonuria and hyperphenylalaninemia.** *M. Grigoriadou[1], H. Kokotas[1], K.H. Schulpis[2], A. Skarpalezou[2], E.D. Papakonstantinou[2], M. Kalogerakou[2], A. Giannoulia-Karantana[3], M.B. Petersen[1].* 1) Department of Genetics, Institute of Child Health, 'Aghia Sophia' Children's Hospital, Athens, Greece; 2) Department of Inborn Errors of Metabolism, Institute of Child Health, 'Aghia Sophia' Children's Hospital, Athens, Greece; 3) Department of Pediatrics, Athens University Medical School, 'Aghia Sophia' Children's Hospital, Athens, Greece.

Phenylketonuria (PKU) is an autosomal recessive inborn error of metabolism resulting from a deficiency of phenylalanine hydroxylase, an enzyme that catalyzes the hydroxylation of phenylalanine to tyrosine, the rate-limiting step in phenylalanine catabolism. If undiagnosed and untreated, phenylketonuria can result in impaired postnatal cognitive development resulting from a neurotoxic effect of hyperphenylalaninemia. Early diagnosis of phenylketonuria, a cause of mental retardation, is important because it is treatable by dietary means. Features other than mental retardation in untreated patients include a 'mousy' odor, light pigmentation, peculiarities of gait, stance, and sitting posture, eczema and epilepsy. To investigate the spectrum of mutations of the PAH gene in Greek patients, we tested 100 patients by Denaturing Gradient Gel Electrophoresis (DGGE) and confirmed the findings by direct sequencing. From our cohort, 56 were referred for PKU of mild to severe degree, 36 had hyperphenylalaninemia (12 were off diet), and one patient had tetrahydrobiopterin (BH4) deficiency leading to hyperphenylalaninemia. Clinical uncertainty characterized seven patients, and therefore no clinical data were provided. Five pairs of siblings were present in our cohort. Two mutations, previously described, were detected in 82 cases, one known mutation and no other finding was present in 4 cases, whilst one known mutation and one other finding of unknown significance were present in 6 cases. One case presented with one finding of unknown significance and no other known finding, while no pathogenic finding was present in four of our cases. Testing was not performed in three of the cases due to unsuccessful PCR amplification. A total of 203 known polymorphisms were found. Our ongoing study will determine the spectrum of the PAH gene mutations in the Greek population and will contribute to the understanding of the clinical manifestations worldwide.

## 3048/F/Poster Board #980

**Knock-down of insulin gene using Adeno-associated virus vector.** *S.H. Pedersen, K. Brusgaard, N. Michelsen.* Biochem., Pharma. and Genetics, Odense University Hospital, Odense, Odense C, Denmark.

Congenital hyperinsulinism (CHI) is a relatively rare disease in western populations with incidences of 1:40.000 newborn/year whereas areas with high consanguinity have shown incidences of 1:2.500-3.000 newborn/year (Ashkenazi-Jews and areas in Saudi-Arabia and Finland). The disease is characterized by hyperinsulinemic hypoglycemia irrespective of blood glucose levels and is associated with a high risk of developing neurological handicaps. Most cases appear neonatal, but appearances in the late infancy and adulthood are seen. It is a heterogeneous disease with respect to phenotype and genetics. Depending on the underlying genetic cause and the severity of the disease, treatment includes conservative and medical management, and alternatively pancreasresection (resection of 60-95% of the organ). Mutations in 7 genes are so far known to be responsible for the development of CHI accounting for approximately 50% of the incidences: the genes 1) ABCC8 and 2) KCNJ11 (encoding the two subunits of the ATP-dependent potassium channel, the sulfonylurea receptor (SUR1) and the potassium inwardly-rectifier channel subunit (Kir6.2)), and the genes encoding the enzymes 3) glucokinase (GCK), 4) glutamate dehydrogenase (GLUD1) and 5) short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD). SUR1, Kir6.2 and GCK have shown to be involved in developing neonatal diabetes. Also, mutations in the HNF4a gene result in hyperinsulinisme lasting for a short or longer period of time - even years. Eventually, these patients will develop maturity onset diabetes of the young (MODY). The latest finding was mutations in the monocarboxylase transporter that is normally only expressed in liver tissue under anaerob conditions during physical exercise. The channel transports pyruvate into the cells. The disease-causing mutations result in expression of the transporter in the membrane of beta cells resulting in active uptake of pyruvate. So, the main problem to solve is hyperinsulinaemic hypoglycaemia. We are going to use silencing RNAs (siRNAs) to knock-down insulin secretion. Synthesis of a siRNA that specifically targets the insulin gene is the aim of this project. We are going to insert the siRNA in to an Adeno-associated virus vector system in order to get a stable and constantly expressed siRNA in Ins-1e cells. The overall purpose is to make a system where insulin synthesis is constantly downregulated post-transcriptionally.

## 3049/F/Poster Board #981

**CYP27B1 and PDCD1 polymorphisms in Polish patients with type 1 diabetes and Addison's disease.** *D. Januszkiewicz[1,3], M. Fichna[1,2], M. Zurawek[1], M. Gryczynska[2], P. Fichna[4], J. Sowinski[2], J. Nowak[1].* 1) Molecular Pathology, Institute of Human Genetics Polish Academy of Scie, Poznan, Poznan, Poland; 2) Department of Endocrinology and Metabolism, Poznan University of Medical Sciences, Poznan, Poland; 3) Department of Paediatric Oncology, Haematology and Transplantology, Poznan University of Medical Sciences, Poznan, Poland; 4) Department of Paediatric Endocrinology and Diabetes, Poznan University of Medical Sciences, Poznan, Poland.

CYP27B1 gene (chr.12q13.1-q13.3) encodes 1alpha-hydroxylase, responsible for conversion of the vitamin D3 precursor into its most active metabolite, involved in the immune function. Its promoter C(-1260)A polymorphism (rs10877012) might affect 1alpha-hydroxylase expression and therefore contribute to autoimmunity. PDCD1 gene (chr.2q37.3) encodes an inhibitory cell-surface receptor, expressed on activated lymphocytes, which plays a role in maintaining immune tolerance. PDCD1 G7146A variant (rs11568821) with putative regulatory function, has previously been associated with various autoimmune disorders. Autoimmune destruction of glandular cells is the main reason of type 1 diabetes (T1D) and Addison's disease (AAD). Both endocrine diseases present complex genetic background with several susceptibility loci. The aim of this study was to investigate the associations of the CYP27B1 C(-1260)A and PDCD1 G7146A polymorphisms with T1D and AAD in the Polish population. The study comprised 215 T1D patients and 101 AAD subjects compared to 251 healthy controls. Mean age of disease onset was 8.3 (±4.3) years for T1D and 35.8 (±12.6) years for AAD. Genotyping was performed by PCR-RFLP method, using TfiI and PstI restriction enzymes, respectively. The CYP27B1 C(-1260) allele appeared significantly more frequent in AAD patients compared to the control group (p=0.020), yielding an OR of 1.53 (95%CI 1.07-2.19). The distribution of C(-1260)A genotypes in AAD vs. control groups also demonstrated statistical significance (p=0.011) with a considerable increase in wild-type homozygotes observed among patients (57.4% vs. 40.2% in healthy individuals). In contrast, no association with CYP27B1 polymorphism was found for T1D (p=0.594 and p=0.989 for alleles and genotypes, respectively). The frequencies of alleles and genotypes at the PDCD1 G7146A polymorphism did not present significant differences between affected subjects and controls (p>0.05). In conclusion, this study confirms the association of the CYP27B1 C(-1260)A polymorphism with AAD in Polish patients, whereas its contribution to T1D risk seems less likely. PDCD1 G7146A does not appear associated with endocrine autoimmunity in Polish subjects. Supported by Ministry of Science Grant N402162533.

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## 3050/F/Poster Board #982

**Corrections in Fabry mice by recombinant adeno-associated virus 2/8-mediated gene therapy.** *U.Y Yu, J.O Choi, S.C Jung.* Department of Biochemistry , School of Medicine. Ewha Womans University, Seoul , Korea.

In fabry disease, genetic deficiency of lysosomal enzyme αα-galactosidase A results in abnormal Gb3 (glycosphingolipids) accumulation in endothelial cells that leads to renal failure, cardiac and cerebrovascular disease. In this study, we generated a pseudotyped rAAV 2/8-hAGA vector and injected it into 15-week-old male fabry mice through tail vein. We characterized the efficacy of the gene therapy at 6, 12 and 24 weeks after treatment. Tissues of liver, spleen, kidney, heart, and brain were examined for Gb3 levels and αα-gal A enzyme activity were analyzed. Treatment of fabry mice with rAAV 2/8-hAGA resulted in the clearance of accumulated Gb3 in tissues such as liver, spleen, kidney, heart, and brain with concomitant elevation ofαα-gal A enzyme activity.αα-gal A enzyme activities (means ± SD) in liver, spleen, kidney, heart and brain from fabry mice of 6 weeks after treatment were 2137.53 ± 80.92, 6413.80 ± 336.88, 4614.68 ± 179.75, 1267.59 ± 30.77 and 310.26 ± 7.48 nmol/h/mg. Enzyme activity was elevated for more than 24 weeks. In addition, fabry mice at 6, 12 and 24 weeks after treatment decreased Gb3 level compared with wild-type mice. Western analyses, expression of the GLA protein identified in the presence of rAAV 2/8-hAGA at 6, 12 and 24 weeks after treatment.. Liver toxicity by rAAV 2/8-mediated gene therapy was not observed. In this study, we have demonstrated the safety and improved efficacy of rAAV 2/8-hAGA mediated gene therapy in fabry mouse model.

## 3051/F/Poster Board #983

**Rhizomelic Chrondrodysplasia Punctata Type 2 Resulting From Paternal Uniparental Disomy of Chromosome 1.** *G.A.M. Nimmo[1], S. Monsonego[1], S. Steinberg[2], N.E. Braverman[1,3].* 1) Montreal Children's Hospital Research Institute, McGill University, Montreal, Quebec, Canada; 2) Kennedy Krieger Institute and Department of Neurology, Johns Hopkins University, Baltimore, Maryland, USA; 3) Departmant of Human Genetics and Pediatrics, McGill University, Quebec, Canada.

Rhizomelic Chondrodysplasia Punctata (RCDP) is a rare autosomal recessive disorder resulting from mutations on one of three genes essential for ether lipid biosynthesis. Affected patients have characteristic features including shortening of the proximal long bones, stippling of the epiphyses, bilateral cataracts, facial dysmorphism, and severe delays in mental development. Here we describe a patient in a nonconsanguineous family with the characteristic clinical features of RCDP. Differential biochemical analysis determined the deficiency is in the peroxisome localized enzyme glyceronephosphate O-acyltransferase (GNPAT), classifying the disease as RCDP type 2. All exons of the *GNPAT* gene, containing 16 exons and spanning 40.2 kb genomic DNA, were amplified using primers in the flanking intronic regions. The PCR fragments were directly sequenced in both directions. We found an apparent homozygous single nucleotide mutation [c.1428delC] causing a premature stop codon. The patient's karytoype was normal: 46, XY. The father was determined to be a heterozygous carrier of this mutation, while direct sequencing of the maternal *GNPAT* genes revealed only wild type sequence. Southern analyses, performed on gDNA from both parents using the full *GNPAT* cDNA sequence as a probe, did not show any evidence for a gene deletion in the mother. A single informative SNP in the 5'UTR of *GNPAT* suggested that no maternal alleles were inherited. Amplification and length analysis of 5 (to date) dinucleotide repeat markers spanning chromosome 1 in the patient and both parents revealed paternal uniparental inheritance. The absence of non-RCDP syndromic clinical features in this patient supports the hypothesis, proposed in other reports of uniparental disomy (UPD), that there are no imprinted genes on chromosome 1. We will discuss the possible mechanisms for the occurrence of the UPD in this patient, as well as an approach for genetic counseling. This is the first description of RCDP resulting from UPD.

## 3052/F/Poster Board #984

**Non-Hematopoietic-Derived TNFα is a Key Modulator of Neurodegeneration in a Mouse Model of Sandhoff Disease.** *H. Abou-Ouf[1], A. Hooper[1], A. Lessan[1], B. Trigatti[3], S.A. Igdoura[1,2].* 1) Department of Biology, McMaster University, Hamilton, ON, Canada; 2) Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada; 3) Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, Canada.

Inflammation cascades appear often to lead the pathogenic process of several neurodegenerative diseases. Tumor necrosis factor-α (TNFα) has been demonstrated as a key modulator of the immune response in the CNS. A variable net effect of TNFα on the pathogenic process has been attributed to the differential profile of microglial distribution and their inflammatory mediators and as such the overall impact of TNFα has been difficult to determine. Sandhoff disease is a lysosomal storage disorder caused by deficiency in hexosaminidase B (hexb). We have previously generated a double knockout of Tnfα and hexb in which we demonstrated that deficiency of TNFα attenuates the neuropathological deficit in Sandhoff disease. In our studies, the involvement of hematopoietic-derived TNFα in the neurodegenerative process was evaluated using bone marrow transplantation (BMT) in a mouse model of Sandhoff disease. Double knockout mice were transplanted with bone marrow from either hexb-/-tnfα+/+ or hexb-/-tnfα-/- mice. We also, transplanted hexb-/-tnf+/+ mice with bone marrow from either hexb-/-tnfα+/+ or hexb-/-tnfα-/- mice. Survival curves and body weights revealed that the double knockout recipients outperformed the hexb-/- recipients regardless of the genotype of the donor. Furthermore, deficiency of non-hematopoietic-derived TNFα in hexb-/- resulted in improved performance in several neurological tests such as wire hang, rotorod and righting response tests when compared to hexb-/- mice receiving single or double knockout BMT. We propose that neurological improvement was achieved through reduced levels of glia/neuronal-derived TNFα rather than reduced levels of blood-derived TNFα. In conclusion, TNFα is an important factor during the progression of neurodegeneration and as a result a potential therapeutic target. However, therapeutics involving TNFα inhibition will require crossing the blood-brain barrier in order to attenuate neuropathogenesis.

## 3053/F/Poster Board #985

**Construction and characterization of new mouse models for Types A & B Niemann-Pick disease.** *X. He, I. Jones, C. Huang, E. Schuchman.* Genetics and Genomic Sciences, Mount Sinai Sch Medicine, New York, NY.

The currently available mouse model of acid sphingomyelinase (ASM) deficiency (ASMKO) is a complete knockout of the ASM gene (SMPD1). While these animals have been extremely useful for the preclinical assessment of treatment for Types A & B Niemann-Pick disease (NPD), they do not express any residual protein and therefore cannot be used to evaluate enzyme enhancement (e.g., "chaperone") approaches. In addition, they develop a rapidly progressive neurological disease, making them a more appropriate model for Type A than Type B NPD. Since all ASM-deficient NPD patients contain point mutations in the SMPD1 gene and express some residual enzymatic activity, we undertook the development of mutation-specific mouse models for this disorder. Two mutations were chosen for these experiments: R496L, a common Type A NPD mutation found in ~40% of Ashkenazi Jewish patients, and ΔR608, a common Type B NPD mutation found in ~20% of patients from North American and Western Europe. Constructs were generated in which the mutations were introduced into the full-length human SMPD1 cDNA, and expression was driven by a fragment derived from the mouse SMPD1 promoter. These constructs were introduced into ASMKO blastocysts, and breeding colonies were generated that express the transgenes on the complete ASMKO background. One founder line for the R496L mutation and three for the ΔR608 mutation have been established. RNA expression analysis in the brains and livers of these animals revealed that the R496L line and two of the three ΔR608 lines had normal levels of SMPD1 RNA expression. As expected, the R496L line had no detectable residual ASM activity, while expression was detected in the ΔR608 lines. Importantly, and in agreement with the ASM activity and patient findings, ΔR608 mice also had markedly improved performance on an accelerating rotorod apparatus as compared to the R496L mice. Studies are currently underway to further characterize the clinical and pathological findings of these animals, and in the future they will be used to evaluate new approaches to enhance residual ASM activity. Funding by NIH grant R01 HD28607 & The National Niemann-Pick Disease Foundation (NNPDF).

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## 3054/F/Poster Board #986

**Mutations of the GLA gene in Korean patients with Fabry disease and frequency of the E66Q allele in Korean newborns.** *S. Heo[1], G. Kim[1,2], J. Park[1], J. Lee[2], B. Lee[1,2], H. Yoo[1,2,3].* 1) Genome Res Ctr/Asan Inst, Asan Medical Ctr, Seoul, Korea; 2) Medical Genetics Clinic and Laboratory, Asan Medical Center, Seoul, Korea; 3) Department of Pediatrics University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea.

Fabry disease is an X-linked recessive lysosomal storage disorder caused by alpha-galactosidase A (GLA) deficiency. To date, more than 400 disease-causing mutations have been identified in GLA gene located on Xq22. We have previously identified 23 distinct mutations (L21F, M42V, H46R, E66Q, I91T, R112C, F113L, C142W, D231G, D266N, S297F, R301Q, T349R, T47X, C90X, Y86X, R220X, R227X, R342X, Q98fsX23, L268delfsX1, I359MfsX32 and T412SfsX37) in GLA in 25 unrelated Korean patients In this report, we observed that E66Q, previously-known as a controversial variant in Fabry disease, shows 40% of wild-type enzyme activity in COS-7 cells by in vitro transient expression assay, indicating that E66Q might be a mild mutation or a functional single nucleotide polymorphism (SNP). In order to investigate the allele frequency of E66Q in Korean newborns, genotype analysis was done using dried blood spots from 803 (459 males, 344 females) newborns by the allele specific TaqMan probe SNP assay. Out of 1,147 alleles tested, 12 alleles (2 male hemizygotes & 10 female heterozygotes) harbor the E66Q variant, subsequently confirmed by sequencing analysis. Therefore, the allele frequency of E66Q turned out to be as frequent as 1.05%.. In summary, the E66Q is highly prevalent in Korean population as well as only identified variant in some Korean Fabry patients. Further study is needed to address the clinical significance of the E66Q variant in the development or modification of Fabry disease phenotype.

## 3055/F/Poster Board #987

**Mutation analysis and genome-wide gene expression profiling on patients with primary lactic acidosis.** *M. Al-Owain[1], D. Colak[1], A. AlBakheet[1], B. AlYounes[1], A. Al-Aqeel[1], F. AlFadhli[1], A. Al-Hashem[1], A. Al-Odaib[1], M. Faiyaz-Ul-Haque[1], Z. Al-Hassnan[1], H. AL-Zeydan[1], Z. Al-Rahbeeni[1], M. Al-Sayed[1], S. AL-Alaiyan[1], K. Abu-Amero[1], P. Ozand[2], N. Kaya[1].* 1) Dept Med Gen, MBC 75, King Faisal Specialist Hosp, Riyadh, Saudi Arabia; 2) Duzen Laboratories, Istanbul, Turkey.

A significant pathology in the newborn period is infants who develop lactic acidosis within few days after birth, and expire within months. The neonatologist remains helpless since no information for its pathogenesis exists precluding a rationalistic therapy or preimplantation or prenatal diagnosis. This study reports findings from clinical, biochemical, molecular genetics and genomics studies performed on a selected cases having developed severe lactic acidosis during their early infancy. Our mtDNA screening indicated that one of the patients has a novel transversion causing conversion of Thr to Pro in ATPase6 gene. Global gene expression profiling experiments, functional pathway analysis, and gene annotation analysis were performed on the whole blood of selected patients and their age and sex matching controls. Significant PANTHER molecular function terms indicated enrichment of genes related to protein folding, nuclear transport, intracellular protein traffic, nitrogen utilization, oxidative phosphorylation, electron transport, coenzyme metabolism, protein complex assembly, mitochondrial transport in the differentially expressed gene clusters. We also investigated biological pathways significantly represented among the differentially expressed genes. The most significantly overrepresented pathways included the ubiquitin proteasome pathway, parkinson disease pathway, cell cycle, TCA cycle, cytoskeletal regulation by Rho GTPase, heme biosynthesis, huntington disease pathway. Based on the gene expression studies, mutation screening of nuclear genes such as MRPS16 were also performed and these screening studies did not gave positive results. Our results will help better understanding of the pathology of this complex disease.

## 3056/F/Poster Board #988

**Identification and characterization of a novel homozygous deletion in the alpha-N-acetylglucosaminidase gene in a patient with Sanfilippo type B syndrome (mucopolysaccharidosis III B).** *K. Champion[1], I. Maystadt[2], A. Destrée[2], P. Vannuffel[2], T. Wood[1], M. Basehore[1].* 1) Molecular Diagnostic Lab, Greenwood Gen Ctr, Greenwood, SC; 2) Centre de Genetique Humaine, Institut de Pathologie et de Genetique, Gosselies, BELGIUM.

Sanfilippo syndrome type B (mucopolysaccharidosis IIIB) is an autosomal recessive disease that is caused by a deficiency of the lysosomal enzyme $\alpha$-N-acetylglucosaminidase (NAGLU). Over 100 different mutations in the *NAGLU* gene have been identified in Sanfilippo syndrome type B patients; however, no large deletions have been reported. Here we present the first case of a large homozygous intragenic *NAGLU* gene deletion identified in an affected child of consanguineous parents. Long range and multiplex PCR methods were used to characterize this deletion which encompasses exons 3 and 4 and is 1,146 base pairs long. We propose that *Alu* element-mediated unequal homologous recombination between an *Alu-Y* in intron 2 and an *Alu-Sx* in intron 4 is the likely mechanism for this deletion, thereby contributing further insight into the molecular etiology of this disorder and providing additional evidence of its genetic heterogeneity.

## 3057/F/Poster Board #989

**Identification of Novel Mutations in Key Regulatory Regions of the Aldolase B Gene that cause Hereditary Fructose Intolerance.** *E.M. Coffee, D.R. Tolan.* Biology, Boston University, Boston, MA.

Hereditary fructose intolerance (HFI) is a potentially fatal inherited metabolic disease. It is caused by a deficiency of aldolase B enzyme activity in the liver and kidney, tissues where fructose metabolism occurs. To date, 43 mutations known to cause the disease have been identified in the protein coding region of aldoB. Here we report the first identification of mutations upstream of the coding portion of aldoB. The promoter, the 5'-untranslated first exon, and the intronic enhancer of several HFI patients revealed single base mutations in each of these key regulatory regions. Two mutations (g.-132G>A, g.-129T>A) are located in the promoter at the consensus C/EBP binding site, one mutation (IVS1+1G>C) is at the exon-1 donor splice site, and four mutations (IVS1+214A>G, IVS1+1873T>A, IVS1+1887G>A, IVS1+2379T>C) are located in the first intron in or near the known transcriptional enhancer. For the promoter mutations, in vivo studies using luciferase reporters suggest that the mutations in the promoter cause a decrease in aldoB expression. In vitro gel-shift assays are described that should confirm a decrease in C/EBP binding at these mutant sites. For the splicing mutation, cDNA synthesized from cells transfected with the exon-1 donor splice site mutant results in aberrant splicing of the transcript. In addition, reporter assays are described that should show cells are unable to synthesize any functional protein when the luciferase is inserted in frame. These data indicate the mutations in the promoter and exon-1 splice junction lead to HFI. Mutations identified in the intronic enhancer region seem likely to be single nucleotide polymorphisms common to HFI patients. The consequences of these single nucleotide changes in the regulatory regions of aldoB are discussed in the context of genotype/phenotype relationships.

## 3058/F/Poster Board #990

**Restoration of biological function of cystathionine beta-synthase mutants by chemical chaperones.** *J. Kopecká, J. Krijt, V. Koich.* Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University, Prague, Czech Republic.

Misfolding and aggregation of mutant enzymes have been proposed to play a role in pathogenesis of homocystinuria due to cystathionine $\beta$-synthase (CBS) deficiency. Chemical chaperones that can stabilize mutants and prevent aggregation have been recently explored as mediators of proper folding of several CBS mutants (Singh, et al., 2007). Our study was aimed at determining the effect of a different set of chaperones on quaternary structure and activity of a large panel of 27 pathogenic mutations representing two thirds of all known patient-derived alleles. Chemical chaperones were used in an E. coli expression system and their effect on folding and catalytic activity of mutants was examined by native western blotting and radiometric and LC-MS/MS assay. Two thirds of mutants was responsive to the presence of either 0.5 mM delta-aminolevulinic acid, 100 mM betaine and/or 750 mM glycerol during expression; these chaperones increased formation of correctly assembled tetramers which was in most cases accompanied by an increase in catalytic activity. Nine mutants (R266K, P49L, C165Y, R125Q, K102N, R369C, V180A, P78R, S466L) were rescuable by all of these three substances and they are potential candidates for chaperone therapy. This study has brought some new aspects of mechanism of CBS folding and suggests that new modality of treatment may become useful for subset of homocystinuria patients. Work was supported by a grant from Grant Agency of Charles University No. 84507 and by Research Project of Charles University No. MSM20620806.

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## 3059/F/Poster Board #991

**Functional complementation in yeast allows molecular characterization of missense argininosuccinate lyase mutations.** *E. Trevisson[1], A.B. Burlina[2], M. Doimo[1], L. Cesaro[3], G. Sartori[3], L. Salviati[1].* 1) Pediatrics Dept - Genetics Unit, University of Padova, Italy; 2) Pediatrics Dept- Metabolic Disorders Unit, University of Padova, Italy; 3) Biochemistry Dept, University of Padova, Italy.

Argininosuccinic aciduria (ASAuria) is an autosomal recessive urea cycle defect due to mutations in the ASL gene, which encodes argininosuccinate lyase. This disorder may present with a severe neonatal onset form, or with a late onset phenotype. To date phenotype-genotype correlations are still not clear because biochemical assays of ASL activity correlate poorly with clinical severity in patients. Moreover, patients are often compound heterozygotes for different mutations, and direct measurements cannot discriminate the role of individual alleles. We employed a yeast-based functional complementation assay to assess the pathogenicity of 12 missense ASL mutations, to establish genotype-phenotype correlations, and to screen for intragenic complementation. The S.cerevisiae ARG4null strain harbours a deletion of ARG4, the yeast homologue of the human ASL gene, and is auxotrophic for arginine. Rather than determining ASL enzyme activity directly, we measured the growth rate in arginine-free medium of the yeast ARG4null strain transformed with individual mutant ASL alleles. Individual haploid strains were also mated to obtain diploid, "compound heterozygous" yeast. We show that the late onset phenotypes arise in patients who harbour either individual alleles retaining high residual enzymatic activity, or because of intragenic complementation among different mutated alleles. In these cases complementation occurs because in the hybrid tetrameric enzyme, at least one active site without mutations can be formed, or because the differently mutated alleles can stabilize each other, resulting in partial recovery of enzymatic activity. Patients with the severe phenotype can harbour mutations of any class; on the other hand, none of the patients with the mild phenotype had two mutations that completely abolish enzymatic activity, indicating that residual ASL activity is necessary, but not sufficient, to ensure a milder phenotype in the patients. Functional complementation in yeast is simple, sensitive, reproducible, and allows the analysis of large numbers of mutant alleles. Moreover, it overcomes the problems inherent to classical biochemical assays, it is not affected by the genetic background of the patient and can be easily adapted for the analysis of mutations in other genes involved in metabolic disorders.

## 3060/F/Poster Board #992

**A new case of ALG8 deficiency (CDG-Ih) with neurological impairment.** *K. Vesela[1], T. Honzik[1], H. Hansikova[1], M.A. Haeuplte[2], J. Semberova[3], Z. Stranak[3], T. Hennet[2], J. Zeman[1].* 1) Department of Pediatrics, First Faculty of Medicine, Charles University, Prague, Czech Republic; 2) Institute of Physiology, University of Zürich, Zürich, Switzerland; 3) Institute for the Care of Mother and Child, Prague, Czech Republic.

Congenital disorders of glycosylation (CDG) represent an expanding group of inherited diseases. One of them, ALG8 deficiency (CDG-Ih), leads to protein N-glycosylation defects caused by malfunction of glucosyltransferase 2 (Dol-P-Glc:Glc1-$Man_9$-$GlcNAc_2$-P-P-Dol glucosyltransferase) resulting in inefficient addition of the second glucose residue onto lipid-linked oligosaccharides. So far, only five patients have been described with ALG8 deficiency. We present a new patient with neonatal onset. The girl was born at $29^{th}$ week of gestation complicated by oligohydramnion. Although the early postnatal adaptation was uneventful (APGAR score 8 and 9 at the $5^{th}$ and $10^{th}$ minutes), generalized oedema, multifocal myoclonic seizures, and bleeding due to combined coagulopathy were present since the first day. Diarrhea progressing to the protein-losing enteropathy with ascites and pericardial effusion developed in the $3^{rd}$ week of life. Pharmacoresistant seizures and cortical, cerebellar and optic nerves atrophy indicated neurological involvement. No symptoms of liver disease except coagulopathy were observed, however steatofibrosis with cholestasis was found at autopsy. The girl died at the age of 2 months due to the progressive general oedema, bleeding and cardio-respiratory insufficiency. Isoelectric focusing of serum transferrin revealed a glycosylation pattern typical for CDG type I. The lipid-linked oligosacharide profile was directing to an ALG8 deficiency (CDG-Ih). Molecular analysis of the *ALG8* gene showed two heterozygous mutations: c.139A>C (p.T47P) and a novel mutation c.1090C>T (p.R364X). The new mutation was found in heterozygous form in the mother, but it was not present on any allele of 150 healthy controls.

*Conclusion:* The prognosis of patients with ALG8 deficiency is unfavorable. Majority of affected children have early onset of the disease with heterogeneous symptoms including multi-organ dysfunction, coagulopathy and protein losing enteropathy. Neurological impairment is not a general clinical symptom, but it has to be taken into consideration when thinking about ALG8 deficiency.

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## 3061/F/Poster Board #993

**Medium-chain acyl-CoA dehydrogenase deficiency - the molecular aspect.** *B.Z. Yang, N.Z. McNeill, J.H. Ding.* Inst Metabolic Disease, Baylor Res Inst, Dallas, TX.

Medium-chain acyl-CoA dehydrogenase (MCAD, E.C. 1.3.99.3) is responsible for the first step in mitochondrial β-oxidation of fatty acids, a process which provides energy, especially during fasting conditions. MCAD deficiency is the most common inherited defect of fatty acid oxidation, characterized by episodes of illness in early childhood. The disorder may present after fasting with symptoms resembling Reye Syndrome, coma, hypoglycemia, hyperammonemia, fatty liver, and sudden death. To investigate the molecular aspect, forty-three unrelated patients with MCAD deficiency were studied recently. The common mutation 985A>G had been tested, which presented only in 57% of all defective alleles in our group. To screen for other mutations within the MCAD gene, all 12 exons and their flanking intronic sequences were amplified. The PCR products were purified and sequenced. A total of 27 different rarer mutations, including eight novel variants, have been detected. Among them, eight mutations have been identified in a few unrelated patients, while the remaining nineteen have each been found in only a single pedigree. The novel mutations, including G275X and R349X were also verified by a PCR/restriction test, but were not detected in the normal control subjects. Our data and others indicated significant phenotypic heterogeneity of MCAD deficiency, even with the same genotype. The low percentage of the common mutation in our group has also been discussed.

## 3062/F/Poster Board #994

**Homozygosity mapping reveals new genes and gene defects in consanguineous families with OXPHOS disease.** *M. Gerards[1,2], B. van den Bosch[1,2], W. Sluiter[3], E. de Wit[3], K. Schoonderwoerd[4], A. van der Kooi[5], I. de Coo[6], H. Smeets[1,2].* 1) Dept Clinic Genet, Maastricht UMC, Maastricht, Limburg, Netherlands; 2) Research School GROW, Univ Maastricht, Maastricht, The Netherlands; 3) Dept Biochem, Mitochondrial Research Unit, Erasmus MC, Rotterdam, The Netherlands; 4) Dept Clin Genet, Erasmus MC, Rotterdam, The Netherlands; 5) Dept Neurol, Academic Medical Center, Amsterdam, The Netherlands; 6) Dept Neurol, Erasmus MC, Rotterdam, The Netherlands.

Mitochondrial or OXPHOS disorders are clinically and genetically highly heterogeneous, making the identification of the underlying gene defect a major challenge. Classification based on clinical or biochemical criteria or gene expression profiling remains difficult and only applicable to small subgroups of patients. Therefore, the most successful approach for finding the genetic defect still remains SNP-based homozygosity mapping and candidate gene analysis, as illustrated for 2 consanguineous families with OXPHOS disease. In the first family with 3 children affected with Leigh syndrome, a homozygous region of 11.5Mb on chromosome 20 was identified containing 8 mitochondrial genes. Patients were homozygous for an amino acid substitution (p.L159F) in C20orf7, an new assembly factor of OXPHOS Complex I. Blue native gel electrophoresis showed in blood a decrease to 30% of mature Complex I in patients compared to controls indicating the pathogenic mechanism of this mutation. A homozygous region on chromosome 1 containing 4 mitochondrial genes was detected in the second family with 3 children with progressive cerebellar ataxia. Mutation screening of CABC1 (the chaperone, ABC1 activity of bc1 complex homologue gene) revealed a homozygous nonsense mutation (c.1042C>T, p.R348X). CABC1 is the homologue of the yeast COQ8 gene, which is involved in the ubiquinone biosynthesis pathway. An additional 4 patients with cerebellar ataxia were screened for mutations in the CABC1 gene. One patient was compound heterozygous for the same c.1042C>T mutation and had an additional nonsense mutation (c.1136T>A, p.L379X). The pathogenic role for both CABC1 mutations is evident as they either lead to a truncated protein missing important protein kinase motifs required for ATP binding or to nonsense mediated mRNA decay. We conclude that homozygosity mapping is the preferred method to identify pathogenic mutations in consanguineous families and maybe also in idividual patients.

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## 3063/F/Poster Board #995

**Characterization of *Caenorhabditis elegans* gene *C17G1.7* - an ortholog of human cystathionine β-synthase.** *R. Vozdek[1], A. Hnizda[1], J. Krijt[1], M. Kostrouchova[1], M. Kodicek[2], V. Kozich[1].* 1) Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague; 2) Department of Biochemistry and Microbiology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology, Prague.

Nematode *Caenorhabditis elegans* could be a suitable model to study metabolic and cellular consequences of homocystinuria due to cystathionine β-synthase (CBS) deficiency. However, metabolism of sulfur amino acids in *C. elegans* is as yet unknown, namely the steps in cysteine biosynthetic pathways. Cysteine can be synthesized either via the transsulfuration pathway which utilizes homocysteine by CBS or via the assimilation pathway which uses sulfide by cysteine synthase (CS).

*In silico* analysis of *C. elegans* database WormBase identified five orthologs of human CBS, namely *F54A3.4*, *ZC373.1*, *C17G1.7*, *K10H10.2* and *R08E5.2*. The aim of this study was to characterize the gene *C17G1.7*. Although this gene is expressed at high level (according to WormBase), our data show that *C. elegans* mutant strain carrying deletion of *C17G1.7* has normal morphology, behaviour, lifespan and egg-laying compared to wild type nematodes. Unaffected phenotype of deletion mutant was also observed at the metabolite level; concentration of aminothiols in crude extracts was undistinguishable from wild type strain. Subsequently, gene *C17G1.7* was expressed in prokaryotic system and purified to homogenity. We determined that purified enzyme has specific enzymic activity for CS reaction; other possible activities were not detected. Recombinant C17G1.7 exhibited $K_M$ values for *O*-acetyl-serine and sulfide of 5.54 and 4.23 mM, respectively, and a turnover number of 139 and 134 $s^{-1}$, respectively.

Our enzymological data suggests that *C17G1.7* gene could play an important role in cysteine metabolism, however, deletion mutant does not lead to an abnormal clinical and biochemical phenotype. This fact supports an assumption that the insufficient activity of one enzyme could be compensated by another paralogs.

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## 3064/F/Poster Board #996

**Identification of a novel mutation of SLC6A19 gene in a Korean patient with Hartnup disorder.** *C. Chong Kun[1], Y. Han Wook[2].* 1) Pusan National University Children Hospital, Geungsang Nam Do , Korea C.K. Cheon; 2) Department of Pediatrics, Asan Medical Center, Children and Adolescent Hospital, University of Ulsan College of Medicine, H. W. Yoo.

Backgound: Hartnup disorder is an inborn error of renal and gastrointestinal neutral amino acid transport with reported clinical association with pellagra-like rash, ataxia and psychotic behavior. Diagnostically,elevated urinary neutral amino acids are the first indication of the disord er. Mutations in SLC6A19, encoding the amino-acid transporter B0AT1, mediating neutral amino acid transport from the luminal compartment into the cells are causative for autosomal-recessive Hartnup disorder. Objective: To report on an 8 year, 5 month old Korean boy with Hartnup disorder who was confirmed by SLC6A19 gene analysis. Results: To confirm the diagnosis of Hartnup disorder, direct sequencing analysis of 12 coding exons and the exon-intron boundaries of SLC6A19 gene wa s performed. The result of sequence analysis sho wed that he was a compound heterozygote possessing two mutations.908C>T (Ser303Leu) and 1787_1788 insG (Thr596fsX73). The former was a novel mutation and also, the latter is a novel frameshift mutation with known 336 polymorphism after stop codon. Conclusion: We experienced one case of Hartnup disorder with a novel mutation of SLC6A19 gene. Although Hartnup disorder is a rare disease in Korea,it should be considered in the differential diagnosis of any infant or child presenting with neutral amin noaciduria and seizure, especially in patients with past history of pella gra-like rash.

## 3065/F/Poster Board #997

**Molecular analysis of two enzymes, HPRT and PRPP synthetase, causing X-linked inborn errors of purine metabolism.** *Y. Yamada, K. Yamada, A. Yamano, N. Nomura, N. Wakamatsu.* Dept Gen, Inst Dev Res, Aichi Human Service Ctr, Kasugai Aichi, Japan.

Deficiency in the activity of a purine salvage enzyme, hypoxanthine guanine phosphoribosyltransferase (HPRT, EC 2.4.2.8; MIM308000), associated with Lesch-Nyhan syndrome (MIM300322) and HPRT-related gout (MIM300323). On the other hand, PRPP synthetase superactivity (MIM300661) is an X-linked inborn error of metabolism in which increased enzyme activity is associated with hyperuricemia and gout, sometimes including neurodevelopmental abnormalities. Human PRPP synthetase (PRPS; EC 2.7.6.1) exists as heterogeneous aggregates composed of two catalytic subunits (PRSI and PRSII) and two associated proteins. Mutations of *PRPS1* (PRSI subunit gene; MIM311850) were found in patients with PRPS superactivity. Recently, in affected males with X-linked recessive Charcot-Marie-Tooth disease-5 (CMTX5; MIM311070), the *PRPS1* mutations responsible for decreased enzyme activity were identified. Furthermore, the mutations resulting in a loss of PRPS activity with hypouricemia were also identified in the patients of Arts syndrome (MIM301835). We have identified a number of HPRT mutations in Asian patients manifesting different clinical phenotypes, by analyzing all nine exons of the HPRT gene (*HPRT1*) from genomic DNA and reverse transcribed mRNA using the PCR technique coupled with direct sequencing. Two mutations were detected in two Lesch-Nyhan families after the last our report (Yamada et al., Nucleosides Nucleotides Nucleic Acids 27: 570-574). One of them, a new single nucleotide substitution (130G>T) resulting in a missense mutation D44Y was detected in the exon 2 of *HPRT1*. The RT-PCR amplification showed not only a cDNA fragment with normal size, but also a small amount of shorter fragment skipping exons 2 and 3. The other missense mutation F74L (222C>A) was detected firstly in a Japanese patient, whereas reported previously in Europe. In four hyperuricemia patients with mild neurological abnormality, no mutations responsible for partial HPRT deficiency were identified on *HPRT1*. In those four patients, we also performed molecular analysis of *PRPS1*, using the PCR and direct sequencing of genomic DNA and mRNA, but any abnormalities as generating PRPS superactivity have not been found.

## 3066/F/Poster Board #998

**Genotype and phenotype of Japanese patients with X-linked adrenoleukodystrophy.** *N. Shimozawa[1], A. Arai[1], N. Kajiwara[1], S. Kozawa[1], T. Nagase[1], Y. Takemoto[1], Y. Suzuki[2].* 1) Life Sci Res Ctr, Gifu Univ, Gifu, Japan; 2) Medical Educat Develop Ctr, Gifu Univ Sch of Med, Gifu, Japan.

X-linked adrenoleukodystrophy (ALD) is a severe neurodegenerative disease associated with accumulation of very-long chain saturated fatty acids (VLCFA) in tissues and body fluids. Patients manifest various neurological disturbances, including intellectual, psychological, visual, hearing, gait, swallowing, autonomic, and sensory disturbances. We have been studying peroxisomal diseases for more than 20 years, and doing biochemical and molecular diagnosis on Japanese patients with peroxisomal diseases. In this study, we document genotype and phenotype of Japanese patients with ALD. We have been identified 46 kinds of ABCD1 (the ALD gene) mutations in 49 Japanese ALD families, including 41 patients with the childhood and adolescent cerebral form, 7 with adrenomyeloneulopathy (AMN), 1 with Addison only, 14 with pre-symptomatic and 40 females with ALD carriers. Most kindreds have a unique mutation, including 31 kinds of missense mutations, 3 of nonsense mutations, 2 of in frame amino acid deletions, 6 of frame shift mutations, 2 of exon deletions and 2 of large deletions. There is no genotype-phenotype correlation and the prognosis of the affected patients can not be predicted, therefore further approaches using these resources are needed to find out another factor that determined the clinical outcome of the ALD patients.

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## 3067/F/Poster Board #999

**Effect of Vitamin D Receptor (VDR) genotypes on risk for osteoporosis in patients with non-neuronopathic Gaucher disease.** *A. Greenwood[1], G. Altarescu[2], A. Zimran[1], D. Elstein[1].* 1) Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel; 2) Genetics Unit, Shaare Zedek Medical Center, Jerusalem, Israel.

The almost universal finding of osteopenia in Gaucher disease and the unpredictable advent of avascular necrosis and/or pathological fractures are clinically relevant issues that are not necessarily reversed by specific enzyme replacement therapy (ERT). However, genetic/epigenetic factors may explain as much as 70% of the variance for bone mineral density (BMD) in the normal population. Quantitative trait loci for BMD have been identified by genome screening and linkage approaches; among the candidate gene polymorphisms, those of the Vitamin D Receptor (VDR) have been correlated with BMD and/or fracture risk in various populations. The purpose of this study is to assess VDR polymorphisms on BMD at the lumbar spine (LS) and femoral neck (FN) in patients with Gaucher disease regardless of ERT use. Helsinki Committee approval was received for this study. DNA was derived from blood samples taken from 74 patients with non-neuronopathic Gaucher disease for whom there was recent BMD data at LS and FN and from 15 healthy individuals. Four VDR polymorphisms (FokI, ApaI, TaqI, and BsmI) were tested. Clinical and demographic data were taken from files. Associations between PAR1 and categorical variables were analyzed by chi-square and Fisher's exact tests; assessment of associations with quantitative variables used ANOVA and Scheffe post-hoc for multiple pairwise comparisons. Non-parametric Kruskal-Wallis ANOVA was used when one category was small. All tests were 2-tailed; p values less than 0.05 was considered statistically significant. FokI was not correlated with any variables tested. ApaI was significantly correlated with Gaucher genotype (N370S/N370S vs others) (p=0.029) but not with the presence of bone disease, need for splenectomy, need for ERT, or BMD. TaqI was significantly correlated with BMD at FN (p=0.03) but with no other variables. There were trends to significance for correlation of BsmI genotype with Gaucher genotype (p= 0.061) and with LS Z-scores (p=0.084), and a significant correlation with the presence of overt bone disease (p=0.018). We conclude that as in other populations, VDR polymorphic genotype, in particular BsmI, may be an independently-sorting modifier in the prediction of bone disease in Gaucher disease. This may have management ramifications.

## 3068/F/Poster Board #1000

**The phenylalanine hydroxylase c.30C>G (p.G10G) synonymous variation is a pathological mutation that functions by creating a common exon splicing silencer motif.** *B.S. Andresen[1], T. Miller[2], S.F. Dobrowolski[3].* 1) Dept Biochem & Molec Biol, Univ Southern Denmark, Odense M, Denmark; 2) Idaho Technology, Salt Lake City, Utah, USA; 3) Department of Pathology, University of Utah, Salt Lake City, Utah, USA.

Correct splicing of exons is determined by a finely balanced interplay between cis-acting regulatory sequences like exonic splicing enhancers (ESE) and exonic splicing silencers (ESS). Mutations that create or disrupt ESS/ESEs may disturb this balance and cause missplicing and disease, irrespective of the perceived consequence dictated by the genetic code. Deleterious mutations in the phenylalanine hydroxylase (PAH) gene may cause Phenylketonuria. In three siblings previously reported to have different clinical phenotypes, but a common PAH genotype defined by RFLP analysis we identified compound heterozygosity for a deleterious c.284-86delTCA variation and a c.30C>G variation. The c.30C>G variant is ostensibly the synonymous variant p.G10G, and would therefore not be expected to be deleterious, and it is cataloged in the Phenylalanine Locus Knowledgebase as a neutral variation, but was not present in 400 control chromosomes. The c.30C>G variation creates a CAGGGT motif within exon 1. We have previously shown that this motif functions as an ESS in other genes and because the 5' splice site of PAH exon 1 is intrinsically weak, we speculated if the c.30C>G variation could cause missplicing and thus be deleterious. The ESS created by the c.30C>G variant was characterized in a splicing reporter minigene, and shown to cause exclusion of an alternatively spliced exon. Moreover, a hybrid minigene harboring the relevant part of exon 1 and part of intron 1 was used to demonstrate that the c.30C>G variation causes aberrant splicing also in its native context. Using nuclear extracts and RNA affinity purification with different wild type and c.30C>G mutant RNA oligonucleotides we demonstrated that c.30C>G creates a binding motif for the splicing inhibitory protein hnRNPH, which can bind to splicing silencers with GGG triplets. The present study provides a new example on how synonymous substitutions can be deleterious by disrupting the finely tuned balance between splicing regulatory elements in constitutive exons. The PAH genotype is valuable diagnostically, to predict disease severity, and to predict efficacy of 6R-tetrahydrobiopterin therapy. Given the critical nature of the PAH genotype, understanding the molecular pathology of variants has significance to patient care. Potential effects on mRNA splicing should therefore always be considered when the consequences of identified sequence variations are evaluated.

## 3069/F/Poster Board #1001

**Early liver transplantation in urea cycle defects: a developmental outcome study.** *P. Pivalizza[1], P.M. Campeau[2], A.A. Tran[2], K.L. McBride[3], G. Miller[4], S. Carter[2], S. Karpen[5], J.A. Goss[6], B.H. Lee[2,7].* 1) Center for Develop. Pediatrics, Baylor College of Medicine, Houston; 2) Human and Molecular Genetics, Baylor College of Medicine, Houston, TX; 3) Center for Molecular and Human Genetics, The Research Institute at Nationwide Children's Hospital, Columbus, OH; 4) Department of Pediatrics, Yale School of Medicine, New Haven, CT; 5) Pediatrics-Gastroenterology, Baylor College of Medicine, Houston, TX; 6) Liver Center, Baylor College of Medicine, Houston, TX; 7) Howard Hughes Medical Institute, Houston, TX.

Patients with urea cycle defects (UCDs) typically have a poor prognosis in terms of survival and neurological outcome when the presentation is in the neonatal period, despite aggressive pharmacological and dietary therapy. Liver transplantation can prevent subsequent metabolic decompensations, but the preexisting neurological status, dependent on the severity of hyperammonemic episodes, is a major factor in the final outcome. Previous studies indicate that most of the children who receive transplants remain significantly neurologically impaired. We performed a prospective developmental outcome study to determine whether aggressive metabolic management of ammonia levels after early referral/transfer to a metabolism center and early liver transplantation would result in better neurologic outcomes. We report on 5 children with UCDs transplanted between 2000-2003; 2 male patients with X-linked ornithine transcarbamylase deficiency and 2 male patients with carbamoyl phosphate synthase deficiency, all of whom had neonatal presentations and underwent liver transplantation before 1 year of age, and 1 female patient with partial X-linked ornithine transcarbamylase deficiency that was intractable to medical therapy, who underwent transplantation at 35 months of age. All children had metabolic cures. There was 1 death 40 months after liver transplantation caused by hepatic artery thrombosis in the immediate post-transplantation period leading to biliary strictures which caused end-stage liver disease. The 6-year survival rate is thus 80%. Developmental testing with the Griffiths scale was performed on average on 5 occasions, 12 months apart, up to 7 years after transplantation. Full-scale indices for the 4 children who underwent early transplantation (now averaging 9 years of age) showed average developmental quotients of 69 (range 51-86), whereas the average developmental quotient for the female who underwent transplantation at 35 months was 80. We conclude that early liver transplantation and aggressive metabolic management improve early neurologic outcomes for children with UCDs.

## 3070/F/Poster Board #1002

**Optic Neuropathy in a Maple Syrup Urine Disease Patient with Poor Dietary Control.** *M. Wick, D. Babovic-Vuksanovik.* Medical Genetics, Mayo Clinic & Foundation, Rochester, MN.

Maple syrup urine disease (MSUD) is an inborn error of metabolism caused by a defect of the branched chain oxoacid dehydrogenase multi-enzyme complex. The defect results in accumulation of the branched chain amino acids (BCAA) leucine, isoleucine and valine. Neurologic toxicity including cerebral edema, cerebrovascular compromise and brain stem herniation is due to leucine and 2-isocaproic acid accumulation. Early disease manifestations include vomiting/feeding difficulties, lethargy and hypertonicity. Untreated, symptoms rapidly progress to seizures, coma and death. Untreated patients who survive are neurologically devastated with severe retardation. Patients treated from the newborn period with dietary restriction of BCAA have normal development and intellectual outcome. However, these patients are at risk for acute decompensation characterized by apathy, choreoathetosis, ataxia, stupor, convulsions and coma. Here we describe a 26 year old patient who presented with optic neuropathy approximately twelve months after self-discontinuation of the MSUD diet. Leucine, isoleucine and valine levels were elevated at 2,165mmol/L, 425mmol/L and 825mmol/L respectively. He was restarted on the protein restricted diet with MSUD Complex supplementation. This resulted in normalization of valine and isoleucine within 2 weeks, while leucine levels remained mildly elevated at 425mmol/L. The patient reported resolution of spells and sense of well being within two weeks. Opthalmologic examination 6 months after re-initiation of diet revealed some improvement in vision. Optic neuropathy is a previously unrecognized finding in MSUD. In our patient it was likely a consequence of his poor metabolic control and his case emphasizes the importance of continuous dietary treatment. Adults with this condition need to remain on BCAA restricted diet to prevent complications, including neurologic, ophthalmologic and other manifestations.

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### 3071/F/Poster Board #1003

**Cervical Cord Compression in Mucopolysaccharidosis Diseases.** *B.M. Diethelm-Okita, C.B. Whitley.* Lysosomal Disease Network, University of Minnesota, Minneapolis, MN.

Background: Cervical cord compression is an important, but probably under appreciated problem in mucopolysaccharidosis (MPS) conditions. The mechanism in most cases is pachymeningitis cervicalis. The standard treatment to date has been decompressive laminectomy, which has uncertain outcomes, the success of which may be syndrome dependant. Many of the published case studies that measure laminectomy outcomes for MPS were completed decades ago, before the advent of current therapies. Hematopoietic stem cell transplantation (HSCT) reduces the GAG level in the CNS, but enzyme replacement therapy (ERT) does not. We sought to assess the outcomes associated with surgical de-compression laminectomy for a cohort of patients with MPS seen at the University of Minnesota, and from the literature. Materials & Methods: We reviewed our records for cases of MPS patients who had undergone surgical laminectomy. Concomitant therapies are also noted. We created a ratings scale from 0 to 4 with 0 being equal to death, 1 equal to a significant loss of function (paresis or hemiparesis), 2 equal to minor loss of function, 3 equal to no change in condition, and 4 to improvement over pre-operative condition. Scores were assigned by physician assessment. Results: Nine subjects were identified in our records: MPS IS, II (2 subjects each), and VI (3 subjects) are presented with outcomes ranging from no complication (rating score =3) to death. Of the 8 patients assessed none had post-surgical improvement, 2 had no complications but no improvement, 1 had minor loss of function, 2 had a significant loss of function, and 2 patients died. Overall, 63% of subjects had less than favorable. Conclusions: Decompressive laminoplasty is a dangerous procedure; especially for those with advanced MPS IS, II or VI disease. Underlying MPS disease predisposes the patient to severe respiratory distress and cardiac morbidity and mortality. Speculation: New approaches to treatment would be important for the quality of life of affected individuals.

### 3072/F/Poster Board #1004

**A case of maculopathy associated with homocystinuria secondary to presumed methylene tetrahydrofolate reductase (MTHFR) deficiency.** *A. Atherton, U. Garg, S. Olitsky, M. Stass-Isern, L. Smith.* The Children's Mercy Hospitals and Clinics, Kansas City, MO.

Homocystinuria, a rare inborn error of metabolism, results from defects in homocyst(e)ine remethylation or transsulferation. The disorders associated with homocystinuria include cystathionine beta synthase deficiency, methylene tetrahydrofolate reductase, folate deficiency and disorders of intracellular cobalamin metabolism. Differentiation of these disorders can be inferred by differing patterns of biochemical markers. We present an unusual case of a 20 month old male child who at birth had a normal expanded newborn screen. At 6 weeks of age, he was admitted to the hospital for feeding difficulties, failure to thrive and loss of developmental milestones. He was noted to be microcephalic and found to have hepatomegaly. Initial work up revealed elevations of plasma and urine homocyst(e)ine with normal to low levels of plasma methionine. There were no elevations of urine methylmalonic acid. Megaloblastic anemia has not been identified. At 2 months of age, he was noted to have horizontal nystagmus. Exotropia was identified at 10 months. Ophthalmologic evaluation at 10 months of age was remarkable for bilateral large atrophic maculopathy. Although complete DNA sequencing of the MTHFR gene failed to identify any mutations, biochemical and laboratory studies are most consistent with a diagnosis of homocystinuria/hyperhomocysteinemia due to MTHFR deficiency. MTHFR enzymatic analysis is currently pending. Nystagmus has been previously described in this disorder as well as central retinal dysfunction and retinal vein occlusion. Salt and pepper maculopathy has been described in CblC deficiency but not in MTFHR deficiency. To our knowledge, this is the first report of large atrophic maculopathy/salt and pepper retinopathy associated with MTHFR deficiency.

### 3073/F/Poster Board #1005

**Growth velocity in patients with Hunter syndrome treated with idursulfase: results from the Hunter Outcome Survey (HOS).** *C. Tifft[1], M. Beck[2], J. Muenzer[3], G. Schulze Frenking[2].* 1) Div Gen & Metabolism, Children's Hosp Natl Med Ctr, Washington, DC; 2) Children's Hospital, University of Mainz, Mainz, Germany; 3) Department of Pediatrics, University of North Carolina, Chapel Hill, NC.

Introduction: The Hunter Outcome Survey (HOS) is a global outcomes database for gathering long-term data on the natural history of Hunter syndrome (MPS II) and the safety and effectiveness of enzyme replacement therapy (ERT) with idursulfase (Elaprase$^{TM}$; Shire HGT, Inc., Cambridge, MA, US). Untreated patients with MPS II have markedly reduced growth velocity after 8 years of age. Methods: As of April 16, 2009, 367 patients were reported as treated with idursulfase in HOS. This analysis provides data on patients who started therapy between 8 and 12 yrs of age (when growth restriction develops and while the patients are generally pre-pubertal) with available height and weight data at start of ERT (baseline) and 1 yr after start of ERT. Results: The eligible population for height and weight were 30 and 36 patients, respectively. At baseline, the mean height for patients was 122.8 cm with a mean Z-score of -2.5 (SD: 10.5 and 1.6, respectively). After one year of ERT, the mean height was 127.7 with a mean Z-score of -2.4 (SD: 12.8, 1.8). For weight, the mean baseline was 30.4 kg with a mean Z-score of -0.6 (SD: 5.4, 1.2). After one year of ERT, the mean weight was 32.6 kg with a mean Z-score of -0.8 (SD: 6.8, 1.3). The mean growth velocity was 4.9 cm/yr and patients seem to be following their own trajectory. Conclusions: We have observed an improvement in the growth of Hunter syndrome patients (8 to 12 years of age) one year after starting ERT based on historical data. Given that typically patients with Hunter syndrome have plateaued their growth by this age, the apparent normal growth is best explained by either a direct effect of ERT on cartilage or by a more general effect of ERT on health and well-being. Ongoing collection of data in HOS, including analysis of the effects of ERT in relation to pubertal development status (Tanner stage), is needed to further elucidate the effect of idursulfase on growth.

### 3074/F/Poster Board #1006

**MPV17-Associated Hepatocerebral Mitochondrial DNA Depletion Syndrome: New Patients and Novel Mutations.** *F. Li, A. El-Hattab, E. Schmit, S. Zhang, W. Craigen, LJ. Wong.* Molec & Human Gen, Balor College Med, Houston, TX.

Mitochondrial DNA depletion syndromes are autosomal recessive diseases characterized by a severe decrease in mitochondrial DNA content leading to organ failure. They are phenotypically heterogeneous, with myopathic, encephalomyopathic and hepatocerebral forms. The later has been associated with mutations in PEO1, POLG1, DGUOK genes and recently with mutations in MPV17 gene which encodes an inner mitochondrial membrane protein where it plays an as yet poorly understood role in mitochondrial DNA maintenance. Mutations in MPV17 gene have been reported in patients with a hepatocerebral mitochondrial DNA depletion syndrome who came to medical attention early in life with liver failure, hypoglycemia, failure to thrive and neurological symptoms. The homozygous p.R50Q mutation was identified in patients with infantile onset Navajo neurohepatopathy. To date 11 different mutations in 19 patients with MPV17-associaated hepatocerebral mitochondrial DNA depletion syndrome have been reported. We report eight new patients and identified seven novel mutations; four missense mutations (p.K88E, p.G94R, p.P98L, and p.A162D), one in-frame deletion (c.271_273 del3), one invariant splice site substitution (c.186+2T>C), and an insertion (c.22_23insC). The p.R50Q mutation, which occurs in CpG sequence, is the most common mutation. Clinically, patients with homozygous p.R50Q and compound heterozygous of p.G94R and p.P98L mutations have a better prognosis, with all the other mutations are associated with early death if not treated by liver transplantation. Localizing the mutations to the predicted MPV17 protein structure showed clustering of mutations in the region of the putative protein kinase C phosphorylation site.

### 3075/F/Poster Board #1007

**Unusual symptoms in a 14-year-old girl with Wilson disease.** *J. Lin[1,2,3].* 1) Pediatric department, Chang Gung Children's Hospital; 2) Chang Gung Memorial Hospital; 3) College of Medicine, Chang Gung University, Taoyuan, Taiwan.

The 14-year-old girl suffered from headache, dizziness, high blood pressure, arthralgia at bilateral wrists, elbows and knees. Blurred vision was complained later on and optic neuritis was diagnosed. Autoimmune disease was suspected first. Series of test was arranged and turned out to be negative. Kayser-Fleischer ring was accidentally found by slit lamp later. Wilson disease was diagnosed by lower serum copper, ceruloplasmin, and excess copper amount of 24 hours urine. Mutations on ATP7B gene exon 8 (PR778L) and exon 13 (P992L) were detected respectively. She developed acute pancreatitis after trientine and zinc acetate were prescribed. Further phenotype and genotype relationship will be discussed and paper reviewed.

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### 3076/F/Poster Board #1008

**20-Year Follow-up of Bone Marrow Transplantation In A Mucopolysaccharidosis VI Patient with Substantial Residual Pathology.** *H.C. Andersson.* Hayward Gen Ctr, Tulane Univ Med Ctr, New Orleans, LA.

Mucopolysaccharidosis type VI (Maroteaux-Lamy Syndrome) is characterized by lysosomal storage causing visceromegaly, corneal clouding, and bone dysplasia (dysostosis multiplex). This rare mucopolysaccharidosis has been treated with bone marrow transplantation (BMT) and, more recently, enzyme replacement therapy (ERT) with galsulfase has become available. Few reports have described the long-term outcome of BMT in MPS VI; such results are critical to determine whether BMT, ERT or a combination is the most appropriate therapy. A 22-year old woman who underwent BMT at age 2y for arylsulfatase B (ASA-B) deficiency (1.60 nM/mg/hr, nl 148-280, leukocyte) is described. The patient presented at age 2 years with facial coarsening, umbilical hernia, splenomegaly, and dysostosis multiplex (broad ribs, platyspondyly). She was transplanted with a male noncarrier allogeneic marrow and had no episodes of rejection. She received bilateral corneal transplantation at age 13years, underwent back surgery for spinal stenosis at 17 yrs and has had 6 sets of PE tubes for recurrent otitis media. At age 22y, her BMT shows 100 percent engraftment, leukocyte ASA-B is normal and urine MPS spot test is negative. Adult height is much less than 5 percentile (50th percentile for 8y6mo female), head circumference is 75 percentile. Spine radiographs demonstrate platyspondyly and the patient has chronic back and neck pain. Echocardiography demonstrates mitral valve prolapse and cardiopulmonary stress test revealed moderate to severe aerobic impairment. Otolaryngologic evaluation for hoarse voice demonstrated incomplete adduction of the vocal cords secondary to thickening of the cords. This patient offers the longest outcome of BMT in a MPS VI and documents the continued disease progression in patients with full engraftment. A comparison trial of ERT vs BMT is necessary to identify the most appropriate therapy in MPS VI patients.

### 3077/F/Poster Board #1009

**Quantificaiton of GM2 and GM3 storage in the brains of Mucopolysaccharidosis I and IIIB canines.** *A.D. Dierenfeld[1], J. Jens[1], E.M. Snella[1], P.I. Dickson[2], N.M. Ellinwood[1].* 1) Animal Sci, Iowa State Univ, Ames, IA; 2) Division of Medical Genetics Los Angeles Biomedical Institute at Harbor-UCLA Medical Center, Torrance, CA.

The Mucopolysaccharidoses (MPSs) are lysosomal storage diseases. Lack of a specific lysosomal enzyme results in accumulation of glycosaminoglycans (GAGs). In addition to GAG storage, neuropathic MPSs also show secondary CNS storage of GM2 and GM3 gangliosides. Our study quantified the amount of GM2 and GM3 storage in MPS I and MPS IIIB canine brains. Gangliosides from dissected cerebral gray matter were extracted and quantified by lipid extraction, fractionation, HPTLC, and scanning densitometry. Bands were calculated as mole %. Comparisons used ANOVA, and Tukey and non-parametric Wilcoxon rank sum post hoc tests. Samples included adult MPS IIIB (n=20), MPS I (n=4), and normal dogs (n=6). MPS IIIB levels were: GM2 3.09±0.23; GM3 8.08±0.55. Levels were statistically ($p<0.0001$) higher than normals: GM2 0.90±0.24, GM3 3.53±0.77. Similarly, MPS I dogs (n=4) had statistical ($p\leq0.0028$) elevations: GM2 3.10±0.43; GM3 11.8±4.59. Normal and affected brains were also evaluated for levels during development, at 1 and 3 weeks, and 2 and 3 months of age. Full analysis of these samples is pending. We also evaluated MPS I dogs receiving weekly intravenous (IV) enzyme replacement therapy from birth for 56-81 weeks with recombinant human iduronidase (rhIDU) at the conventional dosage (0.58 mg/kg, n=4), at a higher dosage (1.57 mg/kg, n=3), and at conventional dosage plus quarterly 0.058 mg/kg intrathecal (IT) injections (n=4). Results. Conventional IV ganglioside levels: GM2 1.95±1.24; GM3 8.95±1.53. High IV therapy levels: GM2 1.41±0.62; GM3 6.84±2.32. Conventional IV/IT levels: GM2 1.93±0.88; GM3 6.78±0.22. No statistical normalization was seen. However for GM3 IT vs. low dose saw improved response in IT dogs that approached significance (p=0.0709). Additionally, GM2 high dose vs. untreated saw a response in high dose that approached significance (p=0.0571). Low experimental numbers and high variability appear to be limitations in using these methods.

Secondary GM2 and GM3 CNS storage in MPS I and IIIB patients is also seen in the canine patients. Treatment of MPS I animals from birth with rhIDU may decrease the secondary storage burden. In this study, conventional dosage does not lead to statistical reduction in ganglioside storage, but IT and high dose treatments may be potential treatments for the CNS components of these disorders, based on the response to therapy of these secondary accumulations. Funded by NIH NS054242 (PID).

### 3078/F/Poster Board #1010

**Evolution and Utility of a Rare Genetic Disease Registry: The Gaucher Disease Registry.** *C. Fairley, G. Frohlich, M. Fitzpatrick.* Genzyme Corporation, Cambridge, MA.

The International Collaborative Gaucher Group (ICGG) Gaucher Registry is an ongoing observational program established in 1991. The goal of the Gaucher Registry is to significantly contribute to the medical understanding of Gaucher disease natural history and treatment outcomes, and to improve the quality of care for Gaucher patients worldwide through active publication of Registry findings and disease management approaches. This goal is achieved via the contribution of longitudinal data from 700+ physicians from 61 different countries. Participating physicians submit data to a centralized, confidential database that is reviewed by an International Board of Advisors consisting of leading medical practitioners for Gaucher disease. Since its inception over 15 years ago, the Gaucher Registry has enrolled over 5000 patients with Gaucher disease. This Registry program is sponsored by the Genzyme Corporation; the inclusion criteria is a confirmed diagnosis of Gaucher disease and there are no exclusion criteria. More than 15 peer review articles have been published from the data collected, and tools for managing patient care have been developed by the Gaucher Registry. One such tool that was developed and subsequently modified is the Recommended Schedule of Assessments for disease management by healthcare providers. These recommendations reflect medical practice patterns and have evolved as the understanding of the disease and treatment outcomes has increased. Examples of changes in recommendations are the addition of echocardiograms, DEXAs, and serum immunoelectrophoresis with the understanding of an increased incidence in Gaucher disease patients of pulmonary hypertension, low bone density, and multiple myeloma respectively. The Gaucher Registry also aims to address unanswered questions about Gaucher disease with the development of the Neurologic outcomes subregistry and the pregnancy subregistry. Finally, the Gaucher Registry provides a forum for data review and disease management discussions for participating medical practitioners through annual meetings in the US, the EU, and Latin America.

### 3079/F/Poster Board #1011

**Neonatal manifestations of Mucolipidosis type II: Longitudinal review of skeletal findings and generalized melanocytosis.** *A.M. Hata[1], C.E. Getman[2], R.A. Kahle[2], R.S. Lackman[3], S.A. Boyadjiev[1].* 1) Genetics, UC Davis, Sacramento, CA; 2) Mercy San Juan Medical Center, Sacramento, CA; 3) Cedars-Sinai Medical Center, David Geffen School of Medicine at UCLA, Los Angeles, CA.

Mucolipidosis type II (I-cell disease) is a rare progressive lysosomal storage disease with a poor prognosis resulting in chronic morbidity and mortality in the first decade of life. Mucolipidosis type II (MLII) is often not diagnosed until 6 to 12 months of age and the clinical manifestations in the early neonatal period have not been well defined. We report a Hispanic male who presented at birth with respiratory distress syndrome due to meconium aspiration. An x-ray revealed skeletal changes of the upper extremities and chest and a genetic consult was requested. An underlying lysosomal storage disease was suspected and testing revealed abnormal excretion of oligosaccharides in the patient's urine. A further assay of lysosomal enzymes showed significant elevation of all lysosomal enzymes, consistent with mucolipidosis type II. Genetic testing revealed a c.25C>T(pQ9X) nonsense mutation and a c.616_619delACAG deletion, both of which have not previously been reported in individuals with MLII but are expected to be disease causing. The patient's family history was non-contributory. Because a diagnosis was obtained in the first two weeks of life, we are able to describe features of disease progression in the early neonatal period, including progression of hepatosplenomegaly, skeletal changes, and overall disease advancement. We observed the natural progression of skeletal changes at ages one week, 3 months, and 9 months that has not been previously described. Vacuolated lymphocytes were observed on peripheral blood smear providing a diagnostic clue for a storage disorder. At six months of age, the patient presented with hyperpigmented lesions on the flank raising suspicion of child abuse. Clinical evaluation and skin biopsy documented melanocytosis, a feature not previously reported with MLII. This case provides an opportunity for longitudinal study to observe the progression of skeletal changes in MLII that have not been previously described.

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## 3080/F/Poster Board #1012

**Novel Endocrine Findings in Two Siblings with Morquio Syndrome Type B.** *L.E. Polgreen[1], M.K. Hordinsky[1], G.E. Hoganson[2], C.B. Whitley[1].* 1) Pediatrics, University of Minnesota, Minneapolis, MN; 2) Pediatrics, University of Illnois at Chicago, Chicago, IL.

Background: Morquio syndrome type B (MPS IVB) is a rare, autosomal recessive, lysosomal storage disease resulting from deficiency of β-galactosidase. It is characterized by severe short stature and dysostosis multiplex. Neither delayed puberty nor the impact of estrogen replacement therapy has yet been reported in MPS IVB. Results: We report two female siblings, ages 14.3 and 18 years, with MPS IVB who developed severe pubertal delay and have atypical skin findings. The diagnosis of MPS IVB was made based on urine keratan sulfate. The children also had diagnostic levels of leukocyte β-galactosidase: 2.8 and 3.6 nmoles/hour/mg protein respectively (normal 66.9-116.3; deficient 0-9.16). Pubertal delay was diagnosed at age 13.8 years in patient 1 and 17.2 years in patient 2. Both of the girls were treated with oral conjugated estrogens at a dose of 0.3 mg daily. Estrogen replacement therapy resulted in the initiation of puberty based on Tanner stage examination within the first 3 months of treatment. Concomitant with this initiation of puberty was an unusually rapid advancement of the bone age, indicating an increased tempo of growth plate closure. Patient one had a bone age advancement of 2 years over 7 calendar months, and patient two had a bone age advancement of 8 years 3 months over 10 calendar months on estrogen replacement therapy. Interestingly, both patients have severe, progressive, biopsy confirmed angiokeratomas on their feet, hands, knees and elbows, which has not been reported in patients with MPS IVB. Conclusions: This is the first case report of severe delayed puberty in MPS IVB. The unexpected rapid closing of the growth plates that occurred following initiation of estrogen replacement therapy suggests an increased sensitivity to estrogen in the growth plates. This rapid closure of the growth plates results in a significant decrease in ultimate adult height. This is particularly important in MPS IVB because of the already extreme short stature that is characteristic in these patients. This sensitivity to estrogen could influence the treatment of delayed puberty as well as growth promoting therapies in children with MPS IVB.

## 3081/F/Poster Board #1013

**Oropharyngeal dysphagia in young infants with Pompe disease.** *Y. Chien[1], S. Peng[2], N. Lee[1], W. Hwu[1].* 1) Departments of Medical Genetics , National Taiwan University Hospital , Taipei, Taiwan; 2) Departments of Radiology, National Taiwan University Hospital , Taipei, Taiwan.

Background: Dysphagia is common in infants with Pompe disease, and most cases finally require gavage feeding. This study investigated the time of onset of dysphagia and its response to treatments in cases diagnosed at very young ages by newborn screening. Material and Methods: From December 2002 to December 2008, 13 infants (5 detected by newborn screening and 8 detected after clinical presentation) with infantile-onset Pompe disease underwent swallowing videofluoroscopic study (VFSS) every three months. All patients received alglucosidase alpha infusion 20 mg/kg every other week. Results: VFSS from the 5 screened cases performed at an age as early as 0.4 month (median 0.8 month, range 0.4-1.1 months) already showed delayed swallow trigger and pharyngeal residues. VFSS from the 8 clinical cases, however, showed severe abnormalities including laryngeal penetration and aspiration. Follow-up studies in the screened cases revealed stabilization of the VFSS scores and all cases remained orally fed, while dysphagia got worse in all but one clinical case. Conclusion: Dysphagia may develop at the first month in infants with Pompe disease. Early enzyme replacement and careful management could stabilize their swallowing.

## 3082/F/Poster Board #1014

**Atypical presentation of Lysinuric Protein Intolerance in an African-American Infant.** *I.A. Larson[1], U. Garg[2], D. Scott[2], A. Atherton[1], L.D. Smith[1].* 1) Metabolic Genetics, The Children's Mercy Hospital, Kansas City, MO; 2) Biochemical Genetics Lab, The Children's Mercy Hospital, Kansas City, MO.

Lysinuric Protein Intolerance (LPI) an autosomal recessive disorder due to defects in SLC7A7, a dibasic cationic amino acid transporter protein. This results in decreased intestinal absorption and decreased renal reabsorption of cationic dibasic amino acids and is most common in the Finnish population. Case Report: We describe a patient of African American descent with no history of Finnish ancestry, who presented at 3 weeks of age with respiratory distress and impending respiratory failure, metabolic acidosis, hyperammonemia and anemia. The patient was previously identified as having alpha thalassemia trait. The baby was asymptomatic while breastfeeding and a transporter defect was suspected after introduction of formula-based feedings led to acute decompensation. Initial biochemical studies were suggestive of LPI, but did not confirm the diagnosis because there was a lack of orotic aciduria and only mild elevations of urine dibasic cationic amino acids. The diagnosis was confirmed by protein challenge. The case was complicated by renal tubular dysfunction although alveolar proteinosis was not present. Anemia responded to iron therapy suggesting that bone marrow involvement was minimal at the time of diagnosis. Since diagnosis and initiation of diet and citrulline management the patient clinically has done well, has had no progression in symptoms and has had excellent catch-up growth. Conclusion: LPI should be part of a differential diagnosis in any patient with hyperammonemia, metabolic acidosis and anemia and that protein challenges can be extremely helpful as a diagnostic tool.

## 3083/F/Poster Board #1015

**Clinical and metabolic characterization of ten individuals with methylmalonic acidemia following solid organ transplantation.** *J.L. Sloan, I. Manoli, C.P. Venditti.* Genetics and Molecular Biology Branch, National Human Genome Research Institute, Bethesda, MD.

Methylmalonic acidemia (MMA) is caused by deficiency of the methylmalonyl CoA mutase apoenzyme or its cofactor cobalamin and is a devastating disorder, recalcitrant to medical management in many cases. Organ transplantation for MMA was first reported in the 1990s and since then several patients have received kidney (KT), liver (LT) and combined liver-kidney transplants (LKT). There is little information on the outcome and metabolic parameters of these patients and there are currently no guidelines regarding indications for transplant in MMA. We describe the medical history and clinical phenotype, obtained by inpatient evaluation, of 10 individuals with MMA that underwent various transplant procedures. Results: Mutation analysis and/or cellular biochemical studies were performed in all participants. Among the individuals with *mut⁰* MMA, there were: 2 KT, 2 LT, and 5 LKT; 1 combined transplant patient received an auxiliary liver graft. One patient with *cblA* deficiency received a KT and her metabolic parameters significantly improved after reinitiating B12 therapy. All 7 patients with *mut⁰* MMA s/p LKT or LT remained metabolically stable since transplantation (40 years combined follow-up). One *mut⁰* patient with KT and poor metabolic control reported repeat hospitalizations with metabolic acidosis. Another *mut⁰* patient who underwent a staged procedure developed renal failure requiring dialysis 1-year post-LT and a worsening movement disorder after liberalizing her diet. All had massively elevated methylmalonic acid in plasma (19-1047 μM, nl <0.4), urine (20-2276 mmol/mol cr, nl<3) and total whole body MMA output (0.32-51.2 mg/kg/day). Studies in this patient group, the largest assembled to date, afford the following conclusions: 1. LT and LKT, but not isolated KT, completely prevent ketoacidotic crises. 2. After LT, patients can develop renal failure and therefore require careful monitoring. There is a theoretical risk of KT and LKT patients developing renal insufficiency. 3. Although solid organ transplantation improves metabolic parameters, nutritional control and cofactor therapy, in B12 responsive patients, are required for optimal management following the procedure due to persistent metabolite elevations and the risk for late complications of the disease. Further studies on the post-transplant state are needed, particularly the examination of genotype and metabolic correlations between the patients pre- and post-procedure and the outcomes achieved.

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### 3084/F/Poster Board #1016

**MAROTEAUX-LAMY SYNDROME OUTCOME IN A SEVERE FORM ASSOCIATED WITH CONGENITAL CARDIOPATHY IN A 3 YEARS OLD PATIENT.** *D. Giovannetti[1], M.A. Acosta[2], M.L. Solano[3].* 1) BioMarin Brasil Farmaceutica, Sao Paulo, Brazil; 2) Human Population Genetics, Titular Profesor in the Medicine Scholl from Universidad del Cauca, Colombia; 3) Hospital and Foundation Cardio-Infantil Bogotá_Colombia.

Mucopolysaccharidosis type VI or Maroteaux-Lamy syndrome is a lysosomal storage disease secondary to the partial to complete deficiency of arylsulfatase B. The patient usually presents coarse facial features, corneal clouding, organomegaly, joint stiffness and multiple dysostosis. Objective: This presentation aimed to report a case of a three years-old patient with MPS VI and a congenital cardiopathy Material and Methods: The patient was born in a small endogamic Indian community named Guambia, in the Department of Cauca - Colombia and have MPS VI symptoms first noted at 6 months old (organomegalia, hearing impairment and umbilical hernia) and a congenital cardiopathy. When she was 10 months old, the cardiac disease decompensate in a congestive cardiac failure due to arteriosus duct patency with great hemodynamic repercussion, severe mitral insufficiency, tricuspid insufficiency and biventricular dysfunction. She underwent a mitral valve replacement with an aortic prosthesis (mitral valve replacement with a biological valve) and a surgical closure of the duct arteriosus, being administered digoxin and antihypertensives in the postoperative period. With 2 years old, she had the MPS VI diagnosis confirmed through enzyme assay: arylsulfatase B activity 2.9 umol/H (reference value: >5.0) Her physical evaluation included coarse facial features, corneal clouding, enlarged tongue, shortened neck, umbilical hernia, hepatomegaly 4 cm. Tomography of cranium with 1 year and 10 months showed a mild ventricular dilatation. Radiographic evaluations dorso-lumbar kyphosis, moderate deformity of lumbar vertebrae caused by anterior wall tapering, bilateral coxa valga and talus varus, metatarsal and metacarpal shortening. Enzyme replacement therapy was started with Galsulfase 1 mg/kilogram/week with 2 years and 11 months and is been delivered weekly without adverse event. An improvement in the respiratory pattern is already noted. Discussion and Conclusions: The congenital cardiopathy's etiology remains uncertain but considering the highly consanguinity rate in the patieny's community, it can be explained by a autossomic recessive inheritance or a multifactorial pattern, and once we don't have any similar case in reported in the literature it's not likely to be related with the MPS VI severity. The ERT associated with early multidisciplinary intervention is safe and have the potential of decrease complications and delay the disease's evolution.

### 3085/F/Poster Board #1017

**Endocrine dysfunction in the mucopolysaccharidoses: a need for further investigation.** *K. Kim[1,2], B. Burton[1,2].* 1) Division of Genetics, Children's Memorial Hospital, Chicago, IL; 2) Department of Pediatrics, Northwestern University, Feinberg School of Medicine, Chicago, IL.

The mucopolysaccharidoses are a group of lysosomal storage disorders with a combined estimated prevalence of 1/22,500. Although highly variable, the disorders in this group are characterized by coarse facial features, skeletal anomalies, short stature, central nervous system abnormalities, cardiovascular disease, and hepatosplenomegaly, due to the progressive accumulation of glycosaminoglycans in various tissues. Little is reported in the literature regarding the endocrine function of MPS patients. Growth hormone deficiency and short stature is a known complication of stem cell/ bone marrow transplantation and has been observed in MPS I patients post-transplantation. Pathology studies post-mortem note accumulation of GAGs in the pituitary gland of MPS patients. One young boy with MPS VI was previously reported in the literature to have significant short stature compared to his affected brother and was found to have growth hormone deficiency. Given that little is known regarding the pituitary function of MPS patients, we examined nine patients with MPS I, II and VI for endocrine disorders and obtained screening labs, including T4, TSH, IGF1, IGFBP3, FSH, LH and fasting AM cortisol. Four patients had no significant findings on exam and normal lab results. Five patients were observed to have one or more of the following: low IGF1, low IGFBP3, premature adenarche, and hypogonadotropic hypogonadism. Given the clinical and laboratory observations in our patient population, we raise the possibility of underlying pituitary dysfunction in some MPS patients and advocate for further study in this area.

### 3086/F/Poster Board #1018

**Vitamin D deficiency and Fabry disease.** *D.P. GERMAIN[1, 2], K. BENISTAN[1], T.C. TRAN[1, 2].* 1) Medical Genetics Unit and Excellence Center for Fabry Disease, Hopital Raymond Poincare, Garches, 92380, France; 2) LGBC, UMR 8159, University of Versailles Saint Quentin en Yvelines, 78035 Versailles, France.

Background: Fabry disease (FD) is an X-linked inborn error of metabolism due to the deficient activity of alpha-galactosidase A, a lysosomal enzyme. FD is a systemic disease characterized by progressive renal insufficiency and added morbidity from cardio-and cerebro-vascular involvement. Vitamin D deficiency is a highly prevalent condition, present in 30% to 50% of the general population [1]. Traditionally, vitamin D has been associated primarily with bone health. However, a growing body of data suggests that low 25-hydroxy-vitamin D [25-OH D] levels may adversely affect cardiovascular (CV) health [1, 2]. Vitamin D deficiency activates the renin-angiotensin-aldosterone system and can predispose to left ventricular hypertrophy (LVH). No data is available regarding vitamin D deficiency in FD but, interestingly, osteopenia has recently been reported in FD [3] a condition in which LVH is highly prevalent. Patients & Methods: Serum 25-OH D levels were investigated in 28 consecutive patients affected with FD (15 M, 13 F) with estimated GFR >60 mL/min/1.73m². Vitamin D insufficiency was defined as 25-OH D serum level <30 ng/mL and vitamin D deficiency as 25-OH D serum level <20 ng/mL. Results: In the study population, the prevalence of vitamin D insufficiency was 82 (n=23) and the prevalence of vitamin D deficiency was 46% (n=13). Seven patients (25%) had serum 25-OH D levels <15 ng/mL while 3 patients (11%) had levels <10 ng/mL (severe deficiency). Normal 25-OH D levels were found in all patients living in the South of France while levels <10 ng/mL were found in all 3 patients with stroke. Discussion: The prevalence of vitamin D insufficiency and deficiency were 82% and 46%, respectively in patients affected with FD. Although FD patients may produce less vitamin D cutaneously because of efforts to minimize sun exposure and of decreased quality of life with indoor lifestyle, our results are comparable with figures found in the general population. Interestingly, very low 25-OH D levels were all associated with cerebrovascular accidents. Vitamin D supplementation is simple, safe, and inexpensive. Larger randomized controlled trials are needed to establish the role of vitamin D status to CV complications in FD but, meanwhile, monitoring serum 25-OH D levels and correction of vitamin D deficiency may prove interesting. 1. Lee JH et al. JACC.2008;52:1949-1956 2. Wang TJ et al. Circulation.2009;117:503-511 3. Germain DP et al. Clin Genet.2005;68:93-95.

### 3087/F/Poster Board #1019

**Surgical intervention in patients with Hunter syndrome: data from HOS - the Hunter Outcome Survey.** *N. Mendelsohn[1], B.K. Burton[2,3], K. Aleck[4] on behalf of the HOS Investigators.* 1) Dept Med Gen, Childrens Hosps Minnesota, Minneapolis, MN; 2) Children's Memorial Hospital, Chicago, IL; 3) Northwestern University Feinberg School of Medicine, Chicago, IL; 4) Saint Joseph's Hospital, Phoenix, AZ.

Hunter syndrome (mucopolysaccharidosis type II) is a lysosomal storage disease characterized by deficiency of the enzyme iduronate-2-sulfatase. Patients with this disorder can present with various signs and symptoms, including dysmorphic facial features and skeletal abnormalities, hepatomegaly and splenomegaly, respiratory problems, progressive hearing loss and cognitive impairment. Some disease manifestations may necessitate surgery. The present analysis was conducted to investigate the surgical interventions typically carried out in patients registered in HOS - the Hunter Outcome Survey - and the age at which these procedures were performed. As of April 2009,, HOS contained data on 681 patients with Hunter syndrome. This study focuses on the 541 HOS patients who were alive at HOS entry. Median age at diagnosis was 3.5 years (1.2-7.1, 10-90th percentile) and median age at last visit was 10.3 (3.7-23.4). In 487 prospective patients for whom surgery data were available, a total of 2067 surgical interventions were reported, with 85% of patients undergoing surgery at least once from birth to last visit in HOS. The most common surgeries were insertion of ear tubes (52%), hernia repair (50%), adenoidectomy (50%), tonsillectomy (35%) and carpal tunnel release (18%). 100 patients (21%) had recurrent hernia repair and 25 patients (5%) had surgery for carpal tunnel syndrome performed more than once. The early surgeries, performed at a median age of <5 years, were hernia (median age: 3.0 years, 0.2-11.5 years), ear tubes insertion (3.2, 1.3-7.5), adenoidectomy (3.5, 1.6-7.4) and tonsillectomy (4.3, 2.0-8.1). Carpal tunnel release was performed at a median age of 8.6 (4.5-19.3). The median times between diagnosis and first surgery were -0.3 (-5.3-7.8), 0.2 (-3.0-3.5), 0.3 (-2.1-3.5), 0.8 (-1.4-4.1) and 4.3 (0.6-11.2) years for hernia repair, ear tubes, adenoidectomy, tonsillectomy, and carpal tunnel release, respectively. These data highlight the early onset of disease manifestations in patients with Hunter syndrome. The frequency and age of which specific interventions were carried out is in line with the natural history of the disorder, with changes to the ears, nose, throat and airway occurring earlier and more frequently than skeletal abnormalities. Whether enzyme replacement therapy can prevent or reverse these manifestations is currently unknown and warrants further investigation.

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## 3088/F/Poster Board #1020

**Abnormal Newborn Screening for Glutaric Acidemia Type I Associated with Infantile Renal Failure.** *G. Arnold.* Div Pediatric Gen, Univ Rochester Sch Med, Rochester, NY.

Purpose: Glutaric Acidemia Type I (GAI) is an inborn error of lysine, hydoxylysine and tryptophan metabolism resulting in profound sensitivity of the basal ganglia to permanent neurological damage from metabolic stress. We report three infants with renal failure who presented with false positive abnormal newborn screening (NBS) for GAI.

Case Reports: Case one was noted to be affected with Eagle-Barrett (Prune Belly) Syndrome and renal failure at birth. Initial and follow-up C5-DC levels are noted in the Table. The infant underwent successful renal transplantation. Case two was also noted to be affected with Eagle-Barrett Syndrome at birth. The infant died of sepsis following renal transplantation. Case 3 was noted to have enlarged cystic kidneys at birth. Newborn screen at 48 hours was normal, but repeat screen was abnormal one week later. Follow-up noted normal glutaric acid levels in blood and urine, normal 3-OH glutarate in urine, but minimal elevation of 3-OH glutarate in blood. Renal failure is slowly progressing. Over all cases, there appeared to be a positive correlation between creatinine and degree of elevation of C5-DC (r=0.68).

**Initial and Follow-up C5-DC in Infants with Renal Failure and Abnormal NBS**

| Patient | Creatinine (mg/dl) | NBS C5-DC (uM) | Ratio C5-DC/C3 | Follow-up C5-DC |
|---|---|---|---|---|
| 1 | 2.7-3.9 | 0.19 (nl <0.15) | 0.1 (nl <0.1) | 0.45-0.68 |
| 2 | 2.4-3.2 | 0.18 | 0.27 | 0.35-0.37 |
| 3 | 1.3-2.2 | 0.18 | 0.25 | 0.18 |

Discussion: Infantile renal failure appears to be associated with false positive newborn screening for glutaric adidemia type I. The mechanism might possibly be associated with poor renal filtration of this metabolite.

## 3089/F/Poster Board #1021

**Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency in Poland - population study.** *D. Piekutowska-Abramczuk[1], J. Wierzba[2], R.K.J. Olsen[3], E. Popowska[1], D. Jurkiewicz[1], M. Borucka-Mankiewicz[1], E. Ciara[1], P. Kowalski[1], A. Tańska[1], W. Gradowska[4], M. Oltarzewski[5], J. Sykut-Cegielska[6], M. Krajewska-Walasek[1], N. Gregersen[3], E. Pronicka[6].* 1) Medical Genetics, Children's Memorial Health Institute, Warsaw, Poland; 2) Pediatrics, Hematology, Oncology and Endocrinology, Medical Academy, Gdansk, Poland; 3) Research Unit for Molecular Medicine, University Hospital, Aarhus, Denmark; 4) Biochemistry, Children's Memorial Health Institute, Warsaw, Poland; 5) Screening Test, Institute of Mother and Child; 6) Metabolic Diseases, Endocrinology and Diabetology, Children's Memorial Health Institute, Warsaw, Poland.

Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency, an autosomal recessive disorder, is the most common defect of the mitochondrial fatty acids β-oxidation pathway in Poland. LCHAD activity is encoded by *HADHA* gene located on chromosome 2p23. 60-86% of reported patients with isolated LCHAD deficiency have a prevalent c.1528G>C substitution in exon 15 of the *HADHA* gene. Majority of them originate from the Baltic populations. Based on the urinary GC-MS organic acid profile, MS-MS blood acylcarnitines profile, and molecular studies 43 Polish patients (40 families) with LCHAD deficiency were identified. Using PCR-SSCP analysis, the common c.1528G>C substitution was revealed on 91% of mutated alleles. A tendency for clustering LCHAD deficient patients in northern part of Poland, especially in Pomeranian voivodeship, was found. The aim of our study was to determine the carrier frequency of the common mutation in various districts of Poland, and to verify the probable correlation between high number of Pomeranian patients and expected high frequency of the c.1528G>C carriers in this region. Up to now, 6015 blood samples collected on anonymous Guthrie cards, have been screened. Fifty six heterozygotes for c.1528G>C substitution (including 41 carriers from the Pomeranian region) were detected. No samples homozygous for the c.1528C allele were identified. These preliminary study confirmed the higher frequency of LCHAD deficiency carriers in northern part of Poland than in other parts of the country (1:73 versus 1:203) and the world. The possibility exist, that the common *HADHA* mutation could originate from this region of Poland. The study was partly supported by the Polish Ministry of Science Project 0678/B/P01/2007/33(N407 0678 33).

## 3090/F/Poster Board #1022

**Newborn screening for Fabry disease by measuring GLA activity using tandem mass spectrometry.** *O. Bodamer[1], A. Dajnoki[2], G. Fekete[2], J. Keutzer[3], J. Orsini[4], V. De Jesus[5], N. Chien[6], P. Hwu[6], Z. Lukacs[7], K. Zhang[3], A. Muhl[8].* 1) University Children's Hospital Salzburg, Austria; 2) 2. University Children's Hospital Budapest, Hungary; 3) Genzyme Inc, Boston, MA, USA; 4) Wadsworth Center, NYS State Department of Health, Albany, NY, USA; 5) Center for Disease Control, Atlanta, GE, USA; 6) National Children's Hospital Taipeh, Taiwan; 7) University Children's Hospital Hamburg, Germany; 8) Centogene Inc, Vienna, Austria.

Background: Fabry disease (FD) is an X-linked inherited lysosomal storage disorder caused by the deficiency of α-galactosidase A (GLA) that leads to progressive storage of neutral glycosphingolipids in vascular endothelium, podocytes and other tissues. The clinical phenotype in both affected females and males may include renal insufficiency, cerebro-vascular insults, cardiomyopathy and other symptoms. Enzyme replacement therapy is available and prompt initiation may improve morbidity and mortality. In order to facilitate high throughput screening we evaluated a tandem mass spectrometry method to measure GLA activity in newborn infants as well as adults. Methods: 3.2 mm punches from dried blood spots (DBS) were incubated with substrate (6-benzoylamino-hexyl)-{2-[4-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-phenylcarbamoyl]-ethyl}-carbamic acid *tert*-butyl ester) and internal standard (6-d5-benzoylamino-hexyl)-[2-(4-hydroxy-phenylcarbamoyl)-ethyl]-carbamic acid *tert*-butyl ester for 22 h. The resulting product and internal standard were quantified using MS/MS. The assay was validated and FD gender specific cut-off values were generated using routine newborn screening and adult samples. Results: GLA activity in DBS from 9 known juvenile and adult males with FD was 0.67+0.38 μmol/h/l.; in 32 female juvenile adult females with FD GLA activity was 1.83+1.14 μmol/h/l, while GLA activity in 6 (19%) females was well above the diagnostic cutoff of 2.6 μmol/h/l. GLA activity in 6 Taiwanese newborn infants with FD identified through newborn screening was 0.88+0.38. In addition, GLA activity levels were measured in routine newborn screening samples: male newborns: n = 5,051, median 9.8 μmol/h/l; mean + SD: 11.2 + 6.4 μmol/h/l, CI 95% 9.67-10.02 μmol/h/l; female newborns n= 4,707, median 10.2 μmol/h/l; mean + SD: 11.7 + 6.3 μmol/h/l, CI 95% 10.02-10.38 μmol/h/l) and normal adult samples. GLA activity was stable for 28 days between 37°C and -80°C. MS/MS carry over was negligible, while intra-run and inter-run variability were less than 10%. Conclusion: The MS/MS method for Fabry disease newborn screening is robust and can be readily multiplexed with other lysosomal disorders such as Pompe, Gaucher, Niemann-Pick, and Krabbe diseases.

## 3091/F/Poster Board #1023

**Diagnosis and prognosis: a model of cooperation in newborn screening for lysosomal storage disorders.** *M. Fuller, J. Tucker, D. Lang, M. Snel, J. Hopwood.* LDRU, SA Pathology at Women's and Children's Hospital, Adelaide, South Australia, Australia.

Lysosomal storage disorders comprise a group of about 50 complex, relatively uncommon inborn errors of metabolism resulting in significant mortality and morbidity. Newborn screening provides an opportunity for pre-symptomatic identification and early intervention to prevent or at least mitigate the pathology associated with these disorders. However, the wide phenotypic variability associated with lysosomal storage disorders poses a major caveat for the implementation of a newborn screening program; how will individuals who remain asymptomatic for subsequent years be managed? We are developing a multiplexed immune-quantification assay that is currently able to measure 14 different lysosomal proteins. Dried blood spots from 102 patients representing ten different lysosomal storage disorders along with 1471 controls were assayed to demonstrate the feasibility of this approach for newborn screening. Of the 102 patients included in the study 93 were positively identified by reduced lysosomal protein levels compared to controls, with 2% false positives observed. To address the need for prognostic information for lysosomal storage disorder patients identified at birth, we are developing a "metabolomics" approach. As lysosomal storage disorders are characterized by the accumulation of incompletely degraded material in lysosomes, this material can easily be measured by mass spectrometry. The primary storage material is usually the substrate for the deficient enzyme in each lysosomal storage disorder, and may consist of glycosphingolipids such as glucosylceramide or trihexosylceramide in Gaucher and Fabry diseases respectively; sphingomyelin in Niemann-Pick A/B; glycosaminoglycans in mucopolysaccharidoses; or glycogen in Pompe disease. For many lysosomal storage disorders, the primary storage has been shown to result in impaired lysosomal function leading to the accumulation of secondary metabolites that are not substrates for the deficient enzyme. We have used mass spectrometry to identify and quantify primary substrates as well as secondary altered metabolites in urine, blood and skin fibroblasts in a variety of lysosomal storage disorder patients. The data generated highlights the opportunity for these metabolites to be used in a predictive manner to determine the course the disease will take.

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## 3092/F/Poster Board #1024

**Screening for Fabry Disease in Japan.** *K. Nakamura, K. Hattori, S. Matsumoto, H. Mitsubuchi, F. Endo.* Dept Pediatrics, Kumamoto Univ Sch Medicine, Kumamoto, Japan.

Fabry disease is an X-linked disorder of alpha-galactocidase A which causes the accumulation of glycolipids in lysosomes. The incidence of the classical type of the disease is approximately 1 in 40,000 males. Recent studies have revealed the late-onset type of the disease to have a higher frequency than previously known. To determine the disease incidence in Japan, we screened newborns to measure alpha-galactosidase A activity in dried blood spots from Japanese neonates. Enzyme-deficient infants were retested, and infants who were double-screening positive were diagnostically confirmed by enzymatic activity and mutation analyses. Ten neonates had a deficiency in alpha-galactosidase A activities and specific mutations, including two neonates with novel missense mutations, and eight neonates with known missense mutations identified previously in late-onset patients. Based on our newborn screening in Japan, the incidence of alpha-galactosidase A deficiency was 1 in 5,600 (1 in 5,600 male). Based on enzymatic activities, the incidence was 1 in 4,000 (1 in 3,100 male). These results suggest that the late-onset phenotype of Fabry disease is underdiagnosed among both males and females in Japan. The recognition of the existence of these patients suggests the need for both early diagnosis and therapeutic intervention. However, ethical issues need to be taken into consideration in terms of when and whom the screening should be performed.

## 3093/F/Poster Board #1025

**Newborn screening for lysosomal storage diseases by MS/MS: Fabry disease.** *C.R. Scott[1], G. Bellamy[2], J. Daiker[2], S. Shaunak[2], R. Jack[3], A.C. Fox[1], F. Turecek[4], M. Gelb[4].* 1) Dept Pediatrics, Univ Washington Sch Med, Seattle, WA; 2) Washington State Newborn Screening Lab, Shoreline, WA; 3) Seattle Children's Hospital, Seattle, WA; 4) Dept Chemistry, Univ Washington, Seattle, WA.

With the advent of enzyme replacement therapy (ERT) for selected lysosomal storage diseases (LSDs), the issue of newborn screening for these disorders has been proposed. We have synthesized novel substrates and internal standards that can be used for the MS/MS assay of enzymes responsible for Gaucher, Fabry, Pompe, Niemann-Pick A/B, Krabbe, MPS-1, MPS-II, and MPS-VI.

To validate the technology for the MS/MS detection of the LDS's, we have initiated a field trial within the Washington State Newborn Screening laboratory for the newborn detection of Fabry disease. After several thousand assays performed on newborn blood spots, the following outcomes are apparent:

--A single 3-mm blood spot can be used for one or more enzyme assays

--The population distribution of enzyme activity for $\alpha$-galactosidase is "log-normal"

--The median activity for $\alpha$-galactosidase from the 3-mm blood spot is 10$\mu$mol/L/hr

--The enzyme activity from the 3-mm blood spot as determined by MS/MS correlates well with our enzyme activity using a fluorometric substrate ($r^2$=0.93)

--The coefficient of variation (CV) is 0.16, 0.22, and 0.10 for low, medium, and high standards supplied by the CDC

--Affected male patients have activity values $<2\mu$mol/L/hr

The limiting factor in adapting the technology to a newborn screening laboratory is the number of pipetting steps as compared to procedures proceeding MS/MS analysis of amino acids or acylcarnitines that are currently being analyzed. Commercial robotic pipettors will be necessary for successful implementation of MS/MS detection of LSD's by enzymatic quantitation.

Sponsored by NICHD HHSN267200603429 and Genzyme Corporation.

## 3094/F/Poster Board #1026

**The CPT1 p.P479L Allele in Northern Canadian Populations: Does it Confer Risk for Infant Mortality?** *S.A. Collins[1], G. Sinclair[2], H.D. Vallance[2], C.R. Greenberg[3], I. Sobol[4], A. Corriveau[5], B. Hanley[6], G. Osbourne[4], T.K. Young[7], L. Arbour[1].* 1) Dept of Medical Genetics, University of British Columbia, Vancouver, BC; 2) Dept of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC; 3) Dept of Pediatrics and Child Health, University of Manitoba, Winnipeg, MB; 4) Health and Social Services, Nunavut; 5) Health and Social Services, North West Territories; 6) Health and Social Services, Yukon; 7) Division of Epidemiology, Dalla Lana School of Public Health, University of Toronto, Toronto, ON.

Introduction: Canada's three Northern territories are home to about 100,000 people, of which nearly 50% are of Aboriginal ancestry. Infant mortality rates increase from west to east, with Nunavut having the highest at 15/1000 live births. Hepatic carnitine palmitoyl-transferase I (CPTI, encoded by *CPT1a*) imports long-chain fatty acids into mitochondria for use in fatty acid oxidation. CPTI deficiency in its severe form may present in infancy with hypoketotic hypoglycemia, seizures, hepatoencephalopathy and sudden death, if not treated. A *CPT1a* variant, p.P479L (c.1436 C>T), is present in British Columbia (BC) First Nations, Canadian and Greenland Inuit and Alaska Natives. Although the association of the variant with infant mortality has not yet been established, a number of homozygous BC First Nations and Inuit children have presented symptomatically. Interestingly, allele frequencies in the Kivalliq region of Nunavut Canada and Greenland Inuit populations are high (0.81 and 0.73 respectively; Greenberg et al. 2009, Rajakumar et al. 2009), but the frequency has not been established in other Canadian Northern populations. Objective: To provide background data of p.P479L allele frequencies in Canada's North and to ultimately determine if the presence of the allele contributes to the excess of infant mortality. Methods: Ethics approval was obtained from university REBs, local research institutes and with consultation with territorial Aboriginal groups. *CPT1a* p.P479L prevalence was determined by genotyping newborn blood spots of infants born in 2006 in Yukon, Northwest (NWT) and Nunavut Territories (n=1584) using the Taqman allelic discrimination RT-PCR assay. p.P479L allele frequencies in the three territories were 0.02, 0.08 and 0.77 in Yukon (n= 325), NWT (n=564) and Nunavut (n=695), respectively. Homozygosity rates were 0%, 3% and 64%. Aboriginal status was available for NWT, with allele frequencies of 0.04, 0.44, 0.00 and 0.01 for First Nations, Inuvialuit/Inuit, Métis and non-aboriginal populations. Conclusion: Although the allele is present in all three territories, the rates of homozygosity are high only in the Inuit populations, suggesting a possible advantage to individuals historically living in the North. Further study is underway to determine if p.P479L variant is associated with infant mortality will provide background information to determine if newborn screening or other preventative health measures are warranted.

## 3095/F/Poster Board #1027

**Newborn screening and early biochemical follow-up in combined methylmalonic aciduria and homocystinuria, cblC type, and utility of methionine as secondary screening analyte.** *J.D. Weisfeld-Adams[1], M.A. Morrissey[2], B. Kirmse[1], B.R. Salveson[1], M.P. Wasserstein[1], P.J. McGuire[1], C. Yu[1], M. Caggana[2], G.A. Diaz[1].* 1) Department of Genetics & Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) 2New York State Newborn Screening Laboratory, Wadsworth Laboratory, Empire State Plaza, Albany, NY.

**Introduction**: Combined methylmalonic aciduria and homocystinuria, cobalamin C (cblC) type, is an inherited disorder of vitamin B12 metabolism caused by mutations in MMACHC. CblC typically presents in the neonatal period with neurological deterioration, failure to thrive, cytopenias, and multisystem pathology including renal and hepatic dysfunction. Rarely, affected individuals present in adulthood with gait ataxia and cognitive decline. Treatment with hydroxycobalamin may ameliorate the clinical features of early-onset disease and prevent clinical late-onset disease. Propionic acidemia (PA), methylmalonic acidemia (MMA), and cblC, cblD and cblF diseases are characterized by elevated propionylcarnitine (C3) on Newborn Screening (NBS). Distinctions can made between these disorders with secondary analyte testing. Elevated methionine is already routinely used as a NBS marker for cystathionine $\beta$-synthase deficiency. Low methionine may be useful as a secondary analyte for specific detection of cbl disorders among a larger pool of infants with elevated C3 on NBS. **Methods**: Retrospective analysis of dried blood spot (DBS) data in patients with molecularly confirmed cblC disease. **Results**: 9 out of 10 patients with confirmed cblC born in New York between 2005 and 2008 had methionine below 0.2 mg/dL on NBS. We incorporated elevated C3, elevated C3:C2 ratio, and low methionine into a simple screening algorithm, providing a specific and novel method of distinguishing cblC from other disorders of propionate metabolism prior to recall for confirmatory testing. **Conclusions:** We anticipate that this algorithm will aid in specific and early detection of cblC, cblD, and cblF, with little additional expense to NBS laboratories screening for organic acidemias and classical homocystinuria.

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## 3096/F/Poster Board #1028

**Amino acids and acylcarnitines concentration in blood spots from low birth weight infants.** *M. Pasquali[1,3], C. Haslip[3], N. Longo[1,2,3], F. Keune[4], H. Randall[4].* 1) Pathology, Univ Utah, Salt Lake City, UT; 2) Pediatrics, Univ Utah, Salt Lake City, UT; 3) ARUP Laboratories, Salt Lake City, UT; 4) Utah Department of Health, Salt Lake City, UT.

Tandem mass spectrometry (MS/MS) is routinely used for the screening of newborns for inherited metabolic disorders. Amino acids and acylcarnitines are extracted from the blood spots and quantified to allow the identification of amino acidopathies, organic acidemias, and disorders of fatty acid oxidation. As in any screening methods, false positive results can occur. The group of infants with the highest rate of false positive results is represented by infants in neonatal intensive care units, many of whom have low birth weight. These infants often require immediate intervention that may affect the values of the amino acids and acylcarnitines measured by MS/MS. We have analyzed the values obtained in three years of screening by MS/MS in Utah according to birth weight (BW). We arbitrarily chose 4 BW groups: < 1000 g, 1000-2000g, 2000-2500g, and > 2500 g and compared the percentile values for all analytes in all groups both in the first screen (collected at < 6 days of age) and in the second screen (7-28 days of age). Our results show that the median values for most analytes, amino acids or acylcarnitines, do not vary significantly among the BW groups. The biggest differences are observed in the highest percentiles (99th and above) of the amino acids. The two lowest BW groups have the highest values for all amino acids. This most likely reflects intravenous hyperalimentation. Interestingly, the long chain acylcarnitines (C16, C18) show the lowest percentile values in the lowest BW groups. This is likely explained by the fact that this group of infants has the highest rate of transfusion, resulting in a pattern of long chain acylcarnitines resembling the "adult" pattern, with lower concentrations of long-chain acylcarnitines. Among the acylcarnitines, C5-carnitine has the highest values in the lowest BW groups. C5-carnitine is elevated in metabolic disorders and during therapy with ampicillin, an antibiotic frequently used in infants admitted to neonatal intensive care units for possible sepsis. Our study shows that the median concentration of amino acids and acylcarnitines does not change significantly with birth weight. However, the distribution of values around the median is affected by medical treatment (intravenous hyperalimentation, transfusions, antibiotic therapy) frequently given to low birth weight infants. Consideration of these artifacts can reduce the frequency of false positive results in low birth weight infants.

## 3097/F/Poster Board #1029

**Positive Newborn Screening for Carnitine Transporter Deficiency: A Marker for Maternal Genetic/Metabolic Diseases?** *C. Prasad[1], J. DiRaimo[1], P. Chakraborty[2], S. Goobie[1], C.A. Rupar[3].* 1) Department of Pedatrics (Genetics), London Health Sciences Centre, London, ON, Canada; 2) Ontario Newborn Screening Program, Children's Hospital of Eastern Ontario, University of Ottawa; 3) Departments of Pediatrics, Biochemistry, and the Children's Health Research Institute, University of Western Ontario, London, Ontario, Canada.

Background: Primary carnitine deficiency is caused by mutations in the carnitine transporter *OCTN2* gene. Primary carnitine deficiency, 3-methylcrotonyl CoA carboxylase deficiency, and glutaric aciduria type 1 are some of the maternal disorders that have been detected secondary to a positive newborn screen for carnitine transporter deficiency. Methods: Ontario Newborn Screening Program referred 120 newborn screen positive infants to the London Health Sciences centre over last 3 years. Out of these 28 were initially positive for carnitine transporter defect (free carnitine level <10uM). None of them were true positives. We present two cases where the mothers had alternative diagnoses to account for their low carnitine levels. Results and Summary: In the first case, a male infant tested positive for carnitine uptake defect with free carnitine level of 7.4uM. Confirmatory testing was normal.The mother's investigations revealed free carnitine level at 0 umol/L (10-30 umol/L). Her urine organic acids showed suberic acid, phenylpropionyglycine, and suberylglycine and increased levels of C6, C8, C10:1 and C10:2. Molecular investigations showed homozygosity for the A985G mutation in the MCAD gene, confirming diagnosis of MCADD. The mother was 26 years of age, asymptomatic and has had two uneventful pregnancies. The second infant (female) tested positive for carnitine uptake defect with an initial free carnitine level of 7.9uM. Confirmatory testing revealed normal results. The maternal history confirmed diagnosis of cystic fibrosis. She is heterozygous for Delta F508 mutation in exon 10, and a rare R347-->P :G1172-->C mutation in exon 7. The mother's free carnitine and total carnitines were borderline low at 13 umol/L (20-53 umol/L) and 22 umol/L (25-73 umol/L) respectively. Conclusions:The pathogenesis in above scenarios is likely due to poor carnitine stores in the mothers leading to a false positive result for carnitine uptake deficiency in the newborns. These cases illustrate the importance of a detailed maternal history and confirmatory screening by acylcarnitine profile, urine organic acids, and carnitine levels in mothers of newborns with positive carnitine transporter deficiency screening results. The detection of asymptomatic MCADD and other maternal genetic metabolic disorders will provide proper management, family screening, and prevention of complications as sudden deterioration can occur with these disorders at any age.

## 3098/F/Poster Board #1030

**G6PD and CAH in Alexandria, Egypt: A high frequency justifying the need for a community- based newborn screening program.** *S. Tayel[1,2], H. Ismail[2,3], H. Kandil[4], I. Marzouk[5], A. Abd Rabou[2], H. Sallam[2,6].* 1) Anatomy Dept, Genetics Unit, Alexandria Faculty of Medicine, Alex., Egypt; 2) Suzanne Mubarak Regional Centre for Women's Health & Development, Alex., Egypt; 3) High Institute of Public Health, Alex., Egypt; 4) Consultant Pediatrician, Ministry of Health, Alex., Egypt; 5) Pediatrics Department, Faculty of Medicine, Alex., Egypt; 6) Obstetrics & Gynaecology Department, Faculty of Medicine, Alex., Egypt.

INTRODUCTION: Neonatal screening program for congenital hypothyroidism has been established at the national level in Egypt in 2000. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most frequent enzyme deficiency. It is a sex-linked genetic disease concerning mostly African, Mediterranean and far-eastern populations. The main clinical expression is a hemolytic anemia which can be acute or chronic. During the neonatal period the disease may manifest as neonatal jaundice. Congenital adrenal hyperplasia (CAH) is increasingly being included in newborn screening programs. The main benefits of screening are avoidance of serious salt loss crises and early proper gender assignment in virilized girls. OBJECTIVES: To determine the prevalence of G6PD deficiency and CAH in Alexandria, Egypt. METHODS: From June, 2008 to May, 2009, a total of 13,000 samples from newborns at 3-7 days of age were screened by the time resolved fluoroimmunoassay method (DELFIA; PerkinElmer) for the quantitative measurement of G6PD activity (6000 samples from male newborns) and for 17-hydroxyprogesterone determination (7000 male and female samples). Any neonate with a value < 2.6 U/gHb and > 30 nmol/l was considered positive for G6PD deficiency and CAH respectively. RESULTS: Of the 6000 male newborns screened, 149 (2.12%) were found to have G6PD deficiency. Of the 7000 male and female newborn screened, 11 (0.16% or 1.6/1000) were affected by the CAH. The mean enzyme activity in G6PD deficient patients was 1.27 +/- 0.4 U/gHb. CONCLUSION: The relatively high prevalence of G6PD deficiency and CAH in Alexandria, Egypt justifies the recommendation to include these two diseases in the national hypothyroidism screening program in Egypt. Efforts are now being undertaken to ensure their nationwide implementation in the newborn screening program.

## 3099/F/Poster Board #1031

**Neuropsychological function in individuals with phenylketonuria treated with Kuvan.** *D.K. Grange[1], S.E. Christ[2], D.A. White[3].* 1) Dept of Pediatrics, Div of Genetics and Genomic Medicine, Washington University, St Louis, MO; 2) Dept of Psychological Sciences, University of Missouri, Columbia, MO; 3) Dept of Psychology, Washington University, St. Louis, MO.

Phenylketonuria (PKU) is a hereditary disorder resulting in disrupted metabolism of phenylalanine (Phe). The profound effects of elevated Phe once associated with PKU, such as mental retardation and seizures, have largely been eliminated through dietary restriction of Phe. However, Phe often remains elevated even in patients considered to be well treated by diet alone. As a result, although more subtle than in the past, PKU patients continue to exhibit neurologic abnormalities and impaired cognition. Kuvan (sapropterin dihydrochloride/BH4) is a pharmaceutical treatment that lowers Phe in BH4 responders and holds promise for improving brain function and cognition. In our study, brain and cognition are examined in PKU patients immediately before beginning treatment with Kuvan (20 mg/kg/day) using MRI and neuropsychological tests of intelligence (IQ), executive abilities, and reaction time (RT). For patients who respond to Kuvan with a reduction of ≥20% in Phe within 4 weeks of beginning treatment, brain and cognition are again examined after 6 months of Kuvan treatment. We hypothesize that improvements in brain and cognition will occur with Kuvan treatment. Here we report results from the baseline neuropsychological evaluation of the first 7 PKU patients enrolled. Patients are from 9 to 20 years of age (M=14, SD=4), with Phe ≥360μmol/L. Patients' neuropsychological performance is compared with that of 10 normal controls from 8 to 22 years of age (M=15, SD=5). Our findings indicate that PKU patients have significantly poorer IQ and executive abilities than controls. The IQ of PKU patients ranged from 75 to 109 (M=92, SD=12), whereas the IQ of controls ranged from 89 to 117 (M=107, SD=9), t(15)=2.7, p<.05. Regarding executive abilities, PKU patients performed more poorly than controls on tests of inhibitory control, working memory, and strategic processing, t(15)≥3.0, p<.01 in all instances. The PKU and control groups, however, were not significantly different on measures of simple RT (p>.05). These results reflect specific impairments in intelligence and executive abilities in PKU patients treated with diet alone prior to treatment with Kuvan. Data collection is ongoing. At the conference, baseline findings from newly enrolled patients will be presented. In addition, findings from 6 month evaluations of BH4 responsive patients will be presented to evaluate whether improvements in brain and cognition are associated with Kuvan treatment.

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### 3100/F/Poster Board #1032

**Overexpression of Arginase in neurons as a potential protective model against nitric oxide mediated neurotoxicity.** *B. Keller[1], T. Bertin[1], B. Lanpher[2], O. Shchelochkov[1], B. Lee[1,3].* 1) Baylor College of Medicine, Molecular and Human Genetics, Houston, Texas, United States; 2) Vanderbilt University School of Medicine, Nashville, Tennessee, United States; 3) Howard Hughes Medical Institute, Houston, Texas, United States.

Nitric oxide (NO) is an important messenger molecule. In the brain it plays an important role in synaptic plasticity and has a role in the regulation of the neurophysiological phenomenon underlying memory and depression. NO has a pivotal role in neuronal development; low levels have been described to be protective in neurons degeneration but high levels of NO cause neuronal destruction. NO is synthesized from aginine by the nitric oxide synthases (NOS) enzymes. However; arginine is also the substrate for arginase to generate ornithine, the last metabolite in the urea cycle. In mammals, there are two isoforms of arginase; the cytoplasmatic isoform, arginase I, functions predominantly in the liver to detoxify the body from harmful ammonia. Arginase II regulates arginine/ornithine concentrations in the cell. The later isoform is located in mitochondria of the kidney and the prostate and at lower levels in mammary glands, macrophages, as well as the brain. Both isoforms compete with the nitric oxide synthases for their rate limiting substrate arginine, leading to decreased formation of nitric oxide. We hypothesize that overexpression of arginase in the brain will be a protective model for nitric oxide mediated neurotoxicity. We generated a transgenic mouse model over expressing arginase I specifically in neurons under the control of the Thy-1 promoter. Founders show an up to 30 fold over expression of arginase I with comparable enzyme activity. The mice survive, have normal growth, are fertile with offspring born in mendelian ratio. H&E Staining and neuron specific Nissl staining show no abnormalities compared to wild type mice. This model will be a valuable tool to further test whether genetic inhibition of nitric oxide production will decrease neurotoxicity in neurologic diseases such as traumatic brain injury (TBI) and amyotrophic lateral sclerosis (ALS).

### 3101/F/Poster Board #1033

**Potential applications of fluorescence-labeled α-L-iduronidase.** *B. Tippin, S. Kan, L. Troitskaya, P. Dickson.* Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, 1124 West Carson St, Torrance, CA 90502.

Mucopolysaccharidosis I (MPS I) is an inherited, lysosomal storage disease caused by lack or low level of α-L-iduronidase (IDU), resulting in progressive accumulation of glycosaminoglycan (GAG) in lysosomes of different tissues including the brain. Some clinical improvement in patients has been observed by enzyme replacement therapy (ERT) with recombinant human IDU (rhIDU; laronidase, Aldurazyme), but the treatment of central nervous system (CNS) involvement is limited due to the difficulty of rhIDU to penetrate through the blood-brain barrier. Thus, cerebrospinal fluid (CSF) delivery is being investigated to apply rhIDU to CNS directly. While rhIDU distributes widely in MPS I dogs at 48 hours following intra-CSF injection, earlier and more precise time estimates of CSF delivery are hampered by the need for serial measurements at different locations. Fluorescent-labeled rhIDU will allow us to study CSF distribution *in vivo* in small animal models, and *ex vivo* using confocal microscopy.

rhIDU was labeled with Alexa Fluor 680 dye (Molecular Probes) to DOL = 3.50 (degree of labeling: moles dye per mole protein). Purified, labeled-rhIDU retained its activity towards the artificial substrate 4MUI *in vitro* when compared to non-labeled rhIDU. Uptake kinetics of labeled-rhIDU into human MPS I fibroblasts ($k_{uptake}$ of 0.72 nM ) matched previously reported values for unlabeled rhIDU ($k_{uptake}$ of 0.7 nM) (Kakkis E. *et al.* 1994 Protein Expr Purif. **5**: 225-32). Furthermore, the labeled-rhIDU was directly detected in MPS I fibroblasts by confocal microscopy, and its subcellular localization was verified within lysosomes by Lysotracker® and antibody against rhIDU. These results suggest that the label does not significantly interfere with the enzyme's ability to enter cells or disturb its active site.

This study demonstrates that fluorescent-labeled rhIDU will be a valuable tool to assess enzyme distribution in CSF and other tissues of interest, with potential for *in vivo* and *ex vivo* applications.

### 3102/F/Poster Board #1034

**Cause of Death in MPS I Registry Patients.** *C. Whitley[1], G. Cox[2], J. Wraith[3], P. Fernhoff[4].* 1) University of Minnesota, Minneapolis, MN; 2) Genzyme Corporation, Cambridge, MA; 3) Royal Manchester Children's Hospital, Manchester, UK; 4) Emory University, Atlanta, GA.

Background: The MPS I Registry is a global, voluntary, observational database that has collected anonymous, longitudinal data on over 700 patients since its inception in 2003. The Registry database is a unique resource to study the factors that impact the timing and cause of death in MPS I patients.

Methods: Registry data were collected prospectively and retrospectively (1979-2008). Two-thirds (67%) of deceased patients were posthumously enrolled. Data were categorized by phenotype, and cause of death. In 32 (22%) deceased patients, more than one cause of death was reported in multiple organ systems.

Results: Twenty percent (n=145) of Registry (N=729) patients were deceased. The majority (88%; 128/145) of deceased patients were clinically categorized with Hurler syndrome, the most severe phenotype of MPS I. Thirty-one percent of all Hurler patients (128/408) were deceased, compared to 8% of Hurler-Scheie (14/171) and 3% of Scheie (2/74) patients. Phenotype was undetermined for one deceased patient. The most common causes of death were respiratory and/or cardiac (46%), unknown (24%), and complications related to hematopoietic stem cell transplantation (HSCT) (13%). In 27 MPS I patients with a known respiratory cause of death, death was attributed to pneumonia (n=7), respiratory failure (n=8), pulmonary hemorrhage and/or edema (n=4), and other respiratory causes (n=8). In the 21 MPS I patients with a known cardiac cause of death, cardiac arrest (n=10) and cardiac failure (n=6) were the most frequently reported causes of death. Cardio/respiratory causes of death in 19 patients included cardiopulmonary failure (n=13), cardiopulmonary arrest (n=5), and severe heart abnormalities/pulmonary edema (n=1).

Conclusions: The most common causes of death in MPS I Registry patients were pulmonary and/or cardiac. Monitoring disease progression in these organ systems may provide important insight into the impact of treatment on morbidity and mortality.

### 3103/F/Poster Board #1035

**Principal components factor analysis of the insulin-like growth factor I (IGF-I) axis reveals a quantitative trait locus on chromosome 16p that is longitudinally associated with insulin and is mediated by IGF binding protein 3 and tumor necrosis factor-α.** *P.B. Higgins, V.P. Diego, S.A. Cole, T.D. Dyer, J.E. Curran, M.P. Johnson, E.K. Moses, L. Almasy, H.H.H. Göring, A.G. Comuzzie, J.W. MacCluer, M.C. Mahaney, J. Blangero.* Dept Genetics, SW Foundation Biomed Res, San Antonio, TX.

The insulin-like growth factor I (IGF-I) axis exerts important influences on insulin metabolism. Recently, the IGF binding proteins (IGFBPs), which are critical components of the IGF-I axis, have been found to exhibit influences on insulin metabolism that could be divided into IGF-I-dependent and -independent mechanisms. We hypothesized that a principal components factor analysis (PCFA) approach would be ideally suited to studying this system because of its ability to extract from the data factors that are correlated within but that are mutually orthogonal. To this end, we performed PCFA on the IGF-I axis, which is represented in our data by IGF-I, IGFBP1, IGFBP3, and the molar ratios of IGF-I to IGFBP1 and to IGFBP3, herein named ratioBP1 and ratioBP3. The latter variables are understood to be rough measures of free IGF-I. While the number of variables is quite small for a usual PCFA, the method is being used for its ability to extract mutually orthogonal factors, which speaks to mechanism in the present case. We found 3 factors, an axis factor comprised of IGF-I, ratioBP1, and ratioBP3, an IGFBP1 factor comprised of IGFBP1 and ratioBP1, and an IGFBP3 factor comprised of IGFBP3 and ratioBP3, which accounted for 45%, 29%, and 22% of the variance, respectively. Using a maximum likelihood variance components (VC) approach, we performed multipoint genome-wide scans of these 3 factors. For the IGFBP3 factor, we found a LOD score of 3.6 on chromosome 16 at the p-terminus, which is the location of the acid-labile subunit (*IGFALS*) gene. This is a significant finding because the vast majority of IGF-I is bound up in the blood stream by a complex comprised of IGFBP3 and IGFALS. Moreover, loss of function mutations in the *IGFALS* gene in humans and animal models are associated with dysregulated insulin metabolism. Bivariate VC analyses of the IGFBP3 factor with a gene expression measure (mRNA levels) of tumor necrosis factor-α (TNF-α) and with fasting insulin (FI) measured 5 years later from when the IGF-I axis variables were measured revealed LOD scores of 3.55 and 3, respectively, again at the p-terminus of chromosome 16. The IGFBP3 factor was significantly positively correlated with TNF-α, and significantly negatively correlated with FI. Together, these results may mean that IGFBP3 and TNF-α act to influence FI, and are pleiotropically regulated by the same locus, which is potentially the *IGFALS* gene.

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### 3104/F/Poster Board #1036

**Accelerated neutrophil apoptosis in a congenital neutropenia syndrome, glucose-6-phosphatase-beta deficiency, is mediated by the mitochondrial stress pathway.** *H.S. Jun, Y.Y. Cheung, J.Y. Chou.* SCD, PDEGEN, NICHD, NIH, Bethesda, MD.

Accelerated neutrophil apoptosis in a congenital neutropenia syndrome, glucose-6-phosphatase-beta deficiency, is mediated by the mitochondrial stress pathway Hyun Sik Jun, Yuk Yin Cheung, and Janice Y. Chou, Section on Cellular Differentiation, Program on Developmental Endocrinology and Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892 Neutrophils, the first line of defense against bacterial or fungal infections, differentiate and mature in the bone marrow (BM). We have previously shown that G6pc3-/- mice lacking glucose-6-phosphatase-β (G6Pase-β or G6PC3) manifest neutropenia and that activated G6pc3-/- neutrophils, isolated from peritoneal exudates, exhibit dysfunction, increased production of endoplasmic reticulum (ER) chaperones, and enhanced apoptosis. Using a negative immunomagnetic depletion system to isolate resting neutrophils from the BM, we now show that G6pc3-/- BM is neutropenic, prior to extravasation, and that the resting neutrophils exhibit impaired respiratory burst, chemotactic, and calcium flux activities. Moreover, G6pc3-/- BM neutrophils exhibit ER stress, oxidative stress, and ultrastructural alterations of the ER. Activation of the protein kinase-like ER kinase (PERK) along with increased expression of phosphorylated eukaryotic translation initiation factor 2α, activating transcription factor 4, and C/EBP-homologous protein demonstrate the involvement of the PERK-mediated ER stress signaling pathway. G6pc3-/- BM neutrophils exhibit increased Annexin V binding and caspase-3 activation, consistent with an increased rate of apoptosis. Bax activation, mitochondrial release of pro-apoptotic effectors and caspase-9 activation demonstrate the involvement of the intrinsic mitochondrial apoptotic pathway. Taken together, the results demonstrate a critical role for G6Pase-β in ER homeostasis and normal neutrophil functions. This research is supported by the Intramural Research Programs of the NICHD, NIH.

### 3105/F/Poster Board #1037

**Enzyme replacement therapy given from birth may prevent brain GAG accumulation in MPS I dogs.** *S. Le[1], N.M. Ellinwood[2], A. Dierenfeld[2], J. Jens[2], E. Snella[2], C. Vogler[3], M. Passage[1], P. Dickson[1].* 1) Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, 1124 West Carson St, Torrance, CA 90502; 2) Department of Animal Science, Iowa State University, 2356D Kildee Hall, Ames, IA 50011; 3) Department of Pathology, Saint Louis University School of Medicine, 1402 South Grand Blvd, St. Louis, MO 63104.

Intrathecal (IT) enzyme replacement therapy (ERT) has been studied in mucopolysaccharidosis I (MPS I) dogs as a way to reduce glycosaminoglycan (GAG) storage in brain. IT ERT in adult MPS I dogs showed reduction in brain GAG to normal levels, while intravenous (IV) ERT alone in adult MPS I dogs showed no significant changes in brain GAG storage to untreated MPS I. We tested IV ERT with recombinant human α-L-iduronidase in MPS I dogs 3-24 days old to determine whether GAG accumulation in the brain could be prevented. 4 MPS I dogs received weekly IV ERT at 0.58 mg/kg/wk, and 4 MPS I dogs received weekly IV ERT at 1.57 mg/kg/wk. 4 MPS I dogs received weekly IV ERT at 0.58 mg/kg/wk with quarterly (every three month) IT ERT at 0.059 mg/kg. Dogs were treated for 56-81 weeks, and were 13-19 mos. old at end-study. 6 age-matched MPS I dogs and 9 normal dogs were used as untreated controls. Whole brain GAG measured by alcian blue binding method in MPS I dogs treated from birth was 5.74 ± 0.160 μg GAG/mg dry weight (0.58 mg/kg IV ERT group, $p = 0.0013$ versus untreated), 4.43 ± 0.269 (1.57 mg/kg IV ERT group, $p = 0.0001$ vs. untreated), and 3.87 ± 0.312 (IT + IV group, $p = 0.0001$ vs. untreated). Brain GAG in MPS I untreated controls was 8.19 ± 0.984, and in normal controls was 5.40 ± 1.82. Tissue pathology by light and electron microscopy is pending. MPS I dogs treated from birth with clinically-used 0.58 mg/kg IV ERT, higher 1.57 mg/kg IV ERT, and IT + IV ERT show brain GAG storage comparable to normal levels. ERT from birth may be an effective strategy to prevent brain GAG accumulation in MPS I disease.

Funded by NIH NS054242. Enzyme donated by BioMarin Pharmaceutical Inc.

### 3106/F/Poster Board #1038

**Immune tolerance induction in enzyme replacement therapy for Pompe disease by anti-CD3 antibody and oral enzyme administration.** *T. Ohashi[1,2,3], S. Ilzuka[2], Y. Eto[3], H. Ida[1,2,3].* 1) Dep of Pediatrics, The Jikei Univ Sch of Medicine, Minatoku Tokyo, Japan; 2) Inst DNA Mecicine, The Jikei Univ Sch of Medicine, Minatoku Tokyo, Japan; 3) Dep of Genetic Diseases, The Jikei Univ Sch of Medicine, Minatoku Tokyo, Japan.

Enzyme replacement therapies (ERT) for 6 lysosomal storage diseases including Gaucher disease, Fabry disease, Pompe disease, mucoplysaccharidosis type I, II and VI, are approved in developed countries. Although their efficacy was proven in many clinical trials, a couple of limitations were turned out. Among them, negative impact of neutralizing antibody to enzyme was one of major limitation in ERT. In fact, we and others already reported negative impact of antibody for therapeutic efficacy of ERT in lysosomal storage diseases. To overcome these obstacles, we tried to induce immune tolerance to infused enzyme. Among 6, lysosomal diseasaes, antibody formation clearly affect the clinical outcome in infantile onset Pompe disease. Because of reason stated above, we choose alpha-Glucosidase (GAA) which is used for Pompe ERT as a model. In this study, we tried two methods to induce immune tolerance. Fist, we administered anti-CD3 antibody. Anti-CD 3 antibody was reported to induce immune tolerance to infused coagulation factor VIII in hemophilia and approved for rejection of renal transplantation in human. We intravenously injected low and high dose of anti-CD3 antibody to Balb/c mice followed by GAA immunization. Low dose anti-CD 3 successfully induced immune tolerance to enzyme but high dose of anti-CD 3 antibody did not. Although anti-CD 3 antibody induced immune tolerance, there are some side effect such as fever and chilling. Ideally, tolerance induction in ERT for lysosomal diseases should be long lasting and antigen specific. So, we tried oral tolerance induction, which might be safe and antigen specific. We orally administrated low and high dose of GAA to Balb/c mice and C57BL/6 mice, followed by GAA immunization. Both orally administered high dose and low dose of GAA successfully induced immune tolerance in both mouse strains. But the stronger immune tolerance was achieved in C57BL/6 mice by high dose oral GAA administration. We currently tested how long immune tolerance persists. In conclusion, parenteral administration of anti-CD 3 antibody and oral administration of GAA might be translated to clinics to induce immune tolerance ERT for lysosomal storage diseases.

### 3107/F/Poster Board #1039

**Proteinase-Activated Receptor (PAR1) Polymorphic variant correlates with thrombocytopenia in Gaucher Disease.** *D. Elstein[1], O. Seror-Burkis[1], A. Zimran[1], G. Altarescu[2].* 1) Gaucher Clinic, Shaare Zedek Medical Ctr, Jerusalem, Israel; 2) Medical Genetics Institute, Shaare Zedek Medical Ctr, Jerusalem, Israel.

Background: Enzyme replacement therapy (ERT) for Gaucher disease is safe and effectively corrects hepatosplenomegaly and hypersplenism. However, thrombocytopenia, not necessarily in severely affected patients nor correlated with splenomegaly, may not normalize. Platelets are involved in (acute and chronic) inflammation and thrombogenesis; both of these functions are mediated by Proteinase-Activated Receptors (PARs). PAR1 expression is down-regulated during systemic thrombin formation induced by inflammation with decreased responsiveness by PAR1 receptors after repeated stimulation. Accumulation of undegraded lipids in macrophages in Gaucher disease induce a chronic state of inflammation. Therefore, the purpose of this study was to describe PAR1 polymorphic genotypes in patients with Gaucher disease and ascertain whether these are correlated with platelet counts. Methods: Blood samples were taken from 80 patients with non-neuronopathic disease, some on ERT, and from 44 healthy controls. PAR1 polymorphisms IVS-14(A/T), -506(I/D) and -1426(C/T) were analyzed by standard methods. Clinical data was collected from the files. Associations between PAR1 and categorical variables were analyzed by chi-square and Fisher's exact tests; assessment of associations with quantitative variables used ANOVA and Scheffe post-hoc for multiple pair-wise comparisons. Non-parametric Kruskal-Wallis ANOVA was used when one category was small. All tests were 2-tailed; p values<0.05 was considered statistically significant. Results: There was a statistically significant difference (p=0.015) between patients and controls for [-1426] genotype but no significant correlations for -1426 with disease severity, need for ERT, need for splenectomy, or presence of bone disease; rather, a significant correlation between lower platelet counts (p=0.0003) and the[-1426TT genotype and a trend for correlation with inflammation markers (p=0.079). There was a statistically significant correlation for -506 genotype with femoral neck bone density (p=0.034). All patients' carried the Wild Type for IVS-14. Conclusion: Since genotype-1426 is the functional variant for PAR1, these provocative findings may suggest a role for PAR1 as an independently-sorting modifier of thrombocytopenia in Gaucher disease, and may have management implications as well such as with newer PAR1 antagonists and/or recombinant aprotinin variants entering clinical development.

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### 3108/F/Poster Board #1040

**Study on dietary effect on plasma lipid changes and feto-placenta outcomes in preeclampsia-like ApoE -/- mouse model.** *M. Sun, R. Ma, Z. Yang, Corresponding author: Zi Yang, zi_yang@email.com.* Dept Ob & Gyn, Peking University Third Hospital, Beijing, China.

Objective: To investigate the effects of dietary factors on the preeclampsia-like development in mouse with lipid metabolism deficiency. Methods:Preeclampsia-like model were established in C57 apoE-/- and wild-type (WT) pregnant mice at early, mid and late gestational stages by injecting nitric oxide synthase (NOS) inhibitor L-arginine methyl ester (L-NAME) subcutaneously. Control groups matched each L-NAME group were received normal saline (NS) simultaneously. Each group was subdivided into standard chow groups (SC) and high-fat diet groups (HF). From day 1 of pregnancy, HF groups were fed a high-fat diet (21% fat, 0.15% cholesterol), and the other group was fed standard chow. All groups were measured blood pressure from day 2 to day 14 and 18 when the fetuses and placentas were removed under anesthesia. Blood pressure and urine protein were measured for identification of mice model, plasma lipid levels were measured, fetal outcomes were compared, liver and placenta histological changes were analyzed among groups. Data were analyzed statistically. Results: In apoE-/- and WT L-NAME groups, blood pressure and urine protein were significantly higher than those in all gestational age matched apoE-/- and WT NS groups ($p<0.05$). In early and mid HF+L-NAME subgroups, plasma concentration of total cholesterol, triglyceride and free fatty acid were all increased, and was the highest in early HF+L-NAME subgroup in apoE-/- mice ($p<0.05$). Morphological examination of placenta showed varying degrees of fibrinoid necrosis and villi interstitial edema in early and mid L-NAME subgroups both in ApoE-/- and WT mice with HF or SC compared to NS controls ($P<0.05$). But there was no significant difference between HF and SC subgroups. Remarkable lipid storage droplets in placenta and liver fatty infiltration were found by Oil Red O staining in early and mid HF+L-NAME subgroups in apoE-/- mice compared to other groups ($P<0.05$). Compared with SC+L-NAME subgroups, fetal weights and placenta weights were significantly decreased in early and mid HF+L-NAME ones ($p<0.05$). Changes in early HF+L-NAME subgroup in apoE-/- mice were striking ($p<0.05$). Conclusion: This study showed that high-fat dietary affected L-NAME preeclampsia-like development on plasma lipid levels and placenta and liver histological changes. High-fat dietary could aggravate the impact of L-NAME preeclampsia-like on pregnant outcomes at early gestational stage especially in ApoE-/- mice.

### 3109/F/Poster Board #1041

**Development of a Mucopolysaccharidosis IVA (MPS IVA; Morquio A Syndrome) Cell Model.** *M.M. Dvorak-Ewell[1], D. Crippen[2], C. Hague[1], M. Vellard[1].* 1) BioMarin Pharmaceutical Inc., Novato, CA; 2) The Buck Institute for Age Research, Novato, CA.

Mucopolysaccharidosis IVA (MPS IVA; Morquio A syndrome) is an autosomal-recessive lysosomal storage disorder caused by deficiency of N-acetylgalactosamine-6-sulfatase (GALNS), an enzyme that degrades keratan sulfate (KS). Clinical manifestations occur primarily in connective tissues rich in proteoglycans, including cartilage, heart valve and cornea, where the endogenously expressed KS accumulates. We produced recombinant human GALNS (rhGALNS) for a potential enzyme replacement therapy, and demonstrated its successful delivery to clinically relevant tissues, including growth plate cartilage and heart valve in the wild-type mice, in vivo. We then established an in vitro model of MPS IVA, using chondrocytes isolated from iliac crest biopsies of two MPS IVA patients (MQCH cells). To induce chondrogenic differentiation and KS production and accumulation, the cells were cultured in alginate beads for 6 - 15 weeks. We confirmed the absence of GALNS activity in MQCH cells by GALNS capture activity ELISA, and observed a resultant 5-10-fold increase in KS accumulation by capillary electrophoresis, in comparison to normal human chondrocytes. Upon treatment with 10nM rhGALNS, we observed lysosomal uptake and restoration of enzyme activity, as well as a decrease in keratan sulfate accumulation in MQCH cells. In an attempt to identify molecular pathways associated with MPS IVA pathophysiology, we used quantitative real-time RT-PCR to profile the chondrogenic gene expression by MQCH cells in comparison to normal human chondrocytes and MQCH cells treated with rhGALNS. We observed aberrant expression of collagens (e.g. 50 - 270-fold increase in collagen I, 10 - 350-fold increase in collagen X) indicating that the cartilaginous extracellular matrix is affected in MPS IVA. Furthermore, expression levels of molecules associated with osteoarthritis and inflammation are also altered (e.g. 22-1700-fold increase in matrix metalloprotease 13, 3-12-fold increase in cathepsin K). rhGALNS treatment attenuated the majority of gene expression changes. As primary human chondrocytes have a limited proliferative capacity in culture, we established immortalized MPS IVA chondrocyte cell lines by stably transfecting MQCH cells with the SV40 viral genome. These cell lines will be used for further characterization of MPS IVA pathophysiology and identification of treatment biomarkers.

### 3110/F/Poster Board #1042

***In vitro* characterization of a NAGLU lysine mutant for potential treatment of Sanfilippo B syndrome.** *S. Kan, L. Troitskaya, S. Dosovitz, K. Haitz, M. Passage, B.L. Tippin.* Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, 1124 West Carson St, Torrance, CA 90502.

Mucopolysaccharidosis IIIB (MPS IIIB, Sanfilippo B syndrome) is an inherited lysosomal storage disease caused by deficiency in the enzyme, α-N-acetyl-glucosaminidase (NAGLU). There is currently no treatment for this disease. Early attempts to produce human recombinant NAGLU (rhNAGLU) enzyme for use as enzyme replacement therapy (ERT) for MPS IIIB failed due to lack of mannose-6-phosphate (M6P) tag addition to the recombinantly produced enzyme. Without the M6P tag, rhNAGLU cannot target and enter the appropiate cells of the body. GlcNAc1 phosphotransferase catalyzes the first step in generating M6P tags during post-translational processing in the Golgi apparatus by recognizing lysine patches on target proteins.

To overcome the lack of MP6 addition, we created a modified rhNAGLU with multiple lysine substitutions (rhLysNAGLU) using site-directed mutagenesis. The sites were chosen by comparative alignment of *NAGLU* genes among different species to maintain conservation of function, while providing a more lysine-rich target for GlcNAc1 phosphotransferase. rhLysNAGLU was expressed in Chinese hamster ovary cells (CHO) using the pCIneo vector (Promega) and the best expressing stable clone was selected for scaled up growth in roller bottles. We purified and characterized the rhLysNAGLU mutant for enzyme activity, stability, pH optimum, cellular uptake, lysosomal targeting, and glycosaminoglycan storage reduction capabilities in Sanfilippo B fibroblasts and compared it to purified rhNAGLU *in vitro*. These pre-clinical studies assess the enhancement and potential therapeutic use of the modified enzyme in ERT for Sanfilippo B syndrome.

### 3111/F/Poster Board #1043

**Enzyme Replacement Therapy in Patients with Hurler Syndrome in Japan.** *M. Kosuga[1], T. Tanaka[2], E. Oda[1], H. Fujita[2], R. Kosaki[2], T. Okuyama[1].* 1) Department of Clinical Laboratory Medicine, National Center for Child Health and Development, Setagaya-ku, Tokyo, Japan; 2) Department of Clinical Genetics and Molecular Medicine, National Center for Child Health and Development, Setagaya-ku, Tokyo, Japan.

BACKGROUND: Mucopolysaccharidosis type I (MPS I, Hurler syndrome) is a lysosomal storage disease caused by a deficiency of lysosomal enzyme, α-L-iduronidase. Recombinant human α-L-iduronidase (laronidase) has been available for patients with MPS I in Japan since 2006. Just about 20 MPS I patients are treated with enzyme replacement therapy (ERT) with laronidase. Because of a small number of treated MPS I patients and the shortness of observing period in ERT, it is important to evaluate the effect and safety of ERT carefully and periodically in MPS I patients. OBJECTIVE: This study aimed to assess the effect and safety of ERT with laronidase in Japanese patients with MPS I. PATIENTS: One male (age 5, Hurler) and two female (age 6 and 11, Hurler and Hurler-Scheie) were followed-up for a mean period of 40 months while undergoing ERT with laronidase. All patients received 0.58mg/kg laronidase i.v. weekly and were examined by means of physical examination, computed tomography of the abdomen, magnetic resonance imaging of the brain, range of motion measurements and measurements of urinary glycosaminoglycans (GAGs) excretion, anti-iduronidase IgG antibodies before ERT and every 6 months after starting ERT. RESULTS: There was clear improvement in the size of liver and reduction in urine GAGs in all patients. The range of motion in all joints was improved in one patient. Two of three patients had a decrease in the number of sleep apnea during ERT. But one patient desaturation index increased gradually and was considered to need continuous positive airway pressure at night and/or adenoidectomy. All patients were positive for antibodies to a-L-iduronidase within 2 months of initiating ERT. However, IgG antibodies were not observed in two of them at 5 months and 2 years post ERT respectively and gradually decreased with time in one patient. Infusion-related reactions consisted of urticaria and wheezing appeared in one patient. The reactions responded to reducing the rate of infusion and antihistamines. The extent of corneal clouding and cardiac function did not change in all patients. CONCLUSIONS: ERT with laronidase was effective significantly in reducing the size of liver and urine GAGs in all patient and ameliorating some clinical symptoms in Japanese patients with MPS type I. There were no critical infusion-related reactions and all patients continue to be treated with laronidase.

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## 3112/F/Poster Board #1044

**UNFOLDED PROTEIN RESPONSE (UPR) IS NOT A MEDIATOR OF APOPTOSIS IN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) FROM FABRY DISEASE PATIENTS.** *P. Rozenfeld, N. De Francesco, C. Fossati.* LISIN, Facultad de Ciencias Exactas, Universidad Nacional de La Plata. Argentina.

The pathophysiology of lysosomal storage diseases (LSD) is not well understood, and it is not solely explained by the burden of storage material. Increased apoptosis has been detected in different LSDs. ER stress/UPR was shown to be a common mediator of apoptosis both in neurodegenerative and non-neurodegenerative LSDs. However, a recent work in neuronopathic Gaucher disease could not demonstrate the involvement of UPR. The aim of this work is to analyze if ER stress/UPR is activated and related to apoptosis in PBMC from Fabry disease patients. We analyzed levels of caspases related to ER stress by western-blotting and expression of genes associated to UPR by quantitative PCR in PBMC from Fabry disease patients. We neither found differences in the levels of ER stress-related caspases, caspase 4 and caspase 12 nor changes in expression of genes CHOP, GADD34, BiP, XBP1, EDEM1, HSP90B1. We conclude that ER stress/UPR is not activated in PBMC from Fabry disease patients, and is not a mediator of apoptosis.

## 3113/F/Poster Board #1045

**Life course analysis of the effects of FTO on BMI in the Northern Finland Birth Cohort 1966 using structural equation modelling.** *M. Jarvelin[1], M. Kaakinen[2], U. Sovio[1], A. Pouta[3], A-L. Hartikainen[2], A. Taanila[2], A. Bennett[4], M.I. McCarthy[4], E. Laara[2], ENGAGE project, HEALTH-F4-2007-201413.* 1) Epidemiology & Public Health, Imperial Collage London, London, United Kingdom; 2) University of Oulu, Biocenter Oulu, Finland; 3) National Institute for Health and Welfare, Oulu, Finland; 4) University of Oxford, UK.

Many studies have shown an association between FTO, the fat-mass and obesity associated gene, and adulthood body-mass index (BMI). More thorough analyses utilising phenotypic data from several time points during the life course may deepen our understanding of the interplay between genetic and environmental factors associated with BMI. We used structural equation modelling (SEM) technique to explore the network of variables associated with BMI from prenatal period until the age of 31 years in 4435 subjects from the Northern Finland Birth Cohort 1966. The association between the FTO rs9939609 risk allele and BMI at 31 years remained robust despite controlling for several relevant factors during the life course (total effect 1.41% difference in BMI levels per allele change corresponding to 0.35 kg/m2 in the mean level, $p<0.0001$). In addition to the association with adult BMI, evidence for associations in the same direction with maternal BMI (0.60%, $p=0.02$), birth BMI (0.30%, $p=0.12$) and BMI at 14 years (0.60%, $p=0.04$) were observed. The total effect of FTO, which takes into account direct and indirect effects through other factors was notably higher than pure direct effect of FTO on adult BMI. However, all the indirect effects came through earlier measurements of BMI and not through environmental exposures, such as diet, physical activity, drinking or smoking. This confirms the previous findings that BMI begins affecting well before adulthood and also suggests that FTO has a pure causal effect on BMI. SEM proved to be an efficient analysis method in estimating complex relationships jointly. The analyses with missing values were easy to incorporate via EM algorithm, allowing us to use the whole potential of the data. SEM could be more utilised in the joint analyses of genetic and environmental exposures.

## 3114/F/Poster Board #1046

**Genome-Wide Linkage Scan for Metabolic Syndrome Related Quantitative Traits in Dominican Families.** *C. Dong[1], A. Beecham[2], S. Blanton[2], S. Slifer[2], T. Rundek[1], R. Sacco[1].* 1) Department of Neurology, University of Miami Miller School of Medicine, Miami, FL; 2) Miami Institute of Human Genetics, University of Miami Miller School of Medicine, Miami, FL.

The metabolic syndrome (MetS) poses a major problem for public health and is characterized by the clustering of multiple correlated metabolic abnormalities. Studies have suggested that the components of MetS may share genetic and environmental determinants. To identify genetic loci for MetS in Hispanics, we performed autosomal genome linkage analyses of 1390 subjects from 100 Dominican families on multiple MetS-related traits: body mass index (BMI), weight, waist circumference (WC), waist-to-hip ratio (WHR), abdominal skinfold, average triceps skinfold thickness, diastolic and systolic blood pressure (DBP, SBP), triglyceride (TG), cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and fasting glucose. A composite factor for MetS was also derived from factor analysis with a high factor loading >0.4 for all traits except HDL. Multipoint variance component analyses were conducted for all traits by adjusting for demographic and lifestyle factors and medication history if applicable. Two regions were identified on chromosome 16 with the most significant or overlapping linkage signals: one is at 16q23 with the highest LOD score of 3.62 ($p<0.00005$) for cholesterol as well as suggestive linkage signals for LDL (LOD=2.65) and average triceps skinfold (LOD=2.32); another is at 16p12 with suggestive linkage signals for cholesterol (1.81), weight (2.26), BMI (2.27), TG (1.84) and MetS composite factor (2.55). Several regions were also found to have overlapping linkage signals of LOD score ≥1.5: chromosome 1 at 9-15 cM for WHR (1.80) and average triceps skinfold (1.88) as well as at 263-268 cM for BMI (2.03) and weight (2.45); chromosome 4 at 32-38 cM for abdominal skinfold (1.94) and fasting glucose (2.43); chromosome 9 at 112-164 cM for abdominal skinfold (1.85), HDL (2.20) and LDL (1.63); chromosome 12 at 125-145 cM for HDL (1.93) and fasting glucose (1.56); chromosome 14 at 84-113 cM for abdominal skinfold (2.17), TG (1.82) and LDL(1.82); chromosome 15 at 77-115 cM for TG (2.60) and WC (1.77); and chromosome 19 at 52 cM for LDL(2.67) and cholesterol (2.15). In addition, other regions with linkage signal of LOD score ≥2.0 for single trait were observed on chromosome 3 (2.33 for SBP at 221 cM), 5 (2.54 for fasting glucose at 114 cM) and 7 (2.56 for fasting glucose at 58 cM). Our results suggest that multiple loci, particularly 16p12 region, may contribute to the development of MetS and the related quantitative traits in Caribbean Hispanics.

## 3115/F/Poster Board #1047

**Desmosterolosis - clinical presentation, neuroradiologic findings, molecular evolution and novel therapeutic considerations.** *C.P. Schaaf[1], P. Katsonis[1], L. Kratz[2], J. Koster[3], R.I. Kelley[2], H.R. Waterham[3], O. Lichtarge[1], M. Shinawi[1].* 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Genetics Laboratories, Kennedy Krieger Institute, Baltimore, MD; 3) Clinical Chemistry & Paediatrics, University of Amsterdam, Amsterdam, The Netherlands.

A sufficient supply of cholesterol is essential for proper development of the human brain. Neuronal differentiation, in particular synaptogenesis, occurs as a cholesterol-dependent process. Cholesterol is a precursor in the synthesis of steroids and other sterols, and it influences the Sonic Hedgehog pathway, an important signaling cascade for brain growth, patterning, and morphogenesis. Several disorders of cholesterol biosynthesis have been associated with structural brain abnormalities.

Desmosterolosis, a rare disorder of cholesterol biosynthesis, is caused by mutations in the enzyme 3β-hydroxysterol-Δ24reductase (DHCR24), a FAD-dependent oxidoreductase. To date, desmosterolosis has been described in only two patients. Here we report a third patient with desmosterolosis who presented with congenital hydrocephalus, mild arthrogryposis, and dysmorphic facial features. Brain MRI confirmed congenital hydrocephalus and revealed thickening of the tectum and the massa indermedia, dysplastic cerebrum with pachygyria, marked underopercularization, and a thin corpus callosum.

The diagnosis of desmosterolosis was established by detection of a significant elevation of plasma desmosterol of 284 μg/ml [nl: 0.82+/- 0.46 μg/mL], and enzyme activity studies in lymphoblasts confirmed the deficiency of DHCR24. The patient was found to be compound heterozygous for a c.281G>A (R94H) and a c.1438G>A (E480K) mutation. We performed structural and phylogenetic analyses of the respective mutations by mapping the DHCR24 sequence on the structure of 2exr (cytokinin oxidase/dehydrogenase from arabidopsis thaliana) and determined that the R94H mutation affects the FAD-binding domain, which is evolutionarily important to the enzyme, as its variations correlate with phylogenetic branching. The E480K mutation occurs in nature, but is accompanied with charge changes in the local protein environment.

We compare the presentation in our patient with the clinical findings of the previously reported cases of desmosterolosis, and propose a list of clinical findings that should instigate a diagnostic evaluation for desmosterolosis. Based on biochemical considerations and previous in vitro studies of mutant DHCR24 in yeast, an experimental therapeutic approach for patients with desmosterolosis is suggested.

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## 3116/F/Poster Board #1048

**A *YARS2* mutation is a novel cause of mitochondrial myopathy characterized by lactic acidosis and sideroblastic anemia.** *L. Riley[1], P.F. Hickey[2,3], S. Cooper[4,5], M. McKenzie[6], M. Ryan[6], A. Compton[7], D. Thorburn[7], J. Rudinger-Thirion[8], E. de Leon[1], M. Bahlo[2], J. Christodoulou[1,5].* 1) Genetic Metabolic Disorders Research Unit, Children's Hospital at Westmead, Sydney, Australia; 2) Bioinformatics Division, The Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia; 3) Department of Mathematics & Statistics, University of Melbourne, Melbourne, Australia; 4) Neurogenetics Research Unit, Children's Hospital at Westmead, Sydney, Australia; 5) Discipline of Paediatrics & Child Health, University of Sydney, Sydney, Australia; 6) Department of Biochemistry, LaTrobe University, Melbourne, Australia; 7) Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, Australia; 8) Unité Propre de Recherche du CNRS, Institut de Biologie Moléculaire et Cellulaire.

Mitochondrial respiratory chain disorders are a heterogeneous group of disorders due to mutations in either mitochondrial or nuclear encoded genes. Identification of causative mutations is important not only for diagnosis but also to understand the pathogenesis of these disorders. We have studied a family where consanguineous parents have had two affected children with persistent lactic acidemia, progressive skeletal myopathy, transfusion-dependent sideroblastic anaemia and deficiencies in complex I & IV activity (MLASA). We undertook multipoint autozygosity mapping of this family using the 250K *Nsp*I Affymetrix Human GeneChip Array to localise the mutated gene. Six regions of interest with a peak LOD score of 2.0 or higher were identified. These candidate regions were subjected to *in silico* analysis using the MitoCarta database which revealed 28 genes encoding proteins with mitochondrial functions. DNA sequencing revealed a missense mutation in one of the candidate genes, *YARS2*, which encodes mitochondrial tyrosyl-tRNA synthetase (mt-TyrRS). Another unrelated patient from the same ethnic group with MLASA was also found to be homozygous for this mutation. This sequence variation was not found in 160 chromosomes from control subjects. The missense mutation leads to a substitution of leucine at residue 52 for phenylalanine (p.F52L). Phe52 lies within the first β-strand of mitochondrial tyrosyl-tRNA synthetase and is highly conserved between species. mt-TyrRS is responsible for attaching Tyr to its cognate tRNA for incorporation into mitochondrial proteins, however the function of the region harbouring the mutation is unknown. Studies were undertaken to determine the expression level, stability and functionality of the mutant protein and its effect on mitochondrial protein synthesis. Preliminary Western blotting results indicate that mutant mt-TyrRS protein is expressed in patient samples, although levels of mt-TyrRS appeared to be lower compared to controls. A mitochondrial protein synthesis assay revealed defects in translation of some mitochondrial proteins. A recombinant form of the mutant protein has been produced to determine the catalytic rate of the enzyme. These functional studies should lead to a greater understanding of the role of the *YARS2* mutation in the pathogenesis of this mitochondrial respiratory chain disorder.

## 3117/F/Poster Board #1049

**Novel Mutations in CYP11B1 Gene and its protein modeling in a Patient with 11β-Hydroxylase Deficiency in Iran.** *M. Abbaszadegan[1], S. Hassani[1], R. Vakili[2], M. Saberi[3], A. Baraderan-Heravi[1], A. A'rabi[1], M. Hasemipour[4], M. Razzaghi-Azar[5], A. Baratian[5].* 1) Division of Human Genetics, Immunology Research Center, Avicenna Research Institute, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran; 2) Departmant of Pediatrics, Imam Reza Hospital, MUMS, Mashhad, Iran; 3) Medicinal Chemistry Department, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, IRAN; 4) Esfahan Endocrine & Metabolic Research Center, Esfahan University of Medical Sciences, Esfahan, Iran; 5) Departmant of Pediatrics, H. Ali Asghar Hospital, Iran University of Medical Science (IUMS), Tehran, Iran.

Background: Mutations in the gene encoding 11β-Hydroxylase (CYP11B1) is the second most common cause of congenital adrenal hyperplasia (CAH), an autosomal recessive disorder characterized by adrenal insufficiency, virilization of female external genitalia, and hypertension with or without hypokalemic alkalosis in about two thirds of patients. Objective: Molecular analysis of CYP11B1 gene in CAH patients with 11β-hydroxylase deficiency. Patients and Methods: DNA analysis was performed in 5 unrelated Iranian families with at least one 11-β hydroxylase deficient child who was presented with precocious pseudopuberty in all male patients. All the patients were detected to have hypertension at the time of diagnosis or later which was controlled by Glucocorticoid treatment except one of them with uncontrolled hypertension and recurrent CVA attacks in spite of both Glucocorticoid and anti-hypertensive regimen and an unsuccessful adrenalectomy procedure. Cycle sequencing of 9 exons in CYP11B1 was performed. To inspect the effects of new mutation on protein structure and function, Three D models for the normal and mutant proteins was developed and their affinity to a previously found effective compound was examined. Models were investigated in SPDBViewer and ViewerLite programs checking amino acids making clashes, Phi-Psi angles, and secondary structure matching the secondary structure prediction. Results: Analysis of the CYP11B1 gene revealed two novel mutations, one as a small insertion in exon 7 (InsAG393) and the other as a single nucleotide deletion in exon 2 (Del G766). In addition to these novel mutations, 3 known mutations in 3 other patients were detected including a T318M, Q356X and R427H in homozygous state. The parents of the patients are consanguineous and heterozygous for the same mutations. Prediction of clinical severity, based on molecular modelling and sequence conservation, correlates well with the clinical diagnosis of the patients carrying these mutations. Conclusion: The novel mutations, Ins AG 393 and Del G766, have not been previously described in the CYP11B1 gene. These mutations predicted to drastically impair enzyme activity resulting in severe classic CAH. The study increases our knowledge of CAH due to 11β-OH deficiency and its various presentations. Prenatal diagnosis for these families is possible utilizing these molecular results.

## 3118/F/Poster Board #1050

**Orthopedic management in the mucopolysaccharidoses.** *K.K. White[1], R. Lachman[2], P. Harmatz[3], S. Turbeville[4].* 1) Children's Hospital and Regional Medical Center, Department of Orthopedic Surgery Seattle, WA, University of Washington Department of Orthopedics and Sports Medicine Seattle, WA; 2) Department of Pediatrics, International Skeletal Dysplasia Registry, UCLA School of Medicine, Radiological Services; 3) Oakland Children's Hospital and Research Center; 4) BioMarin Pharmaceutical, Inc.

The mucopolysaccharidoses (MPS) constitute a family of lysosomal storage diseases resulting from inborn errors of metabolism with subsequent abnormal accumulation of cellular glycosaminoglycans. The musculoskeletal manifestations in MPS range from mild platyspondyly and/or epiphyseal dysplasia to severe life threatening spinal problems and crippling hip deformities. Both joint stiffness and ligamentous laxity are associated with MPS disorders, compounding the problems associated with the skeletal deformities. Dysostosis multiplex, the radiographic constellation of skeletal abnormalities classically seen in MPS, results from defective endochondral growth throughout the skeletal system, including the hips, knees and spine. The molecular physiology of this problem is yet to be understood. Familiarity with these musculoskeletal findings is critical for recognition and diagnosis of these potentially devastating diseases, as well as for determining appropriate treatment options such as surgical procedures of the spine, hips, knees and hands to promote optimal function and gait. Enzyme replacement therapy (ERT) may be beneficial in reducing the burden of skeletal disease when treatment is initiated early. It is has also been shown to improve joint range of motion. While hematopoietic stem cell transplant (HSCT) may be potentially life saving for some MPS diseases, it does not usually alter the course of progressive skeletal disease, although some normalization of articular cartilage, joint range of motion and linear growth may occur. Surgical intervention is often still required in patients with MPS, and early intervention is critical in order to optimize long term function and quality of life. We review the current clinical knowledge-base of musculoskeletal disease in MPS including the impact of newer orthopedic treatment options.

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### 3119/F/Poster Board #1051

**'Missing' enzymes and metabolic pathways: Redefining metabolic pathways with special reference to *Pseudomonas* species.** *D. Perumal[1,2], C.S Lim[1,2], M.K Sakharkar[1,2]*. 1) Advanced Design and Modeling Lab (ADaM),School of MAE, Nanyang Technological University, Singapore; 2) Bio Medical Engineering Research Center(BMERC), Research Techno Plaza,Nanyang Technological University, Singapore.

Comparative metabolic pathway analyses reveal that several pathways in the *Pseudomonas* species show high plasticity and versatility. Computational predictions from multiple sources of information, though useful, may sometimes be inconsistent and further confound the issue of annotation of genes. In such cases, experimental validations may become essential to resolve and reconcile the apparent inconsistencies. We present combined computational experimental analyses on eight completely sequenced *Pseudomonas* species. Potential bypasses in 11 metabolic pathways were identified. Our results for the first time confirm the presence of DNA sequence encoding the enzyme O-acetyl homoserine (thiol) lyase (EC: 2.5.1.49) in *Pseudomonas syringae pv. tomato* which reveals inconsistent annotations in KEGG and the recently published SYSTOMONAS database. These analyses connect and integrate systematic data generation, computational data interpretation, and experimental validation and represent a synergistic and powerful means for conducting biological research. Continued experimental investigation of the metabolic biochemistry of an organism is of importance in (1) to assign pertinent biochemical reactions to the enzymes found in the genome; (2) to validate and scrutinize information already found in the genome; and (3) determination of the presence of reactions or pathways not indicated by current genomic data. Through the comparison of metabolic pathways, intricate and complex relationships between genes that encode enzymes can be studied, and more sophisticated strategies for diagnosis and treatment of complex diseases may become feasible.

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## 3120/F/Poster Board #1052

**Murine bone marrow eGFP- mesenchymal stem cells (bMSC): myogenic potential in dystrophic muscle.** *M. Vainzof, D. Ayub-Guerrieri, P.C.G. Onofre-Oliveira, V.F. Lopes, P.C.M. Martins.* Genetics/Hum Genome Res Ctr, IB-Univ Sao Paulo, Sao Paulo, Brazil.

Muscular dystrophies (MD) are heterogeneous genetic muscle disorders, still devoid of efficient treatment, and the mouse models for these diseases are an important tool for testing putative therapies. With a view to developing treatments for muscular dystrophy, we are testing the myogenic potential of murine bone marrow mesenchymal stem cells (bMSC) following transplantation into dystrophic mice. The bMSC were isolated from transgenic mice carrying a gene expressing enhanced green fluorescent protein (bMSC-eGFP), which constitutes a good marker for tracking them in the injected organism. In our previous studies, we observed that after 30 days post-injection of these cells, treated muscles from mdx mice showed no sign of muscle regeneration, nor any evidence of dystrophin expression. In contrast, non-GFP murine embryonic stem cells were retained. Additionally, human MSCs from adipose tissue were also retained after injection into SJL mouse muscles. To verify if eGFP expressing cells are less tolerated by the dystrophic muscle, a single dose of about $10^6$ bMSC-eGFP cells was injected intra-muscularly into mdx and normal C57Bl animals, and tracked through PCR using primers for the DNA sequence related to the eGFP gene. We detected the presence of eGFP up to 2 days post-injection, in both affected and normal animals. After this time, no traces of the eGFP DNA from transplanted cells were detected, suggesting that the cells had been effectively eliminated from the muscles. These results suggest that, in addition to a possibly adverse environment offered by the dystrophic muscle for the homing and maintenance of injected bMSC stem cells, cells expressing eGFP are poorly tolerated, and rapidly eliminated. Comparative studies of the potential of different stem cells injected into different animal models are important to improve the effectiveness of stem cells in neuromuscular therapies. Funding: FAPESP,CNPq-INCT,FINEP, ABDIM.

## 3121/F/Poster Board #1053

**Low plasma iduronate-2-sulfatase activity and elevated urinary glycosaminoglycans in brothers following stem cell transplantation (HSCT) for mucopolysaccharidosis type II.** *A. Paras[1,2], B.K. Burton[1,2].* 1) Dept Genetics, Children's Memorial Hospital, Chicago, IL; 2) Northwestern University Feinberg School of Medicine, Chicago IL.

The use of HSCT for the treatment of mucopolysaccharidosis type II has been controversial. Its efficacy has been difficult to gauge given the variability of the condition and paucity of data, and HSCT has not been routinely recommended. HSCT has resulted in improvement or stabilization of some aspects of the disease in selected patients, including resolution of hepatosplenomegaly, stabilization of cardiovascular disease, improvement of rhinorrhea and upper airway obstruction, and improvement in joint stiffness. Cognitive outcomes in published cases have been variable, with very limited data on patients transplanted at an early age (<1 year) who would be predicted to have severe CNS involvement based on family history. Intravenous enzyme replacement therapy with Elaprase improves many somatic symptoms including hepatosplenomegaly, joint stiffness, and pulmonary function. ERT is not expected to ameliorate the course of neurocognitive decline. A clear dilemma arises with regard to determining the best treatment strategy. Without clear documented benefits of HSCT for CNS disease, treatment with enzyme replacement therapy appears to provide comparable somatic benefits without the risk of morbidity and mortality associated with HSCT. However, if the diagnosis of MPS II is made in a very young child, the hope remains that HSCT may provide some protection from cognitive decline, as well documented in MPS I (Hurler syndrome). We present two brothers with MPS II who underwent successful unrelated umbilical cord blood stem cell transplantation at ages 11 months and 3y 11m. Assessments of plasma iduronate-2-sulfatase (I2S) have been low in both siblings (approximately 5% and 7% of control) and urinary glycosaminoglycans levels continue to be elevated 9 months post transplant, although a significant decline from baseline was documented in one of the brothers. The biochemical findings are difficult to interpret, and their clinical significance is unclear. The enzymatic findings suggest that plasma I2S is derived from a tissue not fully corrected by HSCT.

## 3122/F/Poster Board #1054

**The difference in effectiveness of herbal medicine between genetic and non-genetic spinocerebellar ataxia.** *T. Okabe.* Dept Integrated Trad Med, Univ Tokoy, Tokyo, Japan.

The spinocerebellar ataxias (SCAs) are clinically and genetically a heterogeneous group of neurodegenerative disorders. At present,we have no effective therapeutic tools. Previous studies have shown for the first time, the successful treatment by medicinal herbs of 2 Japanese patients with genetic spinocerebellar ataxia 6 (SCA6). Recently, we have succeeded in remission induction by the herbal medicine of an another case with CSA6. Ataxia of gait and stance disappeared and the total ataxia score was improved from 26 to 12 on a 100-point semiquantitative International Cooperative Ataxia Rating Scales(ICARS). The results suggest that the herbal therapy would be effective for this genetic spinocerebellar ataxia. In this study, we have examined the effect of the herbal therapy against sporadic spinocerebellar ataxia. Multiple system atrophy (MSA) is a sporadic progressive neurodegenerative disorder characterized by prominent cerebellar and extrapyramidal signs, dysarthria, and dysphagia and symptoms of autonomic nervous system failure (such as urinary incontinence or incomplete bladder emptying, orthostatic hypotension, constipation, erectile failure in men. Three Japanese patients with MSA were treated with the medicinal herbs, to see whether the herbal therapy would be effective against the sporadic spinocerebellar ataxia. Among these symptoms, cerebellar ataxias such as light-headedeness, ataxic gait and swallowing difficulty were slightly improved after the herbal therapy. However, autonomic nervous system failures were not ameliorated by the therapy. The formula used for these cases consisted of different types of herbs such as sedative, anticonvulsive, neuroprotective, neurorefgulatory and DNA binding herbs. Together with the successful treatment of SCA6, the results imply therapeutic potential of the herbal medicine especially for genetic cerebellar ataxia. Extensive studies are required for elucidation of the mechanisms by which medicinal herbs exert their therapeutic activities on this genetic disease of CAG repeat expansion mutation,.

## 3123/F/Poster Board #1055

**A KFC-based approach for rational drug design to treat genetic diseases.** *M. Tang[1], K. Wierenga[2], L. Elsas[1], K. Lai[1,3].* 1) Biochemistry and Mol. Biology, University of Miami Miller School of Medicine, Miami, FL; 2) Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 3) Pediatrics, University of Utah School of Medicine, Salt Lake City, UT.

Traditional drug discoveries rely on experimental, knowledge-based high-throughput screens (HTS) to identify compounds that interact with chosen targets. Advances in high-precision computer modeling software not only allow the accurate docking of the selected compounds from the HTS screens to the targets, but also led to development of virtual fragment-based screens. We propose here that we can combine the results of both the **K**nowledge-based and **F**ragment-based screens with existing **C**rystallographic data of the target to identify more effective compounds to treat genetic disorders. To test our hypothesis, we focused on the potentially lethal genetic disorder called Classic Galactosemia (MIM 230400). Despite improvements in care, mental retardation, premature ovarian failure and other neurological deficits remain common sequelae of this disease. Various lines of evidence indicated that elevated level of galactose-1-phosphate (gal-1P), the product of galactokinase (GALK), is a major, if not sole, pathogenic mechanism in Classic Galactosemia. We hypothesize that elimination of gal-1P production in GALT-deficient cells by inhibiting GALK will significantly relieve them from galactose toxicity. To test this hypothesis, we have screened different chemical libraries composed of over 300,000 small molecules with diverse structural scaffolds for inhibitory properties against activity of purified human GALK. We have identified more than 200 small molecule compounds that inhibit human GALK activity in vitro of at least 86.5 percent. Characterization of selected compounds revealed good potency and considerable efficacy to lower gal-1P in patient cells in culture. However, significant toxicity due to off-target effects was also seen. To improve the potency and selectivity of the compounds, we performed structural studies of GALK-inhibitors using high-precision computer docking programs to establish enzyme-inhibitor interactions. We found that many inhibitors interact with amino acid residues known to be implicated in causing GALK deficiency in humans, and identified new "hotspots" of the GALK protein crucial for catalysis. Additionally, we conducted virtual fragment-based screenings to identify new chemical structures that can bind to the active site of GALK. Using the proposed KFC-based approach, we have generated novel hybrid molecules aimed to surpass the potency and selectivity of either parent compounds identified by the separate screens.

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## 3124/F/Poster Board #1056

**Optic Neuritis and Accommodative Loss after Intravenous Pamidronate in a Child with COL1A1 Variant Osteogenesis Imperfecta and Juvenile Osteoporosis.** *S.C. Benes[1,4], J. Mahan[2], M.E. Nunes[3].* 1) Neuro-Ophthalmology, Nationwide Children's Hospital, Columbus, OH; 2) Nephrology, Nationwide Children's Hospital, Columbus, OH; 3) Genetics, Kaiser Permanente, San Diego, CA; 4) Neuro-Ophthalmology, The Eye Center of Columbus, Columbus, OH.

Case Report: A healthy 11 year old girl (60 inches, 97 pounds) had multiple fractures with sports. She was flexible at several joints and her bone density was consistent with osteoporosis. She underwent 4 intravenous disodium pamidronate infusions every 3 months with the expected headache and flu-like feelings for the 2 days following each of those 3-day infusions, treated with Motrin and rest. A repeat bone density was in the lower limit of normal for age. Following the fifth IV pamidronate infusion, at age 12, her headaches and generalized malaise were more intense than with the prior 4 infusions; on day 3 she had blurred vision in both eyes, pain on eye movement OD, loss of color vision OD, and reported a grey area in her central vision OD. The clinical diagnosis of right retrobulbar optic neuritis was made. She had reduced accommodative amplitude OU. Visual fields, OCT, B-scan ultrasound, visually evoked potentials and MRI testing showed no central nervous system pathology. We suspected this was a toxicity of pamidronate. Blood work was normal except for a low B2 and a single allele mutation COL 1A1 on chromosome 17, exon 8. Her asymptomatic mother shared this mutation. A literature review found only 3 cases of optic neuritis after bisphosphonate therapy in older adult women with osteoporosis. Most common visual complications with bisphosphonates were nonspecific blurred vision, conjunctivitis, episcleritis, scleritis, orbital inflammation, and intraocular inflammation (uveitis). Concerns include: (1) Will her vision fully recover? (2) When bone density normalizes, should the therapy continue? At what dose? Should the children return to sports? (3) Since bisphosphonates as a class have the most consistent rechallenge data on ocular inflammation, is there a safe alternative drug or reduced dose for her in future therapy? (4) Will orthodontia be complicated by excess immobility or mobility, or even osteonecrosis? (5) Are we missing optic neuropathies/ocular inflammations in juvenile pamidronate users due to infrequent questions about vision? (6) What end-points do we "treat to" in patients with juvenile osteoporosis and repeated fractures? (7) Regarding the variant of the COL1A1 allele substitution 613C>G with Pro27 Ala in the triple helix, is there a unique cross-over finding of osteogenesis imperfecta with collagen connective tissue defects (variants of Ehlers-Danlos) and joint hypermobility or a unique sensitivity to bisphosphonates?

## 3125/F/Poster Board #1057

**Enzyme Replacement Therapy for Hypophosphatasia Mouse.** *S. Tomatsu, H. Oikawa, A.M. Montano.* Dept Pediatrics, St Louis Univ Doisy Res Ctr, St Louis, MO.

Background: Hypophosphatasia (HPP) is an inborn error of metabolism caused by deficiency of the tissue-nonspecific alkaline phosphatase (TNSALP), resulting in a defect of bone mineralization. Natural substrates for this ectoenzyme accumulate extracellulary including inorganic pyrophosphate (PPi), an inhibitor of mineralization, and pyridoxal 5-phosphate (PLP), a co-factor form of vitamin B6. Enzyme replacement therapy (ERT) on HPP patients with partially purified plasma enzyme was attempted but with little clinical improvement. TNALP-null mouse model (Akp2-/-) has an infantile form of HPP with a severe hypomineralization and bone deformities. The affected mice die of vitamin B6-dependent seizures within 21 days without vitamin B6 treatment. Attaining clinical effectiveness with ERT for HPP requires delivering functional TNSALP enzyme to bone. There is no established medical treatment approved. Materials and Methods: The C-terminal-anchorless human recombinant TNSALP (hrTNSALP) enzyme derived from Chinese hamster ovary cell lines was purified. TNALP-null mice (Akp2-/-), an excellent model for infantile HPP, were treated from birth using hrTNALP. Long-term efficacy studies (six months) by ERT consisted of every 3 days subcutaneous injections till 28 days old (dose 10 U/g) and subsequently every 3 days intravenous injections for 5 months (dose 20 U/g). We assessed survival and growth rates as well as skeletal manifestations using radiography. Mice were fed with vitamin B6 containing diet. Results: Akp2-/- mice treated grew normally till 2 months and appeared well with a minimum skeletal disease and normal growth as well as absence of epilepsy compared to untreated mice which died by 3 weeks old. Moreover, both male and female treated mice of both genders were fertile. Even if both parents were affected, they provided pups without any complications. Although the treated mice kept active without seizure, after six months of treatment, we noticed moderate skeletal or dental disease with hypomineralization. Conclusions: 1. Enzyme replacement using recombinant form of human TNALP prevents infantile HPP in Akp2-/- mice and improves substantial clinical manifestations with prolonged survival period. 2. Long term efficacy should be carefully reviewed on treated mice. 3. In spite of the fact that treatment started from birth, it may be still challenge to have a complete remission in bone lesions. ERT will be a potential therapy for HPP.

## 3126/F/Poster Board #1058

**Pharmacological intervention for Rett Syndrome.** *J.C. Roux[1,2], E. Dura[1,2], N. Panayotis[1,2], A. Borges-Correia[1,2], A. Spiga[1,2], J. Mancini[3], L. Villard[1,2].* 1) INSERM, U910, Marseille, France; 2) Aix-Marseille University, La Timone Medical School, Marseille, France; 3) Marseille University Hospital, La Timone Children's Hospital, Marseille, France.

Rett syndrome is a severe neurological disorder caused by mutations in the MECP2 gene. Recent breakthrough experiments demonstrated that the reactivation of the Mecp2 gene in the mouse model of Rett syndrome could reverse the symptoms of the disease, thereby providing an exceptional pre-clinical proof of concept to support gene therapy approaches for Rett syndrome. Unfortunately, these approaches are currently out of reach for most genetic diseases and they will be even more complicated to provide to Rett syndrome patients because 1- the brain will be the organ to treat and 2- young children will have to be treated. While several groups have focused their efforts onto the development of these promising approaches, others are trying to develop pharmacological interventions to alleviate part of the symptoms. Using a Mecp2-deficient mouse, we have previously shown that breathing disturbances was associated to noradrenergic deficits at the brainstem level and we demonstrated a significant decrease in the number of noradrenergic neurons in two nuclei involved in the cardio-respiratory control. We subsequently showed that catecholaminergic neurons located in the peripheral nervous system are also affected by the lack of Mecp2 protein. We now have extended our analysis to the locus coeruleus, the main noradrenergic cluster in the brain, where we identified additional noradrenergic deficits, namely a decrease of tyrosine hydroxylase mRNA and protein and a decrease in the number of tyrosine hydroxylase expressing neurones. These catecholaminergic deficits could collectively explain the autonomic dysfunctions observed in the disease and open new perspectives to better understand the consequences of Mecp2 dysfunction. They also strenghten our approach based on pharmacological intervention to stimulate biogenic amine metabolism. We initially validated a pharmacological treatment in the mouse using desipramine, a noradrenergic reuptake inhibitor, and have initiated a phase II clinical trial. We are now testing additional candidate drugs to alleviate the autonomic and motor symptoms of Rett Syndrome using the Mecp2-deficient mouse as a pre-clinical model.

## 3127/F/Poster Board #1059

**Effect of laronidase therapy on myocardial function in a patient with MPS I assessed by two-dimensional ultrasound speckle tracking imaging.** *H. Harada, M. Nakamura, S. Ohno, H. Morita, A. Katoh, H. Ikeda.* Department of Cardiology, Kurume University Medical Center, Kurume, Fukuoka pref., Japan.

Purpose: We assessed whether laronidase, which is recombinant human $\alpha$-L-iduronidase, replacement therapy (LRT) is effective on left ventricular myocardial function in a patient with Sheie phenotype of MPS I. Methods and subject: We assessed left ventricular myocardial function using two-dimensional ultrasound speckle tracking imaging method in a 49-year-old woman with Sheie syndrome. Results: At 6 months after Laronidase ERT, the concentrations of urinary uronic acid gradually decreased (reduction ratio, 78.8%). Simultaneously, hepatosplenomegaly reduced and left ventricular weight decreased from 189 to 152g. These findings suggested that ERT was effective on MPS I. Left ventricular ejection fraction assessed by two-dimensional echocardiogram did not change after ERT. Next, we assessed myocardial function focused on longitudinal and radial strains and torsion obtained from two-dimensional speckle tracking imaging. In the myocardial longitudinal strain (shortening ratio), the mean shortening ratio changed from -13.2 to -17.4%. In the myocardial radial strain (thickening ratio), the mean thickening ratio changed from 26.6 to 83.4%. In the myocardial torsion, left ventricular torsion changed from +6 to +18 degrees. Summary: These indices of myocardial function were improved within normal limits after ERT. Thus, our findings were a first report that ERT had a beneficial effect on left ventricular myocardial function in a patient with MPS I.

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## 3128/F/Poster Board #1060

**Home Therapy with Idursulfase for Hunter Syndrome: The Hunter Outcome Survey Experience.** *B.K. Burton[1], S.A. Jones[2].* 1) Dept Gen,Birth Def, Metab, Children's Memorial Hosp, Chicago, IL; 2) Specialist Registrar in Paediatric Metabolic Medicine, Willink Biochemical Genetics Unit, Royal Manchester Children's Hospital, Pendlebury, Manchester, United Kingdom.

Hunter syndrome is an X-linked metabolic disease caused by a deficiency in the lysosomal enzyme iduronate-2-sulfatase (I2S). Enzyme replacement therapy (ERT) with recombinant human I2S (Elaprase®, idursulfase, Shire HGT) for Hunter syndrome has been available since 2006. Idursulfase is administered weekly by an intravenous infusion that can last 3 to 4 hours. Because of the prolonged infusion time, patients are often forced to miss school or work on the day that they receive their dose. Home infusion offers an alternative, more convenient setting for these weekly infusions. Here we present observations about home infusion from the Hunter Outcome Survey (HOS). HOS is a global, multicenter, long-term observation survey designed to acquire data from Hunter syndrome patients in their normal medical care environment. As of April 16, 2009, 541 prospective patients (ie, alive at HOS entry) from 98 clinics in 23 countries have enrolled in HOS. Of the 367 patients in HOS receiving ERT, 82 (22%) were being treated at home, with most of these patients residing in the United States (n=33 from 13 clinics) or the United Kingdom (n=35 from 3 clinics), with 4 patients each in the Netherlands and Germany, 2 patients each in France and Sweden, and 1 patient each in Canada and Denmark. Their median age at the initiation of ERT was 8.6 years with a median age at transitioning to home therapy of 9.7 years. The median duration of ERT before transitioning to home therapy was 10.9 months (10th-90th percentile: 3.0-38.3 months). In the 65 patients who had not participated in clinical trials of idursulfase, the median age at initiation of ERT was 7.9 years and the median interval before beginning home therapy was 7.8 months (10th-90th percentile: 2.8-17.4 months). Three patients have experienced a total of 7 infusion-related reactions (IRR) after the onset of home therapy. These IRRs were mild-to-moderate in severity and involved fever and rash. In all cases these reactions were successfully managed by slowing or stopping the infusion and/or administration of an antihistamine. Overall, 2 of 28 patients (7%) with at least 12 months of home therapy have reported an IRR. Conclusions: Data from HOS indicate home therapy is feasible and is not associated with a higher incidence of adverse events or IRRs. Thus home therapy is well tolerated and accepted by these families, patients, their physicians, and importantly, by payers and medical insurers.

## 3129/F/Poster Board #1061

**Correction of PKU in the *Pah*$^{enu2}$ mouse by Phenylalanine Ammonium Lyase: Maternal, Biochemical, and Behavioral Studies.** *P. Laipis[1], W. Zeile[1], J. Embury[1], M. Lewis[2], R. Zori[3], D. Musson[4], B. Zhao[4], C. Santamaria[4], S. Bell[4], A. Cheng[4], C. O'Neill[4], L. Tsuruda[4].* 1) Dept Biochem & Molecular Biol, Univ Florida, Gainesville, FL; 2) Dept Psychiatry, Univ Florida, Gainesville, FL; 3) Dept Pediatrics, Univ Florida, Gainesville, FL; 4) BioMarin Pharmaceuticals, Inc. Novato, CA.

Phenylketonuria (PKU) is the most frequent disorder of amino acid metabolism (~1 in $10^4$ births) in populations of European origin. PKU patients accumulate phenylalanine (Phe) to abnormally high concentrations due to a low or absent phenylalanine hydroxylase (PAH), the first enzymatic step in Phe degradation. High Phe results in symptoms ranging from mild cognitive impairment to severe mental retardation. Although deleterious effects can be minimized by a Phe-restricted diet instituted at birth, the diet is demanding and most adult PKU patients are poorly compliant, leading to cognitive and behavioral deficits. Alternate therapies would be valuable, especially for treatment of Maternal PKU Syndrome. We have previously reported that a recombinant phenylalanine ammonium lyase derived from *Anabaena variabilis*, modified by multiple PEGylations (rAvPAL-PEG), is being developed as an investigational enzyme substitution therapy for PKU. Treatment of male BTBR *Pah*$^{enu2}$ mice, an animal model of PKU, with rAvPAL-PEG resulted in long-term correction of Phe levels (> 6 months). Mice were healthy, showed increased body weight and exhibited no adverse effects. Female mice also responded to rAvPAL-PEG, albeit at higher doses than males. Treated female mice showed a correction of the Maternal PKU Syndrome and produced viable pups. We have previously reported histological abnormalities in brains of both male and female PKU mice (Brain Res. 1127:136-50, 2007); rAvPAL-PEG treatment resulted in correction of these abnormalities and subsequent termination of rAvPAL-PEG treatment led to a slow return of these findings. Biochemical analysis showed that tyrosine levels, which are depressed in untreated PKU mice and human patients (Mol Genet Metab 69: 286-294, 2000), remain depressed in rAvPAL-PEG treated PKU mice with physiological Phe levels. This data suggests that not only is the PAH pathway an important input into tyrosine homoeostasis but also that impaired amino acid uptake may not be responsible for the tyrosine depression observed in untreated PKU patients. Based on this observation tyrosine dietary supplementation might be a useful adjunct to rAvPAL-PEG therapy for PKU. Finally, behavioral studies of treated mice show improvements in both activity levels and specific behavior patterns. These results suggest that rAvPAL-PEG therapy may be a viable alternative to the medical diet for PKU patients.

## 3130/F/Poster Board #1062

**Does Enzyme Replacement Therapy (ERT) Reduce the Frequency of Infectious Illnesses in Patients with Hunter Syndrome (MPS II)?** *R.H. Katz[1], B.K. Burton[1,2].* 1) Genetics, Children's Memorial Hospital, Chicago, IL; 2) Northwestern University Feinberg School of Medicine, Chicago, IL.

Individuals with Mucopolysaccharidosis type II (MPS II, Hunter syndrome) display a wide range of symptoms as a result of glycosaminoglycan (GAG) accumulation in tissues. These symptoms include chronic nasal congestion, chronic otitis media, and respiratory distress. Prior to 2006, there was no treatment for Hunter syndrome aside from symptomatic care. Idursulfase (Elaprase®, Shire HGT), an enzyme replacement therapy (ERT), was approved by the FDA and became commercially available in the US in August 2006. Between late August 2006 and January 2007, 6 patients with MPS II, ages 6-13, were started on Elaprase® therapy at Children's Memorial Hospital. Two of these patients have an attenuated phenotype without central nervous system (CNS) involvement, one patient has an intermediate phenotype with mild CNS involvement, and three patients have a severe phenotype with CNS involvement. We examined the medical records of these 6 patients for the two years prior to the initiation of ERT and two years post ERT. We tabulated the number of infectious illnesses diagnosed by their treating physicians, the number of emergency room visits, and the number of hospital admissions during this period of time. In total there were 38 pre-ERT infectious illnesses (14 otitis media, 4 viral infections, 17 upper respiratory infections, and 3 streptococcal pharyngitis), 18 post-ERT illnesses (6 otitis media, 2 viral infections, and10 upper respiratory infections), 2 pre-ERT emergency room visits (both for respiratory distress), 5 post-ERT emergency room visits (all for respiratory distress), 1 pre-ERT hospital admission (pneumonia), and 0 post-ERT hospital admissions. This preliminary data suggests that ERT may reduce the frequency of infectious illness in patients with Hunter syndrome. Further study in a larger number of patients will be needed to confirm these findings.

## 3131/F/Poster Board #1063

**Genz-112638 for Gaucher Disease Type 1: Phase 2 Clinical Trial Results after One Year of Treatment.** *J. Peterschmitt[1], E. Lukina[2], N. Watman[3], E. Avila Arreguin[4], M. Banikazemi[5], G. Pastores[6], M. Iastrebner[7], M. Dragosky[7], H. Rosenbaum[8], M. Phillips[9], M. Kaper[1], S.E. Smith[1], A.C. Puga[1].* 1) Genzyme Corporation, Cambridge, MA; 2) Russian Academy of Medical Sciences, Moscow, Russia; 3) Hospital Ramos Mejia, Buenos Aires, Argentina; 4) Instituto Mexicano del Seguro Social Hospital de Especialidades, Col. La Raza, Mexico; 5) Columbia University Medical Center, New York, NY; 6) New York University School of Medicine, New York, NY; 7) Instituto Argentino de Diagnostico y Tratamiento, Buenos Aires, Argentina; 8) Rambam Medical Center, Haifa, Israel; 9) Sha'are Zedek Medical Center, Jerusalem, Israel.

**Introduction:** Genz-112638, a novel oral small molecule inhibitor of glucosylceramide synthase, is under development for the treatment of Gaucher disease type 1 (GD1). An open-label, multicenter Phase 2 clinical trial to evaluate the efficacy, safety, and pharmacokinetics of Genz-112638 in patients with GD1 is ongoing. Results after 52 weeks of treatment are available. **Methods:** This trial of Genz-112638, given 50 or 100mg twice per day orally, treated 26 adults with GD1 (16F:10M; mean age 34 years, range 18-60; all Caucasian) at 7 sites in 5 countries. Inclusion criteria included splenomegaly (vol.≥10x normal) and either thrombocytopenia (platelets 45,000-100,000/$mm^3$) or anemia (hemoglobin 8-10 g/dL [F] or 8-11 g/dL [M]) and no enzyme replacement or substrate reduction therapy in the prior 12 months. The primary efficacy endpoint was a composite of 52-week improvements in ≥ 2 of 3 parameters: spleen volume (-15%), hemoglobin level (+0.5 g/dL) or platelet count (+15%). Bone status was evaluated by central review of MRI, DXA and X-ray. Long-term treatment and monitoring is ongoing. **Results:** 22 patients completed 52 weeks; 4 withdrew. The primary endpoint was met by 77% (20/26) of all patients and by 91% of 22 patients completing 52 weeks. Mean hemoglobin improved by 1.6±1.4 g/dL and platelet count by 40±37%. Mean spleen volume decreased by 39±11% and mean liver volume by 17±10%. Chitotriosidase and CCL-18 had median decreases of 52% and 55%. Plasma glucosylceramide levels normalized in all patients. No bone crises or reductions in mobility were reported. Femur MRI showed improved dark marrow signal in 7 patients and stable findings in the remaining 13 patients with data. There were no new lytic lesions or infarcts; one pre-existing infarct progressed. Lumbar spine bone mineral density (DXA Z-score) was -1.32±1.02 at baseline and improved by a mean of 0.31±0.48 (p=0.0146; n=18). Genz-112638 was well tolerated with an acceptable safety profile. In all, 91% of AEs were unrelated to drug treatment and most were mild in severity. The most common AEs overall were urinary tract infection and GI symptoms; no single AE occurred in more than 3 patients. Seven drug-related AEs occurred in 6 patients early in treatment and were mild and transient. **Conclusion:** Genz-112638 improved hematologic, visceral and bone manifestations in GD1 and was well tolerated in this Phase 2 study. Phase 3 studies of untreated and Cerezyme-stabilized patients will begin in mid-2009.

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### 3132/F/Poster Board #1064

**Inflammation in the mucopolysaccharidoses and the use of anti-TNF-α therapy.** *C. Simonaro[1], Y. Ge[1], E. Eliyahu[1], K. Jepsen[2], E. Schuchman[1].*
1) Dept Genetics & Genomic Sci, Mount Sinai Sch Medicine, New York, NY; 2) Dept. of Orthopedics, Mount Sinai Sch Medicine, New York, NY.

Enzyme replacement therapy (ERT) is available for three MPS disorders, but has limited effects in the bones and joints. We have investigated the involvement of the toll-like receptor 4 (TLR4) signaling pathway in the pathogenesis of MPS bone and joint disease, and the use of anti-inflammatory agents for the treatment of these diseases. TLR4 knockout (KO) mice were bred to mice with MPS type VII. Double KO animals grew substantially better than MPS VII mice alone, and had longer and thinner faces. The levels of several inflammatory cytokines, including TNF-α, also were substantially reduced in the double KO mice, leading us to evaluate the effects of the FDA-approved anti-TNF-α drug, Remicade, in rats with MPS VI. When initiated pre-symptomatically (1 month of age), intravenous (2x per week for 24 weeks; 3 mg/Kg) Remicade treatment prevented the elevation of TNF-α and other inflammatory molecules in the blood. Importantly, the levels of these markers also were markedly reduced in chondrocytes and synovial fibroblasts from the treated animals. Although the overall growth of these animals was not improved by Remicade treatment, the number of apoptotic chondrocytes were reduced by ~50%, as was the infiltration of synovial tissue into the underlying bone. These results indicated positive effects of Remicade treatment at the sites of pathology. Remicade treatment also reversed the established inflammatory disease in older animals. Thus, these studies reveal the important role of TLR4 signaling in the pathogenesis of MPS bone and joint disease, and suggest that targeting a downstream mediator of this pathway, TNF-α, can have positive effects. Funded by: NIH (R01 DK25759), The National MPS Society and The Isaac Foundation.

### 3133/F/Poster Board #1065

**Resveratrol or SIRT1 activators: new candidate drugs for correction of inborn mitochondrial FAO or RC disorders?** *J. Bastin, F. Aubey, F. Fjouadi.* CNRS FRE 3210, Faculté Necker Enfants Malades, Paris, France.

Resveratrol (RSV) is a natural polyphenol compound found in red grapes and other plants, with potential anti-cancer and cardioprotective effects. RSV acts in part as an antioxidant to reduce cellular oxidative stress, but was also recently proposed to up-regulate mitochondrial oxidative metabolism, through an activation cascade involving SIRT1, a member of the NAD-dependant histone deacetylase sirtuin family, and PPAR Gamma Co-activator 1-alpha (PGC-1α), a key regulator of mitochondrial energy metabolism. This led us to test RSV effects on oxidative stress and residual metabolic capacities in fibroblasts from patients with inborn fatty acid β-oxidation (FAO) or respiratory chain (RC) deficiency. Addition of RSV stimulated residual FAO capacities (3H-palmitate oxidation) in a dose- (20μM to 80μM) and time-dependant manner in cultured patient fibroblasts with the mild form of Carnitine Palmitoyl Transferase 2 (CPT2) or Very Long Chain AcylCoA Dehydrogenase (VLCAD) deficiency. Furthermore, correction of FAO was achieved in cell lines with different CPT2-deficient or VLCAD-deficient genotypes, after treatment by RSV at 80μM for 48h. Quantitative PCR revealed a +30 to +60% CPT2 and VLCAD mRNA increase in response to RSV while western-blot indicated a marked RSV-induced up-regulation of CPT2 or VLCAD protein in the treated patient cells. Studies with DCFH-DA fluorescent probe showed that RSV treatment could also normalize the production of Reactive Oxygen Species (ROS) in FAO-deficient fibroblasts. Exposure of fibroblasts to a SIRT1 activator (CAY10591, Cayman chemicals) could mimic the stimulatory effects of RSV in FAO-deficient fibroblasts, whereas addition of SIRT1 inhibitor Sirtinol prevented the RSV-induced increase in FAO. In parallel, patient cells treated with siRNA directed against PGC-1α exhibited a markedly blunted response to RSV in FAO studies. Finally, preliminary western-blot data indicate that RSV could enhance the expression of subunits or ancillary proteins of all RC Complexes in control fibroblasts, and could increase mutated protein levels in CI- or CIV-deficient cells. Altogether, these initial studies -suggest a potential of natural or synthetic SIRT1 activators for correction of FAO or RC deficiencies due to partial loss-of-function mutations, which are often encountered in these genetic diseases - indicate that the SIRT1/PGC-1α cascade represents a highly relevant new therapeutic target in these disorders.

### 3134/F/Poster Board #1066

**Curcumin partially mitigated the phenotype of Pelizaeus-Merzbacher disease caused by *PLP1* point mutations in mouse.** *K. Inoue[1], L-H. Yu[1], T. Morimura[1], H. Iwashita[1], R. Yamamoto[1], K. Deguchi[1,2], B. Antalfy[2], N. Inoue[1], H. Osaka[3], M. Itoh[1], Y. Goto[1].* 1) Dept MR & BD Res, Natl Inst Neurosci, NCNP, Kodaira, Japan; 2) Dept of Pathology, Texas Children's Hospital, Houston, US; 3) Neurology, Clinical Research Institute, Kanagawa Childeren's Medical Center, Yokohama, Japan.

Natural compounds have great advantage in rapid and safe clinical application if therapeutic effects against genetic diseases, especially in pediatric patients, can be established. Pelizaeus-Merzbacher disease (PMD) is a devastating X-linked inherited neurodevelopmental disease characterized by a failure of myelination in the central nervous system (CNS). PMD is caused by *PLP1* mutations, including genomic duplication, deletion, and point mutations, each of which lead to CNS dysmyelination through different molecular mechanisms. Nevertheless, no effective therapy for PMD is available to date. *PLP1* point mutations, mainly leading to amino acid substitutions, cause improper protein folding and accumulation in the endoplasmic reticulum (ER), resulting in disruption of ER proteostasis and apoptosis of oligodendrocytes. A natural compound curcumin has been reported to mitigate manifestations of some protein-misfolding-diseases, such as cystic fibrosis and demyelinating peripheral neuropathy, in mice. Here we examined if curcumin can also alleviate phenotype of *msd*, a mouse model of PMD. We treated *msd* mice with oral administration of curcumin (180mg/kg/day) dissolved in milk and found that curcumin significantly extended the life span of *msd* mice. Curcumin also inhibited apoptosis of oligodendrocytes in the *msd* brains. In contrast, no improvement in mylin formation or myelin-protein expression was observed. Although the exact molecular mechanism for curcumin in reducing the cellular toxicity caused by misfolded mutant protein still remains to be resolved, our data suggest that curcumin can be a good candidate for the treatment of PMD patients with *PLP1* point mutations.

### 3135/F/Poster Board #1067

**Pharmacological antagonism of TGFβ signaling improves skeletal muscle repair in mice with sarcopenia.** *T.N. Burks, E. Andres-Mateos, J.L. Simmers, E.M. MacDonald, C. van Erp, R. Rattner, R.D. Cohn.* McKusick-Nathans Institute of Genetic Medicine, Department of Pediatrics and Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland.

We have previously demonstrated that increased TGFβ signaling causes abnormal muscle repair and regeneration in various inherited myopathies. Moreover, antagonism of TGFβ in dystrophin-deficient mdx mice and fibrillin-1 deficient Marfan syndrome mice attenuated myopathic disease progression and improved muscle regeneration and repair. Critical loss of muscle mass is observed in various systemic conditions as well as in the physiological process of aging (sarcopenia). This often not only increases morbidity and mortality, but also increases the incidence of pathologic fractures, functional deterioration and institutionalization. The molecular mechanisms underlying sarcopenia are only poorly understood, but it has recently been demonstrated that an increase in TGFβ signaling contributes to impaired satellite cell function and muscle repair in old skeletal muscle. We therefore evaluated whether antagonism of TGFβ signaling via administration of TGFβ-neutralizing antibody (TGFBNab) or the angiotensin II type1 receptor blocker, losartan, has any beneficial impact on the muscle repair process of aged mice in vivo. To assess muscle regeneration capacity, muscle injury was induced by administration of the snake venom cardiotoxin and skeletal muscles of aged mice were analyzed at 4 and 18 days after injury induction. We find that systemic TGFβ antagonism via TGFBNab or losartan significantly improves the muscle regeneration process in response to injury. Aged mice demonstrate an increase in regenerating muscle fibers at 4 days after injury induction. Furthermore, after 18 days, mice treated with TGFBNab or losartan exhibit significantly less of fibrotic tissue formation and an overall more homogeneous appearance of regenerated skeletal muscle. Thus, pharmacological antagonism of TGFβ signaling may be an interesting target for the management of age-related loss of muscle mass. Further studies of long-term administration of losartan in aged mice over a prolonged period of time are currently in process in order to establish whether a beneficial impact on steady-state architecture and muscle function can be determined.

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## 3136/F/Poster Board #1068

**New approaches to the therapy of keratinisation disorders: 3D skin model with a disturbed epidermal barrier function.** *K.M. Eckl[1,2], G. Weindl[3], K. Ackermann[3], M. Schäfer-Korting[3], H.C. Hennies[1,4].* 1) Dermatogenetics, Cologne Center for Genomics, University of Cologne, Germany; 2) Institute of Neurophysiology, University of Cologne, Cologne, Germany; 3) Div. for Pharmacology, Institute for Pharmacy, Freie Universität Berlin, Berlin, Germany; 4) Center for Molecular Medicine, University of Cologne, Cologne, Germany.

Hereditary keratinisation disorders are a clinically and genetically heterogeneous group of skin diseases. They are characterised by abnormalities in terminal keratinocyte differentiation. Clinical features may include marked keratosis, more or less intense scaling of the skin and mild to extensive erythema. A disturbed epidermal barrier function is often seen, which leads to secondary symptoms such as increased trans-epidermal water loss, imbalances in nutrition but also often severe eczema and allergies. To investigate the epidermal barrier function and to assess specifically designed therapeutics we have developed a 3D skin model which mimics congenital ichthyosis, a rare and severe keratinisation disorder. The model consists of an underlying dermal equivalent and a fully stratified perfectly organised epidermal part, with a well established basement membrane zone in the dermal-epidermal junction area. For model preparation we use keratinocytes which have been specifically knocked down for single disease related genes. The model has been validated following OECD guidelines for artificial skin using reference substances and is now being used to investigate the ability, effects, and toxicity of the application of new drugs for therapeutic approaches, e.g. gene products and metabolites that are missing in the patient epidermis. To improve our model we are investigating the effects of long time cultivation and media components as well as the effects of melanocytes and Langerhans cells (dentritic cells) in the patient epidermis mimicking model. To improve cultivation duration the knock down process is now being changed from transient to lentiviral mediated. Our model is the first human artificial skin model showing an epidermal barrier functional defect, which is highly similar to biopsy samples from patients suffering from congenital ichthyosis. The models can be routinely manufactured, used for drug and cosmetic screening, is easily established and shows a high reproducibility from batch to batch. The European patent application for this model has just been filed.

## 3137/F/Poster Board #1069

**Management of ADHD in a population of Children with Genetic Learning Disability.** *A.D. Rasalam[1], J.C. Dean[2].* 1) Learning Disability Team, Royal Aberdeen Childrens Hospital, Aberdeen, United Kingdom; 2) Department of Clinical Genetics, Aberdeen Royal Infirmary, Foresterhill, Aberdeen, United Kingdom.

In North East Scotland (Population : 500,000; Estimated population with learning disability : 15,000), any child of school going age who has a recognised learning disability has their education provided through a special school or through a Special Needs Base within a mainstream school. The psychiatric and psychological needs of all these children are provided through the Learning Disability Team at the Royal Aberdeen Children's Hospital. All children referred to the team are offered genetic testing including Karyotype, Fragile X and Telomere MLPA to evaluate an underlying cause for their learning difficulties. Children are also offered an appointment with the geneticist as appropriate or when clinical suspicion of particular disorders exists. Among the children seen by the team 56 fulfilled the diagnostic criteria for Attention Deficit (Hyperactivity) Disorder. The diagnosis was made based on the presence of hyperactivity, impulsivity or problems of attention that is significantly in excess of that expected for developmental stage and evident in multiple settings. These children and their families are then offered psychological, behavioural and pharmacological interventions. Among these 56, an underlying genetic disorder which was identified in eleven (3 girls, 8 boys). The disorders diagnosed include Fragile X Syndrome, Neurofibromatosis Type 1, Myotonic Dystrophy, Hyperprolinemia, Incontinentia Pigmenti, Telomere deletion or duplication and one child with an apparently balanced reciprocal translocation. The balanced translocation involved chromosomes 9 and 10 and segregated with behaviour disturbance in the family. There were 3 Telomere duplications and one telomere deletion. One child had inherited 16q duplication from a parent who also has clinical features of attention deficit and impulsivity and had attended special school. The other patients had an 11q duplication, 12q duplication and 12q deletion. All the children had a beneficial response to treatment and are on either Methylphenidate or Atomoxetine or a combination of the two. Four patients responded to Methylphenidate, three to Atomoxetine, three to a combination of the two, while one family chose to try behavioural methods. The families and schools of these children have noticed a significant improvement in quality of life and academic progress in these children. Standard therapy including drug treatment appears to be of value in patients with ADHD due to an underlying genetic cause.

## 3138/F/Poster Board #1070

**Dose-dependent improvements of the hypophosphatasia phenotype in *Akp2$^{-/-}$* mice following the administration of ENB-0040.** *H. Landy[1], I. Lemire[2], L. Blond[3], T.P. Loisel[2], G. Boileau[4], J.L. Millan[5], P. Leonard[2], P. Crine[2].* 1) Enobia Pharma, Cambridge, MA; 2) Enobia Pharma, Montreal, Canada; 3) University of Montreal, Dept. of Clinical Sciences, Montreal, Canada; 4) University of Montreal, Dept. of Biochemistry, Montreal, Canada; 5) Burnham Institute for Medical Research, La Jolla, CA.

Hypophosphatasia (HPP) is a rare inherited form of rickets or osteomalacia caused by inactivating mutations in the gene encoding tissue nonspecific isozyme of alkaline phosphatase (TNSALP). The prevalence of the disease is about 1:100,000 for the severe form. Severity is inversely related to the age at symptom onset. Since no established medical therapy for HPP is presently available, we have designed a soluble recombinant form of TNSALP (ENB-0040) for enzyme replacement therapy. ENB-0040 comprises the catalytic domain of the human TNSALP, human immunoglobulin Fc region and a deca-aspartate peptide to target the enzyme directly to bone. We have used the *Akp2$^{-/-}$* mouse model to evaluate the effect of systemic administration of ENB-0040 on skeletal defects and survival of HPP mice. We have previously shown that daily subcutaneous (SC) administration of ENB-0040 prevents bone mineralization defects of the feet in *Akp2$^{-/-}$* mice. We have now evaluated the dose response relationship of ENB-0040 SC administration on survival and bone mineralization defects of the feet, rib cages and pelvic limbs. Newborn *Akp2$^{-/-}$* mice received daily SC injection of ENB-0040 at 0.5, 2.0 and 8.2 mg/kg for 43 days. The primary endpoint was extent of mineralization defects; secondary endpoints included mortality, body weight and femur and tibia length. Bone mineralization defects were evaluated by radiographs and classified in a blinded fashion by a qualified veterinarian radiologist. Animals were classified as Normal or Abnormal. Abnormal individuals have at least one missing bone structure including secondary ossification center. There is a clear relationship between administered daily dose of ENB-0040 and improvement of mineralization defects of feet, rib cages and pelvic limbs with $r^2$=0.993, 0.986 and 0.999, respectively. According to the dose response model, the ED50 was 0.9, 0.8 and 0.5 mg/kg/day for feet, rib cages and pelvic limbs. Long bones seem to respond to lower daily doses of ENB-0040. There was also a clear relationship between dose and survival with $r^2$=0.989. Median survival was improved from 19 days in Vehicle-treated *Akp2$^{-/-}$* mice to 24, 31 and >44 days with increasing doses of ENB-0040. Body weight and bone length were also improved in a dose dependent manner. These dose response relationships strongly support the pharmacological activity of ENB-0040 and provide support for the estimation of the range of human effective doses.

## 3139/F/Poster Board #1071

**Hydroxycobalamin dose escalation optimizes metabolic control in cblC.** *N. Carrillo-Carrasco[1], J. Sloan[1], D. Valle[2], A. Hamosh[2], C.P. Venditti[1].* 1) NHGRI, National Institutes of Health, Bethesda, MD; 2) Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Cobalamin C (cblC), a form of combined methylmalonic acidemia and hyperhomocysteinemia, is recognized as the most frequent inborn error of intracellular cobalamin metabolism. This condition is detected by expanded newborn screening and although the phenotype is variable, many patients have an acute neonatal presentation that is life-threatening if not suspected and treated promptly. Intramuscular hydroxycobalamin (OH-Cbl) is the main treatment for patients with cblC, but formal dosing guidelines do not exist. Clinical improvement and a decrease of plasma methylmalonic acid (MMA) and total homocysteine (tHcy) levels, and an increase in methionine are typically observed soon after its initiation. However, despite treatment, long-term complications such as developmental delay and progressive visual loss may develop. We describe the biochemical response of a 13-year-old male with cblC and worsening metabolic parameters despite strict adherence to a conventional treatment regimen. He is homozygous for c.271dupA mutation, the most common disease-causing allele. He was diagnosed at 3 weeks of life, responded appropriately to conventional treatment with IM OH-Cbl 1 mg/day, betaine, folic acid and carnitine. At the time of assessment at 13 years of age, he had normal growth, pigmentary retinopathy with severely constricted visual fields, and mild developmental delay. tHcy was 112 $\mu$M (normal 0-13 $\mu$M), plasma MMA 25 $\mu$M (normal <0.27 $\mu$M), propionylcarnitine 3.92 nM (normal <0.8 nM), methionine 17 $\mu$M (normal: 7-47 $\mu$M). We progressively increased the OH-Cbl dose from 1 to 20 mg per day with no other modifications in his regimen. We observed a dose-dependent response with an 80% reduction of plasma MMA (25 to 5.14 $\mu$M) a 55% reduction of tHcy (112 to 50 $\mu$M) and a greater than two-fold elevation of methionine (17 to 36 $\mu$M). This was accompanied by a reduction in the levels of propionylcarnitine, 2-methylcitrate and cystathionine. Our observations show that higher OH-Cbl doses might be required to achieve an optimal biochemical response in cblC patients. Whether this intervention may slow or eliminate other complications in cblC is unknown. The safety of parenteral administration of much higher doses of OH-Cbl has been previously established. Future clinical trials to determine the benefits of this intervention in patients with cblC and other disorders of intracellular cobalamin metabolism should be planned.

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### 3140/F/Poster Board #1072

**Simvastatin and brain cholesterol turnover in children with Smith-Lemli-Opitz syndrome (SLOS).** *L.S. Merkens[1], J.B. Roullet[1], A.E. DeBarber[2], A.S. Pappu[2], D. Lütjohann[3], J.A. Penfield[1], J.M. Jordan[4], W.E. Connor[5], R.D. Steiner[1,6].* 1) Dept Pediatrics, Oregon Health & Science Univ., Portland, OR; 2) Dept Physiology and Pharmacology, Oregon Health & Science Univ., Portland, OR; 3) Dept of Clinical Pharmacology, Univ. of Bonn, Bonn, Germany; 4) Oregon Clinical and Translational Research Institute, Oregon Health & Science Univ., Portland, OR; 5) Dept of Medicine, Oregon Health & Science Univ., Portland, OR; 6) Dept Molecular and Medical Genetics, Oregon Health & Science Univ., Portland, OR.

Smith-Lemli-Opitz syndrome is an autosomal recessive disorder caused by a defect in the final step in cholesterol (CH) synthesis. Mental retardation is one nearly universal feature. Cholesterol deficiency and accumulation of cholesterol precursors may be causative. One treatment objective is to raise plasma CH and lower precursors such as 7-dehydrocholesterol (7DHC). Dietary CH is widely used as potential treatment, but since it does not cross the blood brain barrier (BBB), it is unlikely to impact development in affected children. Statins inhibit HMG-CoA reductase, a key proximal enzyme in cholesterol synthesis. Lipophilic statins (e.g. simvastatin; simv) cross the BBB and may be therapeutic by reducing 7DHC while increasing brain CH synthesis (Hum Mol Genet: 15:839). 24S-hydroxycholesterol (24S) is formed in the brain from CH and crosses the BBB into the circulation where it serves as a marker of brain cholesterol turnover. Thus simv treatment may increase 24S in SLOS. We measured plasma (pl)-24S in 6 SLOS children (simv group, age: 1.3-12.5 yr) on high cholesterol diet before and after treatment with simv (mean treatment duration: 1.4.yr; dose 0.1-0.4 mg/kg) and in 6 SLOS children on a high cholesterol diet (control group; age 0.3-14.4 yr) at two visits separated by 1.9 yr on average (approximately the same as the simv treatment period). Sterols were measured by GC and 24S by GCMS or LCMS. The two groups had similar initial parameters: pl-CH and 7DHC concentrations (mg/dl) were respectively $115 \pm 17$ and $7.8 \pm 3.1$ (simv) and $91 \pm 23$ and $6.3 \pm 2.3$ (control) (mean ± SE). 24S changes were expressed as the ratio of 24S to pl-CH (ng/mg; PNAS 93:9799). This ratio decreased similarly in the two groups, by 22% on average (before and after treatment) in the simv group and by 30% in the control, cholesterol only group (change between the two visits; NS). We confirmed the effect of age on the 24S-to-CH ratio ($p < 0.02$) but did not find any significant relationship between this ratio and either the severity scores, pl-CH, or 7DHC concentrations. We conclude that since simv did not change 24S from treatment with dietary cholesterol alone, simv treatment does not alter brain CH turnover in SLOS and is therefore unlikely to be therapeutic under the current experimental conditions. Studies with a larger number of subjects and/or studies with optimal dosing of simv will be necessary to further establish the potential benefit of statin therapy in SLOS.

### 3141/F/Poster Board #1073

**Familial Dysautonomia: Therapeutic efficacy of kinetin and luminescence-based splicing assay.** *R.S. Shetty[1, 2], Y.T. Chen[1, 2], J. Mull[1, 2], M.M. Hims[1, 2], L. Liu[1, 2], M. Leyne[1, 2], J. Pickel[3], S.A. Slaugenhaupt[1, 2].* 1) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) National Institute of Mental Health, National Institutes of Health, Bethesda, MD.

Familial dysautonomia (FD), a congenital sensory neuropathy having widespread sensory and autonomic dysfunction, is caused by aberrant splicing of *IKBKAP* gene. All FD patients carry an intronic splice site mutation (IVS20+6T>C) in the *IKBKAP* gene, which results in tissue-specific partial skipping of exon 20 and subsequent reduced production of IKAP protein. Although FD is a recessive disease, homozygous mutant cells express wild-type *IKBKAP* transcripts, and produce normal IKAP protein. We are targeting the plasticity of the splicing machinery for the development of therapeutics for FD that would improve the production of the wild-type *IKBKAP* transcript and potentially lead to dramatic phenotypic improvements. We have previously identified kinetin, a plant cytokinin, as a modulator of *IKBKAP* mRNA splicing in FD lymphoblast cell lines. Recently, we tested the ability of kinetin to alter *IKBKAP* splicing in humanized FD *IKBKAP* transgenic mouse models, which carry the intronic *IKBKAP* (IVS20+6T>C) splice mutation and exhibit similar tissue-specific aberrant splicing pattern as FD patients. Treatment with kinetin modifies *IKBKAP* splicing in all major tissues assayed in the transgenic mice. The wild-type *IKBKAP* mRNA and IKAP protein levels were also significantly higher in the kinetin-treated mice suggesting that dietary supplementation of kinetin holds great promise as a potential therapeutic for FD. In parallel, we are developing new luminescence-based *IKBKAP* splicing assays using our previously designed *IKBKAP* minigene constructs to measure levels of wild-type *IKBKAP* transcripts in cells. These assays will be useful for high-throughput screening for drugs that can be used for treatment of FD, and perhaps even other human genetic diseases caused by splicing disorders.

### 3142/F/Poster Board #1074

**Drug Development for Rare and Neglected Diseases.** *P. Terry[1], K. Zonno[1,2], S. Terry[2].* 1) GRAND Therapeutics Foundation, Washington, DC; 2) Genetic Alliance, Washington, DC.

Rare and neglected diseases suffer not only the burden of the condition, but a lack of adequate incentives to develop therapies. At present, there is clear evidence of the insurmountable difficulties associated with the complex technical, scientific, and business aspects of successfully delivering treatments for over 7,000 genetic diseases. A recent IOM report declared: "... drug sponsors can spend over 13 years studying the benefits and risks of a new compound, and several hundred millions of dollars completing studies before seeking FDA's approval. About 1 out of 10,000 chemical compounds initially tested for their potential as new medicines is found safe/effective, and approved by FDA..." This sobering assessment is reflective of the active drug development enterprise for common disease, and doesn't consider the more severe failure rates for rare disease. There appear to be four major barriers, or 'walls', blocking routine drug development: assay development, preclinical/medicinal chemistry, natural history studies/clinical cohort development and regulatory approval. A public-private partnership has been created to advance creative solutions to navigate through these four walls. The effort is focused on helping organize and fund applied research, technology development, intellectual property management, and product development for rare and neglected diseases. The activities will focus on genetic disease mechanisms and directly assist in the develop of screening assays, secondary functional assays, screening compounds, re-purposing approved drugs, and creating mechanisms to get treatments into patients through the early clinical trial phases. Multiple entry points for various diseases will be created to allow each stakeholder group to capitalize on their ability to contribute based on expertise, state of the science, and possible serendipity from discoveries in industry, academia, and federal activities across the drug development continuum. An analysis and outline of the current rare and neglected disease product development opportunities will be presented on this initiative.

### 3143/F/Poster Board #1075

**Correcting SMN2 splicing with tailed antisense oligoribonucleotides: a potential therapeutic strategy for Spinal Muscular Atrophy.** *N. Owen[1], H. Zhou[2], F. Muntoni[2], I. Eperon[1].* 1) Biochemistry, University of Leicester, Leicester, United Kingdom; 2) Dubowitz Neuromuscular Unit, Hammersmith Hospital, Imperial College, London, United Kingdom.

Childhood onset Spinal Muscular Atrophy (SMA) is caused by homozygous deleted or mutation of the survival of motor neuron (SMN1) gene in over 95% of cases. A nearly identical copy gene, SMN2, lies in the same chromosomal location but is only found in humans. A single coding nucleotide difference between the genes (C to T; +6 position of exon 7) causes predominant exclusion of exon 7, resulting in a less stable truncated protein product. SMN2 cannot wholly compensate for the loss of SMN1, but is able to modulate the disease phenotype through the low amount of full length protein produced. Two models have been proposed for the loss of exon 7 in SMN2 transcripts; loss of an Exonic Splicing Enhancer (ESE) or gain of an Exonic Splicing Silencer (ESS) sequence. With the first model the loss of an ESE would result in the loss of interaction of the SR protein SF2/ASF and the skipping of exon 7. As a potential therapy we have developed bifunctional oligonucleotides consisting of an RNA antisense moiety, able to base-pair with the SMN transcript, and a tail sequence with consensus motifs that bind SF2/ASF. Such Targeted Oligonucleotide Enhancers of Splicing (TOES) increase the inclusion of exon 7 both in vitro and in SMA patient derived cell lines. We have investigated the effect of chemical modification along with tail length to improve both efficacy and to minimise toxicity. The most effective TOES oligonucleotide to date comprises of LNA (locked nucleic acid) and 2'O-methyl RNA demonstrating low toxicity and increased inclusion of exon 7. In addition, we have tested alternative target annealing sites in exon 7 to determine whether the tail is sufficient to result in inclusion of the exon independent of exonic enhancer sequences. The results show that the most conserved exonic enhancer is required in addition to the TOES oligo. Further work is being directed to making self-sufficient trans-enhancers.

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### 3144/F/Poster Board #1076

**Human Rhodopsin(P23H)-GFP: a Mouse Model to Examine Gene Therapy Strategies to Treat Autosomal Dominant Retinitis Pigmentosa.** *B.A. Price[1,2], T.G. Wensel[2], J.H. Wilson[1,2].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX.

Retinitis pigmentosa (RP) is a disorder that is typified by progressive retinal degeneration affecting 1:4,000 people worldwide. Patients with RP experience night blindness early in the course of the disease, which suggests rod cell dysfunction. As the disease progresses, patients experience a loss of peripheral vision and ultimately become blind. Inheritance of RP is heterogeneous; patterns of autosomal dominant (AD), autosomal recessive (AR), and X-linked recessive have all been documented. Many genes are known to cause RP upon mutation, but the most commonly affected is rhodopsin. Over 100 different mutations within rhodopsin can cause either ADRP or ARRP, with ADRP being more prevalent. Mutations causing ADRP are found throughout the entire rhodopsin protein-coding region and primarily consist of missense and nonsense mutations. The most common in North America is a cytosine to adenosine transversion that occurs in codon 23 causing a change from proline to histidine (P23H). The exact mechanism of pathogenesis associated with this mutation remains ambiguous. Ultimately, we aim to treat this disorder using a gene knockout approach. We have engineered a mouse that expresses human Rhodopsin(P23H)-GFP (P23H-GFP) from the endogenous mouse locus. These mice express P23H-GFP at low levels to allow us to follow correction, but not necessarily high enough to cause retinal degeneration. Most autosomal dominant gene therapy strategies involve targeting a specific mutation, but due to the heterogeneous nature of ADRP this would be costly and time-consuming. Gene knockout could provide a strategy to treat multiple individuals with distinct mutations that cause ADRP.

### 3145/F/Poster Board #1077

**Therapeutic RNAi for Dominant Muscle Disease.** *J.R. Chamberlain, J. Wei, E.E. Finn.* Medical Genetics, Department of Medicine, University of Washington, Seattle, WA.

Developing therapies for dominant genetic disease requires approaches aimed at eliminating the toxic gene product, either protein or mRNA. We are developing methods based on the endogenous RNAi pathway to destroy expressed mRNAs that are involved in dominant muscle disease pathogenesis. Our goal is targeted knockdown of expression of either protein or mRNA expressed from microsatellite mutations that cause disease. To test the effectiveness of RNAi as a potential muscle therapy, we targeted *lac*Z mRNA that is constitutively expressed in tissues of the ROSA26 mouse. we expressed short-hairpin RNAs (shRNAs) from mU6 that targeted *lac*Z mRNA and delivered them to muscle via tail vein injections in the context of rAAV6. We observed a 50-fold and 30-fold reduction in β-gal activity from cardiac and skeletal muscle, respectively, that most likely reflects differing levels of transduction of the vector. Our original shRNA expression constructs led to toxicity in heart and liver tissues that was mitigated by shortening the recognition sequence from 21nt to 19nt. We have since redesigned our RNAi expression cassettes to express miR30-based hairpins from U6 or shRNAs from the muscle-specific creatine kinase promoters CK6 and CK7. The main focus for testing the therapeutic potential of RNAi *in vivo* is a mouse model of muscular dystrophy, the *HSA*$^{LR}$ model of myotonic dystrophy (DM). The pathogenic repeat-expanded mRNA responsible for DM is expressed in skeletal muscle from the human skeletal actin gene (HSA). We are using recombinant adeno-associated virus, serotype 6 (AAV6), for muscle delivery of the RNAi expression cassettes targeting the pathogenic repeat expanded DM mRNA. We have observed widespread transduction of the RNAi expression cassettes and concomitant changes in both splicing patterns associated with RNAi delivery *in vivo* and in electromyographic analyses of the mouse muscle that is different from the disease state that most likely reflective of phenotypic improvement due to RNAi. Therapeutic targets for facioscapulohumeral muscular dystrophy (FSHD), cause by macrosatellite repeat contraction, include *FRG1* and *DUX4*, both implicated in FSHD pathology. The ability to decrease β-gal activity in the ROSA26 mouse and to affect dominant disease in a mouse model of muscular dystrophy suggests that rAAV6 vectors delivering siRNAs could be used for treatment of dominantly inherited diseases of muscle.

### 3146/F/Poster Board #1078

**Lethal Murine Model of Methylmalonic Acidemia Rescued in Excess of 12 Months by Gene Therapy.** *R.J. Chandler[1,2], C.P. Venditti[1].* 1) National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA; 2) Institute for Biomedical Sciences The George Washington University, Washington, District of Colombia, USA.

Methylmalonic acidemia (MMA) is a genetic disorder caused by deficient activity of methylmalonyl-CoA mutase (MUT). MMA patients exhibit increased methylmalonic acid levels in the plasma and urine. Clinical complications of this disease include lethal metabolic decompensation, growth retardation, renal failure, metabolic strokes and neurological complications. These clinical problems occur even when the patients are being properly managed and treated. Because of the high mortality and poor long-term prognosis associated with MMA, new therapies are needed. To investigate the efficacy of gene therapy for MMA, a recombinant adeno-associated virus serotype 8 carrying the murine *Mut* cDNA (rAAV8-m*Mut*) was injected directly into the liver of newborn *Mut*$^{-/-}$ mice. Greater than 95% of the 27 *Mut*$^{-/-}$ mice injected with 1 or 2x10$^{11}$GC of rAAV8-m*Mut* have survived for over 1 year. All the untreated (n=58) and rAAV8-*GFP* treated (n=18) *Mut*$^{-/-}$ mice perished before day of life 72 with most of these mice dying in the first few days of life. Hepatic *Mut* RNA levels decreased from 37-72% at 90 days post-injection to 10-15% at one year post-treatment. Significant levels of *Mut* RNA were also detected in the skeletal muscle, brain and heart. Protein was detectable in the liver and skeletal at 90 days. Plasma methylmalonic acid levels in the treated *Mut*$^{-/-}$ mice were significantly reduced at 24 and 60 days after treatment and remained stable one-year post-treatment, indicating that substantial MUT enzymatic activity was restored and maintained. Whole body MUT enzymatic activity, indirectly measured by *in vivo* conversion of 1-$^{13}$C-sodium propionate into $^{13}CO_2$, was detectable in *Mut*$^{-/-}$ mice one-year post-treatment. To more accurately simulate a treatment scenario in humans, three untreated *Mut*$^{-/-}$ mice that survived 20 days were treated with an intraperitoneal injection of rAAV8-m*Mut*. At the time of treatment, these *Mut*$^{-/-}$ mice were growth deficient, lethargic and exhibited plasma methylmalonic acid levels of 1,100+/-118 μM. Following treatment the mice *Mut*$^{-/-}$ exhibited a rapid increase in activity, growth, reduction of plasma methylmalonic acid levels to 361+/-38 μM and an increase in MUT activity. These experiments provide the first evidence that gene therapy has clinical utility in the long term and acute treatment for methylmalonic acidemia and provide proof of principle evidence to support the development of gene therapy for other organic acidemias.

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## 3147/F/Poster Board #1079

**Complete genetic correction of induced pluripotent stem cells from Duchenne muscular dystrophy using a human artificial chromosome.**
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Induced pluripotent stem (iPS) cells made with defined factors have great potential for gene therapy, as such cells can be generated from an individual's tissues, and when re-introduced can contribute to the specialised function of any tissue. Homologous recombination has been used for the gene restoration of various genetic defects in embryonic stem (ES) or iPS cells. However, gene defects with unknown sites of mutation and those involving large deletions cannot be restored by homologous recombination. Duchenne muscular dystrophy (DMD) is caused by dysfunction of the dystrophin gene. Although several vectors have been developed for DMD gene therapy, no episomal vector containing the entire dystrophin genomic region has been reported, due to the extremely large size of this region (2.4 Mb). Human artificial chromosomes (HACs) offer several advantages as gene therapy vectors, including stable episomal maintenance that avoids insertional mutations and the ability to carry large gene inserts including regulatory elements. As a proof of concept, we herein report the correction of a genetic deficiency in iPS cells derived from DMD model (mdx) mice and human DMD patient, using a HAC with a entire genomic dystrophin (DYS-HAC) which we have recently developed (Hoshiya et al.,Mol. Ther.2009). The DYS-HAC was transferred to the mdx- or DMD patient-specific iPS cells via microcell-mediated chromosome transfer (MMCT). FISH analyses showed that the DYS-HAC was present as an individual chromosome in the iPS cells. Multiplex PCR and PCR analyses showed the transferred DYS-HAC corrected the deletion of dystrophin in the iPS cells. Next, the iPS cells containing the DYS-HAC were injected into SCID mice to assess the capacity of the differentiation and the expression in the teratoma. The transplanted DMD patient- and mdx-specific iPS cells with DYS-HAC gave rise to differentiate to three germ layers and the human dystrophin expression was detected in the tissues. Furthermore, chimeric mice from the mdx-iPS (DYS-HAC) were produced to assess the capacity of differentiation and human dystrophin expression in vivo. The DYS-HAC was detected in the all tissues examined and the dystrophin was detected in the sarcolemmal membrene of the chimeric muscle. Therefore, the combination of patient-specific iPS cells and HAC containing defective gene(s) provides a powerful tool for gene and cell therapies.

## 3148/F/Poster Board #1080

**Human FVIII expression using a HAC vector toward stem cell-mediated gene therapy for hemophilia A.**
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Hemophilia A, an X-linked hemorrhagic disorder due to mutations in the gene that encodes Factor VIII (FVIII), affects 1 in 5000 males. Clinically, severe hemophilia is characterized by spontaneous and prolonged hemorrhage that can result in disability and death. Current therapies include fixed-dose FVIII prophylaxis, factor replacement therapies, and most recently, gene therapy using viral vectors. These therapies are limited by incomplete efficacy, high cost, restricted availability, and the possible tumorigenesis in treated patient. Human artificial chromosome (HAC) is stably maintained as an independent chromosome in host cells and should be free from the potential insertional mutagenesis problem of conventional transgenes. Therefore, HAC has been proposed as an alternative implement to cell-mediated gene therapy. In this study, we sought to elucidate the potential of HAC vector carrying the human FVIII cDNA for gene therapy of Hemophilia A. We have demonstrated the production and secretion of FVIII using the HAC vector carrying multi-copies of FVIII, and that HAC vector can be maintained as an independent mini-chromosome without integration into the host genome in CHO cells. We observed the expression level of FVIII depends on the copy number on the HAC in CHO cell. Next, the FVIII-HAC was transferred from the CHO hybrids into human immortalized (hi) MSC using the microcell-mediated chromosome transfer (MMCT) technique. We also observed that FVIII was expressed in hiMSC with the FVIII-HAC as a function of copy number, i.e., 4 copies < 16 copies. These results suggest that the HAC vector is useful for regulated expression of transgenes in stem cell-mediated gene therapy.

## 3149/F/Poster Board #1081

**A novel non-integrated human artificial chromosome vector carrying a conditional centromere.**
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**Background** Conventional gene transfer techniques using a viral vector, plasmid, P1 phage-derived artificial chromosome (PAC), bacterial artificial chromosome (BAC) and yeast artificial chromosome (YAC) integrate randomly into the host chromosome. Whenever the gene expression becomes unnecessary, it is difficult to remove the transgene because of insertion into host chromosomes. Conditional gene expression system without host chromosomal integration will be useful for physiological analysis. **Results** Human artificial chromosome containing tet-oprerator sequence in the centromeric region (tetO-HAC) had previously produced (Nakano et al., Dev. Cell 2008). The function of centromere can be controlled w/ or w/o doxycycline after introducing tet-repressor rtTA gene. To be useful for gene delivery, we inserted a loxP cassette into the tetO-HAC by homologous recombination. By using the Cre/loxP recombination, we introduced the EGFP gene into the tetO-HAC, resulting in stable expression at least 2 weeks. The tetO-EGFP-HAC was eliminated by introduction of rTA with high efficiency. This elimination system with the tetO-HAC could not affect host chromosome stability. **Conclusion** Here, we established a conditional gene expression system using a non-integrated human chromosome vector. Hence, a non-integrated artificial chromosome vector will be a useful tool for the conditional gene expression.

## 3150/F/Poster Board #1082

**Adeno-associated virus (AAV) type8 mediated systemic neonatal gene therapy for hypophosphatasia.**
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Hypophosphatasia (HPP) is an inherited systemic skeletal disease caused by the deficiency of tissue-nonspecific alkaline phosphatase (TNALP). TNALP is attached to the outside plasma membrane via a GPI anchor and expressed in mainly liver, bone and kidney. Absence of TNALP activity results in extracellular accumulation of a natural substrate, inorganic pyrophosphate (PPi), which is a strong inhibitor for mineralization. HPP is clinically classified into four forms based on the age at onset and its severity. TNALP knock-out mice (Akp2-/-) phenotypically mimic to infantile HPP. They are born with a normally mineralized skeleton but develop apparent richets and die within 3 weeks, suffering severe skeletal hypomineralization and epileptic seizures. Recently, Millan et. al., demonstrated that Akp2-/- mice could be treated by daily subcutaneous injection of a bone-targeted form of TNALP in which a deca-aspartate (D10) sequence is linked to the C terminal end of soluble TNALP. We are currently trying to develop gene therapy approaches for HPP. In this experiment, we studied the feasibility of adeno associated virus (AAV) mediated systemic gene therapy of neonatal mice. We generated AAV vector expressing human soluble TNALP with a D10 sequence (AAV8-TNALPD10). In vitro experiments using culture cells showed that TNALP-D10 has high affinity to hydroxyapatite and enhances mineralization. Next, we injected AAV8-TNALPD10 (1x1012 vg/body) into the jugular vein of neonatal AKP2-/- mice at day 1. Sustained expression of TNALP activity in plasma (10.47 ± 4.3 U/ml) was detected in treated mice for at least 2 months. Mature bone mineralization was confirmed by X-ray analysis. Most significantly, treated HPP mice lived for more than 27 weeks with normal physical activity and healthy appearance, while non-treated mice died by 3 weeks. Ectopic calcification and abnormal calcium metabolism were not detected in treated mice. Furthermore, the mice treated with 1x1011 vg/body AAV8-TNALPD10 lived at least 9 weeks (n=5), while the mice treated with 1x1010 vg/body died within 3 weeks (n=7) as nontreated mice. These results suggest that AAV mediated systemic neonatal gene therapy is safe and effective to cure the infantile form of hypophosphatasia.

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### 3151/F/Poster Board #1083

**Adeno associated virus (AAV)-mediated neonatal gene therapy of metachromatic leukodystrophy.** *N. Miyake, K. Miyake, M. Yamamoto, T. Shimada.* Dept of Biochemistry and Molecular Biology, Nippon Medical School, Tokyo, Japan.

Lysosomal storage diseases (LSDs) are important targets for enzyme replacement and gene therapy. The success of gene therapy for LSDs with neurological involvement such as metachromatic leukodystrophy (MLD) depends on the development of efficient delivery of lysosomal enzymes and/or vectors across the blood-brain barrier (BBB) to achieve wide distribution of enzyme throughout the brain. Since both the immune system and the BBB are developmentally immature during the perinatal period, neonatal gene transfer may be a highly promising strategy to treat genetic neurological disorders. In this experiment, we studied the feasibility of adeno associated virus (AAV) mediated systemic neonatal gene therapy of MLD mice. First, we examined biodistribution of serotype 8 AAV vector expressing GFP (AAV8/GFP) after injection into the jugular vein of neonatal mice (C57BL/6). Strong GFP expression was detected in the liver, muscle, and heart one month after injection. In addition, widespread transduction throughout the brain including the cortex, cerebellum, olfactory bulb, and brainstem was observed after neonatal injection, indicating that systemic neonatal injection of AAV is an effective strategy to cross the BBB for gene transfer into the central nervous system, which is tightly protected from virus infection in adult mice. To treat MLD mice, we generated serotype 8 AAV vector expressing human arylsulfatase A (AAV8/ASA) and IV injected into neonatal MLD mice. ELISA analysis showed that sustained expression of ASA was detected in the liver, muscle, heart and brain for more than one year. Furthermore, in the behavior test, AAV8/ASA treated mice showed a significant improvement in their ability to traverse narrow balance beams, as compared to non-treated MLD mice (Latency: $8.6 \pm 0.4$ vs. $13.0 \pm 1.3$ sec, $P<0.05$; Slips: $2.9 \pm 0.3$ vs. $4.3 \pm 0.4$ times, $P<0.05$). These data suggest that systemic neonatal injection of AAV is an effective strategy for treatment of genetic neurological diseases such as MLD and Krabbe disease.

### 3152/F/Poster Board #1084

**Adenoassociated virus-derived vector mediated correction of Mucopolysaccharidosis IVA.** *A.M. Montaño[1], C.J. Almeciga-Diaz[2], S. Tomatsu[1], L.A. Barrera[2].* 1) Dept Pediatrics, St Louis Univ, St Louis, MO, USA; 2) Instituto de Errores Innatos del Metabolismo, Pontificia Universidad Javeriana, Bogotá, Colombia.

Morquio A disease (MPS IVA) is an autosomal recessive disorder caused by the deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS), leading to accumulation of keratan sulfate and chondroitin-6-sulfate mainly in bone and cornea. The disease is characterized by generalized skeletal dysplasia and normal intelligence. So far there is no effective therapy available for Morquio A patients. In this study we have used adenoassociated virus-derived vectors (AAV) to mediate the gene transfer of the human GALNS gene in-vitro and in-vivo. We used three different promoters to drive GALNS gene expression, the human cytomegalovirus major immediate early promoter/enhancer (CMV), human alpha-antitrypsine (AAT) and elongation factor 1-alpha (EF1). In HEK293 cells, the eukaryotic promoters AAT and EF1 induced similar expression levels to those obtained with the CMV promoter. Transfection of human MPS IVA fibroblasts produced GALNS enzyme levels of 36, 54 and 15% compared to normal human fibroblasts, for CMV-GALNS, AAT-GALNS and EF1-GALNS vectors, respectively, while in murine MPS IVA chondrocytes values were around 40 and 70% of normal values. These enzyme activity levels were increased between 1.5 and 4.0-fold when cells were co-transfected with an AAV vector carrying the human Sulfatase Modifying Factor-1 (SUMF1) gene, and allowed to raise normal enzyme levels in MPS IVA fibroblasts. Seven to eight week-old MPS IVA mice were intravenously infused with AAT-GALNS and CMV-SUMF1 vectors for 12 weeks. GALNS activity in plasma and tissues was around 20 and 40%, respectively, of wild-type levels. These enzyme levels allowed a complete clearance of glycosaminoglycans (GAGs) in liver of MPS IVA treated animals. However, no difference in urinary GAG levels was observed between MPS IVA untreated, treated and wild-type animals, showing the need to use a different biomarker or quantification method. Finally, no silencing of CMV promoter was observed neither in-vitro nor in-vivo, pointing towards an effect of the vector type over this silencing mechanism. Altogether these results are the first evidence of the enzymatic correction in a murine model of MPS IVA by gene therapy and show the advantage of the co-expression with SUMF1 in order to obtain therapeutic levels of enzyme activity.

### 3153/F/Poster Board #1085

**Long-term expression of human Factor IX in rhesus macaque following catheter-based delivery of HDAd.** *P. Ng[1], P.M. Patel[2], N.C. Grove[1], D. Palmer[1], A. Beaudet[1], C. Mullins[2], N. Brunetti-Pierri[1].* 1) Dept Molecular & Human Genetics, Baylor Colllege of Medicine, Houston, TX; 2) Dept Pediatric Cardiology, Baylor College of Medicine, Houston, TX.

Helper-dependent adenoviral (HDAd) vectors result in long-term transgene expression without chronic toxicity and therefore, they hold tremendous potential for liver-directed gene delivery for the treatment of inborn errors of hepatocyte metabolism including hemophilias. The rhesus macaque is an excellent nonhuman primate animal model for preclinical testing of hemophilia B gene therapy. The administration of first generation adenoviral (FGAd) vectors in rhesus macaques resulted in short term human Factor IX (hFIX) expression and the induction of high-titer, neutralizing anti-hFIX antibodies. The formation of neutralizing anti-hFIX antibodies by FGAd is a serious concern for gene therapy applications and therefore it should be carefully evaluated in the preclinical studies for HDAd vectors. Towards this goal, we have administered an HDAd expressing hFIX to four rhesus macaques through a balloon catheter strategy that we have previously developed. In this approach a balloon catheter is percutaneously positioned in the inferior vena cava to occlude hepatic venous outflow and $1x10^{11}$ or $1x10^{12}$ vp/kg of an HDAd expressing hFIX was injected into the liver via a hepatic artery catheter. To date, all four animals have exhibited sustained, therapeutic levels of hFIX and absence of neutralizing anti-hFIX antibodies. These results suggest that in contrast to FGAd, HDAd vectors can drive long-term FIX expression and do not result in neutralizing anti-hFIX antibody formation. Therefore, HDAd are attractive vectors for hemophilia B gene therapy.

### 3154/F/Poster Board #1086

**Efficient dystrophin restoration in mdx mice skeletal muscles is induced by ZM2 nanoparticles-AON complexes.** *P. Rimessi[1], M. Fabris[1], E. Bassi[1], S. Falzarano[1], P. Sabatelli[1,2], G. Vattemi[3], D. Perrone[4], F. Gualandi[1], N.M. Maraldi[5], L. Merlini[1], K. Sparnacci[6], M. Laus[6], P. Bonaldo[7], P. Braghetta[7], A. Ferlini[1].* 1) Dept Medical Genetics, University of Ferrara, Ferrara, Italy; 2) IGM-CNR, Unit of Bologna c/o IOR, Bologna, Italy; 3) Department of Neurological Sciences and Vision, Section of Clinical Neurology, University of Verona, Verona, Italy; 4) Department of Biology and Evolution, University of Ferrara, Ferrara, Italy; 5) Department of Human Anatomical Sciences, University of Bologna and Laboratory of Cell Biology, IOR, Bologna, Italy; 6) Department of Environmental and Life Sciences INSTM, University of Piemonte Orientale, Alessandria, Italy; 7) Department of Histology, Microbiology, and Medical Biotechnology; University of Padova, Padova, Italy.

Therapeutic approaches for Duchenne muscular dystrophy (DMD) represent now a realistic hope. However, obstacles must be overcame as optimising the non-toxic effective doses, improving the delivery system, reaching all affected tissues, obtaining a sustainable therapeutic effect, finding an easy-handling way of administration in view of life-long treatment. In this study we describe novel core-shell nanoparticles (marked as ZM2) we designed and prepared. These cationic nanoparticles are made up of a core of N-isopropil-acrylamide+ (NIPAM), and surrounded by a shell bearing cationic groups. The adsorption of 2'-O-methyl full-length phosphorothioate (2OMePS) AON onto ZM2 nanospheres, resulted in a high loading value, corresponding to 90 μg of AON per mg of nanoparticles. We injected intraperitoneally the ZM2/AON compound (7.5 mg/Kg of AON) in mdx mice for 7 weeks (1/week), and 1 week after the last administration we performed tissues analysis. Since counting positive fibers is considered a fundamental parameter for evaluating the dystrophin restoration efficiency, we compared two independent methods, the first manual and the second represented by a novel semi-automated and semi-quantitative using a software we developed in cooperation with Nikon (NIS-Elements 3.0 AR imaging program, Nikon). The two different approaches gave quite similar results, with dystrophin positive fibers ranging from 40% and 35%.RT-PCR and exon specific Real time RT-PCR assays showed an exon 23 skipping percentage of 10% in skeletal muscles. Western blotting analysis showed the presence of high molecular weight dystrophin protein in ZM2-AON compound treated mdx mice. In conclusion, this novel ZM2-AON compound greatly enhances the dystrophin restoration in the skeletal muscles of the mdx mice, confirming that these NPs represent a very promising vehicle for systemic delivering of AONs. The combination of slow release and depot effects, together with the protection from degradation/sequestration and the high AON binding capacity of this novel ZM2 NPs could be responsible for the relevant efficacy and efficiency. ACKNOWLEDGMENTS The Telethon Italy grants GGP05115 and GUP07011 (both to A.F.). Thanks are also due to Prof. A. Medici (Dept of Chemistry, University of Ferrara) and to the Industria Chimica Emiliana (ICE Reggio Emilia) Grant (to AF), to TREAT-NMD Network of Excellence of EU FP7 n. 036825 (to LM and Telethon-Italy).

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## 3155/F/Poster Board #1087

**Manipulating Gene Splicing to Rescue Common Mutations in Metabolic Disorders.** *L.M. Vincent, S.G. Ziegler, R. Hess, W. Westbroek, W.A. Gahl, M. Huizing.* Med Gen Br, NHGRI/NIH, Bethesda, MD.

Certain rare disorders, including the Hermansky-Pudlak (HPS), Griscelli (GS), and Chediak-Higashi syndromes (CHS), result from defects in the biogenesis of lysosome-related organelles (LROs), such as melanosomes in melanocytes, lytic granules in lymphocytes, and delta granules in platelets. Some clinical characteristics of LRO disorders include albinism, bleeding diathesis, infections, neurological impairment, and fatal pulmonary fibrosis. Directed therapy is difficult since little is known about the protein functions in LROs. In view of successful advances in therapeutic exon skipping (e.g., Duchene Muscular Dystrophy), we applied anti-sense morpholino oligonucleotides (MOs) to patients' cells to induce in-frame skipping of one or more exons carrying deleterious mutations that result in HPS type 1 (HPS-1) or CHS. RT-PCR analysis confirmed that all our MOs had at least 75% efficacy in removing their exon targets. Immunofluorescence (IF) staining against the melanosomal protein TYRP1 detected melanosome trafficking defects (lack of accumulation in dendritic tips) that are typical for HPS-1. MOs targeting *HPS1* exons 12 and 13 induced this phenotype in normal melanocytes and consequently failed to rescue the phenotype in patients' cells. Interestingly, an *HPS1* exon 15 MO, which targets the major 16bp duplication Puerto-Rican founder mutation, achieved partial rescue of the HPS-1 phenotype. Ongoing immunoblot assays against HPS1 and HPS4, which interact in the Biogenesis of Lysosome-related Organelles Complex (BLOC)-3, will further verify cellular functionality of these exon-skipped HPS1 isoforms. We also targeted CHS-causing mutations in the ARM/HEAT repeats of the gene *CHS1/LYST*. IF staining against lysosome associated membrane protein-3 (LAMP-3) of patients' fibroblasts detected the enlarged lysosomes typical for CHS. Removal of ~120kDa of CHS1, through concurrent skipping of exons 4, 5, and 6, induced a severe CHS phenotype in normal fibroblasts indicating significant loss of CHS1 function, while skipping of exons 11 and 12 only induced a mild loss of CHS1 function. Ongoing studies will determine if the skipping of exons 11 and 12 can rescue patients' fibroblasts. The transition from normal to defective HPS1 or CHS1 function can be followed using MOs and cell biological techniques to investigate the basic defect in these diseases. Thus, MO-induced exon skipping has the potential for determining the mechanism of action and treatment of genetic disorders.

## 3156/F/Poster Board #1088

**Antisense RNA/ethylene-bridged nucleic acids chimera induces exon skipping and restores dystrophin expression in myocytes of Duchenne muscular dystrophy.** *M. Yagi, Y. Yamauchi, H. Awano, Y. Takeshima, M. Matsuo.* Dept Pediatrics, Kobe Univ Graduate Sch Med, Kobe, Japan.

Duchenne muscular dystrophy (DMD) is a progressive muscle wasting disease, succumbing at twenties and caused by mutations in the dystrophin gene. Nearly two thirds of DMD cases are found to have exon deletion mutations in the dystrophin gene and shift the reading frame of dystrophin mRNA, thereby creating a stop codon. As a result, dystrophin is deficient in their muscle. On the other hand, mild Becker muscular dystrophy (BMD) is shown to have in-frame deletion mutation is the dystrophin gene, enabling truncated dystrophin production. It is supposed that transformation of an out-of-frame mutation into an in-frame mutation is a way of DMD treatment. We have long proposed that induction of exon skipping by antisense oligonucleotides is the most plausible therapy for DMD. Previous studies have demonstrated antisense phospholothioate oligonucleotides induced exon 19 [Pramono et al. 1996] and artificial induction of exon 19 skipping led to muscle dystrophin expression in vitro and in vivo [Takeshima et al. 2001][Takeshima et al. 2006]. Taken it into consideration that a 2'-O, 4'-C-ethlene-bridged nucleic acid (ENA) is highly nuclease-resistant and thermodynamically stable, we reported that an antisense RNA/ENA chimera was 40 times as effective as corresponding conventional phosphorotioate oligonucleotides in inducing exon 19 skipping [Yagi et al., 2004]. To develop the broad therapeutic applicability of this exon skipping strategy, it is necessary to identify the specific antisense oligonucleotides that can accurately and efficiently skip exons located in the deletion hot spots in the dystrophin gene. We focus to induce skipping of exon 45 that is located in one of the deletion hot spots. We designed many antisense RNA/ENA chimeras against exon 45 and examined their ability to induce exon skipping. The best antisense RNA/ENA chimera could induce exon 45 skipping in cultured myotubes from DMD patients with different types of deletions mutations neighboring exon 45. Remarkably, 100% of the dystrophin mRNA obtained from cultured myotubes with the antisense oligonucleotides treatment lacked exon 45. The production of dystrophin in treated myotubes was also confirmed. Our results make dystrophin expression possible in a broader spectrum of DMD cases.

## 3157/F/Poster Board #1089

**Evaluation of gene therapy for Hereditary Inclusion Body Myopathy (HIBM) using GNE-Lipoplex.** *T. Yardeni[1,4], C. Ciccone[1], J. Poling[1], L.M. Vincent[1], K. Patzel[1], I. Manoli[1], S. Hoogstraten-Miller[2], T. Ferrine[2], D. Darvish[3], Y. Anikster[4], J. Nemunaitis[5], P. Maples[5], C. Jay[5], W.A. Gahl[1], M. Huizing[1].* 1) Medical Genetics Branch, NHGRI/ NIH, Bethesda, MD; 2) OLAM, NHGRI, NIH, Bethesda, MD; 3) HIBM Research group, Encino, CA; 4) Tel-Aviv University, Sackler Faculty of Mdicine, ISRAEL; 5) Gradalis Inc., Dallas, TX.

HIBM is an adult-onset, progressive neuromuscular disorder, caused by *GNE* mutations. *GNE* encodes the ubiquitously expressed, key enzyme in sialic acid (SA) synthesis, UDP-GlcNAc 2-epimerase/ManNAc kinase. We created an HIBM mouse model, mimicking the Persian-Jewish founder mutation M712T. Mutant mice unexpectedly died before day 3 of life (P3) from severe glomerulopathy due to hyposialylation. Administration of the SA precursor ManNAc led to improved glomerular sialylation and survival of mutant pups beyond P3. We are pursuing a therapeutic trial for HIBM using ManNAc, which has yet to be approved by regulatory authorities. In addition to this proposal to manipulate compounds in the SA pathway, delivery of an intact *GNE* gene may be a feasible treatment option. We created a human non-viral GNE-plasmid embedded in cationic liposomes (*h*GNE-lipoplex), which was administered via intramuscular injection (biceps and extensor carpi radialis longus) to one HIBM patient. The patient showed no adverse effects and her arm muscle strength improved moderately. To proceed with *h*GNE-lipoplex intravenous (IV) or intrahepatic (the major organ of SA synthesis) injections in patients, we tested these treatments in our HIBM mice. *h*GNE-lipoplex was injected IV (retro-orbitally) and intra-hepatically in litters at age P1. Survival beyond P3 was the initial outcome parameter. The major tissues of treated mice were assessed at P5 for *h*GNE expression, glomerular disease, sialylation, and histology. Intrahepatic treatment yielded survival of all wild type (+/+) and heterozygous (+/-) pups, but just one of 8 mutants survived beyond P3; that pup had increased sialylation of the glomerular glycoprotein podocalyxin at P5. IV injections resulted in no surviving mutants beyond P3; however, some +/- pups died as well, indicating possible lethality of the injection method. Surviving +/+ showed *h*GNE expression in liver and kidney at P5, indicating no toxicity of *h*GNE-lipoplex and effective gene delivery to these tissues. It is possible that *h*GNE-lipoplex treatment at P1 (untreated mice die at P3) may not allow enough time for *h*GNE protein to be expressed and to produce enough SA, whereas the ManNAc treatment immediately delivers the substrate, bypassing the critical GNE processing step, allowing for faster production of SA. Further animal studies are planned to elucidate the timing and efficacy of GNE-lipoplex therapy.

## 3158/F/Poster Board #1090

**AAV2/8-Mediated Gene Therapy Provides Prolonged Protection Against Biochemical Induction of Acute Attacks and Improves Motor Function in Acute Intermittent Porphyria Mice.** *M. Yasuda[1], D.F. Bishop[1], M.E. Fowkes[2], S.H. Cheng[3], L. Gan[1], R.J. Desnick[1].* 1) Gen & Genomic Sci, Mount Sinai Sch Med, New York, NY; 2) Pathology, Mount Sinai School of Medicine, New York, NY; 3) Genzyme Corporation, Framingham, MA.

Acute Intermittent Porphyria (AIP) is an autosomal dominant inborn error of hepatic heme biosynthesis due to the half-normal activity of hydroxymethylbilane synthase (HMB-synthase). Patients with AIP are prone to life-threatening acute neurological attacks that are precipitated by various factors (e.g., fasting, cytochrome P450 inducing drugs, menstruation, alcohol, etc.) that induce hepatic heme biosynthesis and result in increased plasma and urinary $\delta$-aminolevulinic acid (ALA) and porphobilinogen (PBG). The acute attacks are currently treated with intravenous hematin, but a more continuous therapy is needed, particularly for patients experiencing frequent attacks. Thus, a recombinant AAV8-based serotype vector expressing murine HMB-synthase driven by the liver-specific $\alpha$1-microglobin enhancer and $\alpha$1-antitrypsin promoter was generated, and its effectiveness to prevent the biochemical induction of an acute attack was evaluated in an AIP mouse model with ~30% residual HMB-synthase activity. Intraperitoneal administration of the AAV vector resulted in a rapid and dose-dependent increase of HMB-synthase activity, most marked in the liver. Administration of 7.6 x $10^{11}$ viral particles achieved wild-type or greater levels of HMB-synthase activity that were stably sustained for over 40 weeks. Treated mice were resistant to the characteristic accumulation of urinary ALA and PBG when periodically challenged with intraperitoneal injections of phenobarbital, a potent inducer of hepatic heme biosynthesis, thus indicating that the expressed enzyme was functional *in vivo*. Notably, the treated mice performed significantly better on rotarod and footprint analyses compared to untreated controls at 32 weeks of treatment, although AAV therapy did not prevent the development of peripheral motor neuropathy. In conclusion, these studies demonstrated successful long-term correction of the hepatic metabolic abnormalities and improvement of motor function, and thus provide the rationale for the development of AAV-mediated therapy for patients with recurrent AIP attacks.

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## 3159/F/Poster Board #1091

**Development of Functionalized Mesoporous Silica Nanoparticles as Potential Hepatic Theranostic Agents.** *Y.H. Zhang[1], B.L. Huang[1], M. Liong[2], N. Henderson-MacLennan[1], J. Zink[2,3], E.R.B. McCabe[1,3,4].* 1) Dept Pediatrics, UCLA Sch Medicine, Los Angeles, CA; 2) Dept Chemistry and Biochemistry, UCLA, Los Angeles, CA; 3) California NanoSystems Institute, UCLA, Los Angeles, CA; 4) Department of Human Genetics and Bioengineering, UCLA, Los Angeles, CA.

Mesoporous silica nanoparticles (MSNPs), functionalized with tissue-specific ligands, iron oxide cores and therapeutic drugs, have been used for targeting, imaging and drug therapy in culture (Liong et al. ACS Nano 2:889-896, 2008). Lactobionic acid (LA) is galactose chemically linked to gluconic acid, and is reported to be a specific ligand for the hepatocyte-specific asialoglycoprotein receptor (ASGPR). LA-conjugated particles have been used in culture and in vivo (Kamruzzaman Selim et al. Biomaterials 28:710-716, 2007), but not with MSNPs. Our goal is to develop a hepatocyte-specific delivery system for improved diagnostics (e.g. enhanced MRI) and therapeutics (e.g. small molecules, DNAs and proteins), referred to as "theranostics." We coupled MSNPs with LA (LA-MSNPs) and tested intracellular uptake and toxicity. Preliminary cell culture experiments showed that LA-MSNPs were internalized into HepG2 cells. We are evaluating the uptake of LA-MSNPs into HepG2 cells compared with MCF7 (breast adenocarcinoma) cells. We are also comparing the uptake of LA-MSNPs with uptake of unmodified MSNPs. The preliminary experiments indicated that the LD50 for LA-MSNPs, as a measure of cytotoxicity, was 2000ug/ml despite substantial, receptor-mediated association with HepG2 cells. These data suggest relatively low toxicity. We are testing the LA-MSNPs in cell lines before testing these in vivo to optimize the functionalized MSNPs before investing the time and money on whole animal experiments. We speculate that theranostic nanoparticles will gain broad application in the diagnosis and treatment of genetic diseases.

## 3160/F/Poster Board #1092

**Clinical phenotype of Becker muscular dystrophy patients with deletions generated by exon 51 skipping.** *A.T.J.M. Helderman-van den Enden[1], C.S.M. Straathof[2], A. Aartsma-Rus[1], J.T. den Dunnen[1], B.M. Verbist[3], E. Bakker[1], J.J.G.M. Verschuuren[2], H.B. Ginjaar[1].* 1) Center for Human and Clinical Genetics Leiden University Medical Center, Leiden, Netherlands; 2) Department of Neurology of the Leiden University Medical Center; 3) Department of Radiology of the Leiden University Medical Center.

Mutations in the dystrophin-encoding DMD gene on the X chromosome result generally in Duchenne muscular dystrophy (DMD) if the mutation is out-of-frame and in Becker muscular dystrophy (BMD) if the mutation preserves the reading frame. Consequently, in BMD patients an altered form of dystrophin is present that is partially functional, whereas in DMD patients dystrophin is virtually absent in muscle fibers. DMD patients may benefit from antisense-mediated skipping of exon 51 to restore the reading frame, which would result in the production of a shortened, Becker-like dystrophin protein. The course and prognosis of DMD patients could then be converted into that of BMD, if these antisense oligonucleotides (AON) can be successfully delivered to all skeletal muscles. Local intramuscular treatment with AON was successful in a DMD patient with an exon 50 deletion. Systemic antisense-mediated exon skipping is currently tested in phase I/II clinical trials. We describe the clinical features in two adult Becker patients with a deletion of exons 45-51 and in one schoolboy with a deletion of exons 50-51 of the DMD gene. They all had minimal complaints and no restrictions in daily life. The sharp contrast in clinical features between the severe exon 50 related DMD phenotype and the mild exon 50-51 BMD phenotype suggests that early AON treatment of DMD patients could have a major impact on clinical outcome.

## 3161/F/Poster Board #1093

**Novel approaches for characterizing molecular networks involved in the maintenance of skeletal muscle mass.** *E. Andres-Mateos[1], T.N. Burks[1], R. Marx[1], J.L. Simmers[1], E.M. MacDonald[1], T.G. Marr[3], D.K. Vaughan[4], R.D. Cohn[1,2].* 1) Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Pediatrics and Neurology, Johns Hopkins University School of Medicine, Baltimore, MD; 3) Hiberna Corporation, Boulder, CO; 4) Department of Biology and Microbiology, University of Wisconsin Oshkosh, Oshkosh, WI.

A plethora of genetic disorders is associated with significantly decreased skeletal muscle mass and concomitant muscle weakness and atrophy. These conditions include, but are not limited to, primary skeletal muscle disorders such as muscular dystrophies, structural myopathies and metabolic myopathies. Loss of skeletal muscle mass can also be observed in a variety of peripheral and/or central nervous system disorders such as the mitochondriopathies, spinal muscular atrophy and amyotrophic lateral sclerosis. The underlying pathogenetic phenomena can vary but are generally thought to be the net result of perturbed satellite cell function accompanied by increased muscle proteolysis and decreased protein synthesis. In stark contrast, hibernating mammals have evolved to survive prolonged periods of immobilization without any significant loss of muscle mass. The molecular mechanisms underlying this phenomenon are poorly understood, and we therefore explored the molecular pathways involved in the maintenance of muscle mass in the hibernating 13-lined ground squirrel (Spermophilus tridecemlineatus). Comparative analysis of various skeletal muscles from hibernating and non-hibernating squirrels reveals no significant differences in the morphology or size of the muscle fibers, but in hibernating animals, we do observe a switch from fast-type muscle fibers to slow-type fibers, which are more resistant to injury. Further molecular analysis during hibernation demonstrates a downregulation of muscle atrophy pathways that involve FOXO3a, independent of the activation of Akt. Interestingly, we also observe an up-regulation of AMPK and an increased expression of PGC-1$\alpha$. Moreover, we find an increased expression of anti-apoptotic proteins during hibernation. In summary, our results demonstrate for the first time that a remarkable pattern of decreased proteolysis is accompanied by increased mitochondrial metabolism and protection against apoptosis,in a physiological animal model of prolonged immobilization. Thus, our data provide new insights into the molecular pathways involved in maintenance of muscle mass and protection against atrophy. This approach will enable us to identify the key regulatory molecules responsible for these pathway alterations, opening new avenues for therapeutic interventions in patients with a variety of inherited and acquired forms of skeletal muscle atrophy and degeneration.

## 3162/F/Poster Board #1094

**Use of intravenous ketamine in pain management in NF1-associated glomus tumors: A case report.** *S. Coombes[1], A. Mannes[2], D. Stewart[1].* 1) NHGRI, Bethesda, MD; 2) NIH Clinical Center, Bethesda, MD.

Glomus tumors are small, benign tumors that arise from the glomus body, a thermoregulatory shunt concentrated in the fingertips. Glomus tumors are associated with the monogenic tumor-predisposition disorder neurofibromatosis type 1 (NF1). Glomus tumors often present with a triad of tenderness, cold insensitivity and paroxysmal pain. In our experience with 4 adults with NF1, surgical excision is often, but not always, curative. Pain recurrence could not always be attributed to identifiable recurrent tumor burden. We present a case report of a 37-year-old Hispanic female with NF1and multiple glomus tumors of the fingers. At the time of her presentation, she had a 5-year history of severe pain in the 3rd, 4th and 5th digits of both hands that left her unable to work. Her symptoms and imaging of the hands were consistent with glomus tumors and the complex regional pain syndrome (CRPS). Over a period of 2 years, she underwent three surgeries (including glomus tumor excision and neurectomy) on her fingers, however, after a short period of relief, her pain would return. Conventional treatment strategies, including gabapentin, pregabaline, local blocks, topical lidocaine, hydromorphone and methadone did not provide adequate relief. Multiple stellate ganglion blocks provided only temporary relief. Given the intractability of her pain, we administered low-dose (10 $\mu$g/kg/hr) intravenous ketamine over three days. She also received serial bupivacaine blocks of the left fourth digit. Prior to treatment, she reported 10/10 pain, especially in the fourth finger of the left hand. She also had hyperesthesia, allodynia, and flushing of the left hand. The fingertip of the left fourth finger was visibly swollen. After 3 days of intravenous ketamine, she reported 3/10 pain in the left fourth digit. The swelling of the left fourth digit had resolved. At eight weeks of follow-up, she reports that her fingers remain essentially pain-free. She also reports that her frequent migraine headaches and sciatica symptoms have also resolved since the ketamine infusion. Treatment with intravenous ketamine appears to be successfully controlling this patient's severe, refractory pain secondary to CRPS.

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## 3163/F/Poster Board #1095

**Landscape analysis of biobanks and registries as a tool to enhance translational research systems.** *S.F. Terry, J. Bialick, K. Zonno, L. Horn.* Genetic Alliance, Washington, DC.

Clinical data registries and biospecimen repositories are crucial for enabling translational research by providing samples for gene discovery, study of disease biology and pathways, and development of new tests and targeted therapies. Redundant repositories and registries for specific diseases are abundant and new registries and biobanks are proliferating around institutions, communities and states. The registry and biobank landscape is highly variable, both in purpose and process. In an attempt to understand the variability and discern best practices, we undertook a landscape analysis in May 2009 to examine registries' and biorepositories' structures and processes. A comprehensive survey regarding the following aspects of registries and biobanks was sent out within the field: collection and storage of clinical data and specimens, confidentiality, privacy, and security of data and samples, regulatory compliance, as well governance, management and finances. Using the listserv of the professional society of biobank directors, as well as direct contact with many biobank directors, we were able to survey them. Results showed a wide variety of methods for data collection and storage, highly variable consenting processes, generally poor privacy protections, very little donor-centric focus and disparate regulatory compliance. Furthermore, none of the biobanks surveyed indicated that they had any ability to collect patient data from an EMR/PHR system despite the fact that nearly half stored their information in a secure online server. Of great concern in a general lack of interoperability, and little attention to standardized language. The data gathered led to registry and biobank best practices and recommendations. These are useful to both the registry and biobanking industry and also to policy makers as these repositories become more ubiquitous.

## 3164/F/Poster Board #1096

**Brain delivery of recombinant acid sphingomyelinase by ICAM-1-targeted nanocarriers.** *C. Garnacho[1], R. Dhami[2], E. Schuchman[2], S. Muro[1,3].* 1) Center for Biosystems Research, U. Maryland Biotechnology Institute, College Park, MD; 2) Dept. of Genetics and Genomics Sciences, Mount Sinai School of Medicine, New York, NY; 3) Fischell Dept. of Bioengineering, U.Maryland College Park, College Park, MD.

Genetic deficiency of acid sphingomyelinase (ASM) causes types A and B Niemann-Pick disease (NPD), leading to aberrant lysosomal accumulation of sphingomyelin and cholesterol, multi-organ dysfunction, and premature mortality, which in Type A NPD is due to neurological defects. Enzyme replacement therapy (ERT) by intravenous infusion of recombinant ASM shows promising therapeutic potential, yet this strategy is suboptimal for enzyme delivery to the brain. As an example to overcome this obstacle, we coupled ASM to polymer nanocarriers targeted to intercellular adhesion molecule-1 (ICAM-1), a surface protein abundantly expressed in diseased brain endothelium, as well as neuronal and glial cells. ASM was absorbed on 180 nm poly(lactic-co-glycolic acid) nanocarriers coated with anti-ICAM (anti-ICAM/ASM NCs). C57Bl/6 and ASM knockout mice were injected with anti-ICAM/125I-ASM NCs vs naked 125I-ASM, and the isotope content was determined from blood and brain 30 min post-injection. Data were calculated as percent of the injected dose (% ID) and brain/blood localization ratio (LR, %ID per gram of tissue / %ID per gram of blood). Brain targeting was also visualized by fluorescence microscopy using polystyrene anti-ICAM/ASM NCs or control IgG/ASM NCs. Although naked 125I-ASM was detected in the brain (0.32±0.06 %ID), a high fraction of the enzyme was in circulation 125I-ASM at that time (32.7±5.9 %ID). Hence, the brain/blood localization ratio for 125I-ASM was 0.04±0.01. In contrast, anti-ICAM/125I-ASM NCs were detected in the brain (0.3±0.02 %ID) but the fraction of carriers in circulation was significantly lower (12.2±0.06 %ID), and the brain/blood localization ratio was 0.12±0.01. Therefore, brain-tissue targeting of anti-ICAM/125I-ASM/NCs was ~3 fold over that of the naked enzyme. Fluorescence microscopy confirmed specific targeting of anti-ICAM/ASM NCs in the brain. Anti-ICAM/ASM NCs had a different distribution pattern at 5-15 min versus 30-60 min post-injection. At earlier time points anti-ICAM/ASM NCs looked scattered on the brain vasculature, whereas the carriers formed ring-like structures at the later time points, suggesting endocytic transport to perinuclear lysosomes. Therefore, ICAM-1-targeted nanocarriers hold promise to enhance brain targeting of enzyme replacement for NPD and, likely, other lysosomal disorders.

## 3165/F/Poster Board #1097

**Improved delivery of alpha-galactosidase to Fabry disease endothelial cells by ICAM-1-targeted nanocarriers.** *J. Hsu[1], D. Serrano[2], C. Garnacho[3], S. Muro[1,3].* 1) Fischell Dept. of Bioengineering, U. Maryland College Park, College Park, MD; 2) Cell Biology and Molecular Genetics, U. Maryland College Park, College Park, MD; 3) Center for Biosystems Research, U. Maryland Biotechnology Institute, College Park, MD.

Fabry disease is a lysosomal storage disorder (LSD) characterized by genetic deficiency of $\alpha$-Galactosidase A ($\alpha$-Gal), which causes aberrant intracellular storage of globotriaosylceramide (Gb3). It mainly affects vascular endothelial cells (ECs), resulting in vascular lesions, myocardial ischemia, hypertension, cerebrovascular damage, thrombosis, and premature death. Enzyme replacement therapy (ERT) by infusing recombinant $\alpha$-Gal in circulation is an available treatment for Fabry disease. However, lack of specific affinity of this enzyme to ECs results in rapid clearance from the bloodstream. To overcome this obstacle, we propose to target $\alpha$-Gal to intercellular adhesion molecule 1 (ICAM-1), an EC surface molecule highly expressed in diseased endothelium. To evaluate this strategy we coupled model coffee bean $\alpha$-Gal and anti-ICAM to the surface of 100 nm, FITC-labeled polystyrene prototypes or biodegradable PLGA nanocarriers (anti-ICAM/$\alpha$-Gal NCs), and tested their binding, endocytosis, lysosomal transport, and Gb3 degradation (vs that of naked $\alpha$-Gal) by fluorescence microscopy in macro- and micro-vascular ECs, either control or treated with deoxygalactonojirimycin and TNF$\alpha$ to mimic Gb3 accumulation and inflammation in Fabry disease. Anti-ICAM NCs bound specifically to both EC types, validating the adequacy of this strategy for targeting these two distinct vascular beds. Targeting was similar for control cells and the Fabry model, and was not compromised by coating $\alpha$-Gal along with anti-ICAM on the carrier surface. ECs internalized >80% anti-ICAM/$\alpha$-Gal NCs within 1 h, which was mediated via amiloride-sensitive ICAM-mediated endocytosis, distinct from the clathrin mechanism utilized for classical Fabry disease ERT. Anti-ICAM/$\alpha$-Gal NCs were transported to Texas red dextran-labeled lysosomes and co-localized with intracellular Gb3 by 3-5 h after their uptake by ECs. Finally, incubation of cells with chloroquine (to neutralize the lysosomal pH and inhibit endogenous lysosomal enzymes while permitting activity of the coffee bean $\alpha$-Gal) and anti-ICAM/$\alpha$-Gal NCs resulted in the reduction of Gb3 accumulation to control values in Fabry EC models, vs naked Gb3 which only partially attenuated Gb3 accumulation. These results indicate that ERT assisted by ICAM-1-targeted nanocarriers has the potential to evolve into a new ERT for Fabry disease and, perhaps, other LSDs.

## 3166/F/Poster Board #1098

**Enhanced delivery of lysosomal enzyme replacement therapies by targeting ICAM-1 versus receptors of clathrin and caveolar endocytic pathways.** *M. Meng[1], C. Garnacho[2], S. Muro[2,3].* 1) Dept. of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Center for Biosystems Research, U. Maryland Biotechnology Institute, College Park, MD; 3) Fischell Dept. of Bioengineering, U. Maryland College Park, College Park, MD.

Genetic deficiency of acid sphingomyelinase (ASM) causes Niemann-Pick disease (NPD), leading to excess lysosomal storage of sphingomyelin and cholesterol, neurologic and pulmonary pathology. Enzyme replacement therapy (ERT) by infusion of recombinant ASM is based on the presence of mannose-6-phosphate on this enzyme, which target ASM to cell receptors mediating lysosomal delivery via clathrin pits. However, the efficacy of this strategy is suboptimal. Targeting ASM to the cell adhesion molecule ICAM-1, by coupling ASM to anti-ICAM nanocarriers, switched the uptake pathway from clathrin pits to non-classical cell adhesion molecule (CAM) endocytosis and improved ASM delivery in vitro and in vivo. To define parameters which provide such an enhanced delivery, we compared the accessibility of ICAM-1 to that of receptors of classical endocytosis, and explored how NPD lipid storage affects classical vs CAM endocytosis required for intracellular ASM delivery. Isotope tracing in mice showed that antibodies to transferrin receptor and PV1 (the protein of caveolae diaphragm), associated to clathrin- and caveolar- pathways, only accessed their targets from the circulation when administered as free counterparts (12.5±3.5 and 17.4±0.3 % injected dose, ID, in lungs) but not when coupled to carriers (0.8±0.02 and 3.5±0.4 %ID in lungs). Contrary, ICAM-1 was accessible to both free anti-ICAM (6.5±0.2 %ID in lungs vs 1.2±0.2 % for IgG) and anti-ICAM nanocarriers (25.5±1.6 %ID in lungs vs 1.3±0.3 % for IgG nanocarriers). In cell culture, fluorescence tracing showed that clathrin-mediated uptake of transferrin was decreased in NPD fibroblasts (71±3 % of control), which also displayed altered clathrin recruitment to transferrin binding sites (17±2% co-localization in NPD cells vs. 69±9% in normal cells). Disease cells also showed decreased caveolar-mediated uptake of cholera toxin (70±9 % of normal cells) and abnormal recruitment of caveolin-1 to cholera toxin binding sites (7±2% co-localization in NPD cells vs. 83±3% in normal cells). Contrary, CAM-mediated endocytosis of anti-ICAM nanocarriers was not affected in NPD fibroblasts (102±7% of control). Hence, both high accessibility of ICAM-1 to anti-ICAM nanocarriers and efficient CAM endocytosis, as opposed to determinants and pathways of classical endocytosis, support enhanced ASM delivery by anti-ICAM nanocarriers and may represent a valuable means to improve ERT for NPD and, likely, other lysosomal storage disorders.

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## 3167/F/Poster Board #1099

**Disabling autophagy in skeletal muscle permits fully effective enzyme replacement therapy in a lysosomal storage disease, Pompe Disease.** *N. Raben, C. Schreiner, R. Baum, S. Takikita, P. Plotz.* NIAMS, ARB, NIH, Bethesda, MD.

Autophagy, a major pathway for delivery of proteins and organelles to lysosomes, has been implicated in many cellular and developmental processes and in several human diseases, including Pompe disease, a lysosomal storage disorder caused by deficiency of acid alpha-glucosidase (GAA). The enzyme is responsible for the breakdown of glycogen within the lysosomes. Clinically the disease manifests as a profound cardiac and skeletal muscle myopathy. The current enzyme replacement therapy (ERT) with recombinant GAA (Myozyme, Genzyme Corp.) clears glycogen effectively in the heart, but substantially less so in skeletal muscle. In our GAA KO model and in patients with Pompe disease, we have observed an upregulation of autophagy and large pools of autophagic debris in skeletal muscle cells. In the Pompe mice, the autophagic buildup and the resistance to therapy are linked and limited to fast fibers. Since autophagy is a presumed mechanism of glycogen delivery to the lysosomes we tried to reduce both the amount of lysosomal glycogen and the autophagic accumulation by knocking out either Atg5 or Atg7, two critical autophagic genes, in the skeletal muscle of Pompe mice. Suppression of autophagy alone resulted in a somewhat diminished glycogen load. Following ERT, however, the skeletal muscle glycogen was reduced to normal or near normal levels. This successful clearance of lysosomal glycogen has never been observed in Pompe mice with genetically intact autophagy. Furthermore, following ERT, these glycogen-free lysosomes became functionally competent, as evidenced by a dramatic reduction in the level of ubiquitinated proteins. Because this therapeutic approach leaves muscle cells deficient in autophagy, we analyzed muscle-specific autophagy-deficient wild type mice. We have found that disabling autophagy in skeletal muscles of wild type mice has no significant effect on their apparent strength, mobility, weight, or lifespan. We are currently exploring the feasibility of autophagy suppression in skeletal muscle on a temporary basis as a therapeutic approach. It has recently become clear that the upregulation of autophagy is a common feature in a variety of lysosomal storage disorders; therefore, the suppression of autophagy may have a broad application to these and other classes of diseases with autophagic dysfunction.

## 3168/F/Poster Board #1100

**Cystine Depletion of Cystinotic Fibroblasts by Cystinosin.** *J. Thoene, M. Witcher.* Pediatrics, D5420 MPB0718, Univ Michigan, Ann Arbor, MI.

Cystinosis results from defective lysosomal cystine transport, leading to lysosomal cystine storage. Cystinosin, encoded by CTNS, at 17p13.3, functions to move cystine from the lysosome. To determine if cystinosin administration to cultured cystinotic fibroblasts could correct cystine storage, the human cystinosin gene was subcloned into pFastBacHTA vector and the resulting construct transformed into E.coli DH10Bac to generate recombinant Bacmid. Positive colonies were selected by Gm, Tet and Kan, followed by PCR identification. The recombinant Bacmid was extracted and then transfected into Sf9 cells to generate the baculovirus, P1 viruses were amplified, and the target protein identified by Western blot using a recombinant antibody to an added 6-His tag on the protein N-terminus. Reactivity was found to remain mainly in the cell pellet, and not seen in the medium.This portion of the work performed under contract by GenScript Corporation. Removal of medium after 5 days incubation on transfected Sf9 cells, dialysis into Ham's F12 and addition of this dialyzed medium (Cys) to cystinotic fibroblasts produced a 63% cystine loss over 4 days compared to negligible loss induced by media from outside the dialysis membrane(Contr).See table. Results are per cent of initial values for two experiments in cell line GM 00008 and 1 in GM 00046. Initial cystine content was 4.56 and 3.48 nmol cystine /mg cell protein for GM00008, and 2.99 in GM 00046. We speculate that cystinosin in the medium is either too dilute, or modified such as not to be seen on Western.

| T(h) | 0 | 24 | 48 | 96 |
|---|---|---|---|---|
| Cys | 100 | 70 | 52 | 37 |
| Contr | 100 | 84 | 102 | 78 |

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## 3169/F/Poster Board #1101

**Down syndrome, Ts65Dn, mice benefit from neural progenitor cell treatment.** *K.B. Bjugstad[1], S.K. Cornelius[1], E.Y. Snyer[2], K.N. Maclean[1].* 1) Pediatrics, University of Colorado Denver, Aurora, CO; 2) Stem Cells & Regenerative Medicine, The Burnham Institute for Medical Research, La Jolla, CA.

Down syndrome (DS) is the most common cause of genetic mental retardation. It results from the triplication of human chromosome 21. The genes found at 21q21-21q22.3 are associated with the cognitive deficits of DS and may reveal the etiology of Alzheimer's disease (AD). All DS brains examined have amyloid plaques, neurofibrillary tangles, and neuronal loss, decades before the general population. The genes carried on chromosome 21 that might be involved in AD include the amyloid precursor protein, superoxide dismutase 1, and carbonyl reductase. The Ts65Dn mouse is a model of DS /AD, and carries a partial triplication of mouse chromosome 16, the portion that is analogous to 21q21-21q22.3. The triplication of these genes produces a mouse that has many of the cognitive deficits associated with pediatric development and later AD. The trisomic mouse was used to determine if disomic neural precursor cells (NPC) could attenuate cognitive dysfunction and preserve the neuronal population during aging. Young (2m) and aged (10m) trisomic and disomic littermates were implanted bilaterally into the hippocampus with murine-derived NPC (C17.2 line). Three months post-transplant, mice underwent cognitive behavior testing. Young (now 5m) and Aged (now 13m) mice were tested in conditioned taste avoidance (CTA) where learning is determined by the avoidance of a novel flavor. Aged mice were further tested in the Morris water maze (MWM), a task of spatial learning. At the end of behavior testing, brains were examined for preservation of host hippocampal neurons using immunohistochemistry against microtubule associated protein 2(MAP2). Trisomic mice were impaired in the CTA, final MWM latencies, and increased MWM search perseveration. The implantation of NPC improved groups' CTA performance and shortened the trisomic group's final MWM latencies, but had no effect on perseveration. Trisomic mice had fewer CA1 and CA3 neurons than disomic littermates. Aging potentiated neuronal loss in CA3 and dentate gyrus (DG) of trisomic mice. The implantation of NPC increased cell numbers in all aged mice but in the DG, only the trisomic mice benefited. CA1 appeared unaffected by NPC presence. While trisomic mice suffered from various cognitive deficits and possessed fewer hippocampal neurons, implantation of NPC rescued some cognition and preserved neurons in CA3 and DG. These NPC induced effects in both young and aged models of DS, support their potential use as a treatment for DS/AD.

## 3170/F/Poster Board #1102

**Systemic transplantation of Human Adipose-Derived Stem cells into the Golden Retriever Dystrophic dog.** *N.M. Vieira[1], C.R. Bueno Junior[1], V. Brandalise[1], M.P. Brolio[2], A.L. Reginato[2], E. Zucconi[1], M. Secco[1], M.M. Camargo[3], M. Vainzof[1], M.A. Miglino[2], M. Zatz[1].* 1) Human Genome Research Center, Biosciences Institute, Univ Sao Paulo, Sao Paulo, Brazil; 2) School of Veterinary Medicine and Animal Science, Department of Anatomy, Univ Sao Paulo, Sao Paulo, Brazil; 3) Department of Immunology, Instituto de Ciências Biomédicas, Univ Sao Paulo, Sao Paulo, Brazil.

Progressive muscular dystrophies (PMD) are a group of disorders characterized by progressive degeneration of skeletal muscle caused by the absence or defective muscular proteins. The possibility to restore the faulty muscle protein and improve muscular performance by cell therapy is a promising approach for the treatment of PMD. Different animal models are available for pre-clinical studies; however the only animal model that reproduces the full spectrum of human Duchenne Muscular Dystrophy pathology is the Golden Retriever Muscular Dystrophy (GRMD) dog. Affected animals carry a splice site mutation in intron 6 of the dystrophin gene. This mutation predicts a premature termination codon in exon 8 and a peptide that is 5% the size of normal dystrophin. These dogs present clinical signs within the first weeks of life involving the limbs as well as masticatory muscles. Diaphragmatic and intercostal muscles impairment leads to progressive respiratory failure. Death occurs from bronchopneumonia and cardiac arrest, usually before 2 years of age. Here we have injected human adipose-derived stem cells (hADSC) intravenously, without immunosupression, into 2-months old GRMD male dogs. These animals received 5 . $10^7$ cells . kg-1 for six months, weekly in the first month and then monthly. This methodoly was used aiming to compare the results with our previous observed data in the SJL mice model, using the same protocol. We are currently evaluating their ability to: engraft into recipient dystrophic muscle after systemic delivery; form chimeric human/canine muscle fibers; express human muscle proteins in the dystrophic host and improve muscular performance. The results may have important applications for future therapies in patients with different forms of muscular dystrophies. SUPPORTED by FAPESP, INCT, CNPQ and FINEP.

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